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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

(steroidogenesis/steroid hormones/testis/ovary/Leydig cells)

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ABSTRACT **Observations of patients deficient in the steroidogenic enzyme 3β-hydroxy-Δ^5-steroid dehydrogenase/isomerase (3βHSD) have suggested the presence of distinct 3βHSD structural gene(s) that are expressed at peripheral sites, possibly the liver. We now report the isolation of cDNA clones representing three forms of 3βHSD from mouse Leydig cell and liver libraries. The three forms share significant identity but differ from each other by 5–10% within their coding regions. RNA that hybridizes to radiolabeled 3βHSD probes is present in the gonads, adrenal glands, liver, and kidneys of both sexes. Ribonuclease protection analysis using antisense probes derived from each of the three forms demonstrates that one form, 3βHSD I, is restricted to steroidogenic tissues. Two other forms, 3βHSD II and III, are expressed in liver and kidney but are not detected in steroidogenic tissues. A polyclonal antibody raised against the human placental form of 3βHSD recognizes a 42-kDa protein in gonadal and adrenal tissue and a 45-kDa protein in liver. The antibody recognizes a 42-kDa protein in kidney only weakly. 3βHSD enzyme activity is present in testicular, adrenal, hepatic, and renal tissue, with adrenal tissue possessing the highest specific activity. When expressed as total 3βHSD activity for whole organ mass, activity is greatest in the liver. The results demonstrate that the mouse liver is a significant site of 3βHSD activity and demonstrate the existence of multiple 3βHSD structural genes in the mouse.**

The enzyme 3β-hydroxy-Δ^5-steroid dehydrogenase/isomerase (3βHSD; EC 1.1.1.145) catalyzes the conversion of the 3β-hydroxy-Δ^5-steroids pregnenolone and dehydroepiandrosterone to the 3-keto-Δ^4-steroids progesterone and androstenedione, respectively. Progesterone is a precursor of the adrenal steroids cortisol, corticosterone, and aldosterone; androstenedione is the precursor of the gonadal steroids testosterone and estrogens. Therefore, 3βHSD activity is essential for the production of all steroid hormones and is found in all steroidogenic tissues, including adrenal glands, testes, ovaries, and human placenta. Evidence for the presence of 3βHSD activity in nonsteroidogenic tissue has been provided by several investigators. 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 the source of activity in peripheral tissue may be the product of distinct 3βHSD structural genes (6–10). These reports include male and female patients who exhibit adrenal hyperplasia and various degrees of male pseudohermaphroditism but 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. The recent availability of a polyclonal antibody raised against the purified human placental 3βHSD protein (11) and the cloning of the human placental 3βHSD cDNA (12, 13) have made it possible to investigate the existence of multiple 3βHSD genes and their tissue-specific expression in the mouse.‖

MATERIALS AND METHODS

cDNA Libraries and Isolation of cDNA Clones. A Leydig cell cDNA library was constructed with poly(A)$^+$ RNA isolated from Leydig cells of adult CD-1 mice. Purification of Leydig cells and preparation of poly(A)$^+$ RNA have been described (14–16). The library was constructed in λgt11 by using a Riboclone cDNA synthesis kit (Promega) and *in vitro* packaging extracts (Stratagene). A male CD-1 mouse liver cDNA library in λUni-ZAP was a gift from John Gearhart (Johns Hopkins University, Baltimore). An adult male BALB/c mouse liver cDNA library, both random and oligo(dT)-primed, was purchased from Clontech.

A 1200-base-pair (bp) *Eco*RI–*Sac* I restriction fragment of a human placental 3βHSD cDNA (12), radiolabeled by the random hexanucleotide primer method (17, 18), was used to screen 6×10^4 plaques of the Leydig cell cDNA library. Nitrocellulose filters were hybridized for 12–16 hr with probe (10^6 cpm/ml) at 54°C by standard techniques. A 906-bp *Sac* I–*Bgl* II restriction fragment of the mouse clone 3βHSD I was used to screen 2.4×10^5 plaques of the mouse λUni-ZAP liver library and a 296-bp *Xba* I–*Eco*RI fragment containing 266 bp from the 5′ region of the 3βHSD I clone and 30 bp of the vector was used to screen 1.8×10^5 plaques of the BALB/c mouse library. Standard procedures were used in manipulating the clones and in generating maps of restriction sites (16).

DNA Sequence Analysis. Sequencing was performed using a Sequenase kit (United States Biochemical) (19), pBluescript (Stratagene), and double-stranded templates (20). Subclones for sequencing were prepared by creating deletions with convenient restriction enzymes or by the creation of

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Abbreviations: 3βHSD, 3β-hydroxy-Δ^5-steroid dehydrogenase/isomerase; DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone.

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‖The sequences reported in this paper have been deposited in the GenBank data base [accession nos. M58567 (3βHSD I), M75886 (3βHSD II), and M77015 (3βHSD III)].

nested deletions by limited exonuclease III digestion (21). Some regions were sequenced using synthetic primers.

Analysis of RNA. Male and female CD-1 mice were killed at ≈60 days of age by CO_2 asphyxiation. Tissues were removed, quickly frozen on dry ice, and stored at −70°C until needed. Total RNA was isolated from frozen tissues and analyzed by Northern blotting (15, 18).

Ribonuclease protection analysis was performed as described (22). Antisense probes were transcribed with T7 RNA polymerase from templates subcloned into pBluescript KS and were labeled with [α-^{32}P]CTP. The full-length type I probe was 114 nucleotides long, including 104 nucleotides corresponding to the type I sequence beginning at the *Sac* I site of 3βHSD I (nucleotide 233; Fig. 1) and extending to an *Nco* I site at nucleotide 129. The full-length type II probe was 185 nucleotides long, 138 of which corresponded to the type II sequence and extended from the *Bgl* II site at nucleotide 688 to an *Rsa* I site at nucleotide 550. The full-length type III probe was 243 nucleotides long, including 188 nucleotides corresponding to the type III sequence and extending from the *Sph* I site at nucleotide 570 to a *Stu* I site at nucleotide 382. The probes were hybridized at 48°C for 16 hr to RNA extracted from tissues of male and female mice. Hybridization mixtures were treated with ribonucleases A and T1 (40 and 2 μg/ml, respectively) for 40 min at 30°C. Probe fragments protected from ribonuclease digestion were resolved in a denaturing 6% polyacrylamide gel.

Immunoblot Analysis. Samples of frozen tissue were homogenized in 50 mM potassium phosphate-buffered saline (150 mM NaCl/50 mM potassium phosphate, pH 7.4) and were then adjusted to an SDS concentration of 1%. Leydig cell lysates were prepared by treatment with 1% SDS in 50 mM sodium phosphate-buffered saline. After electrophoresis, the proteins were transferred to a nitrocellulose membrane as described (23). Blots were probed with primary antibody (IgG fraction of a rabbit antiserum raised against the

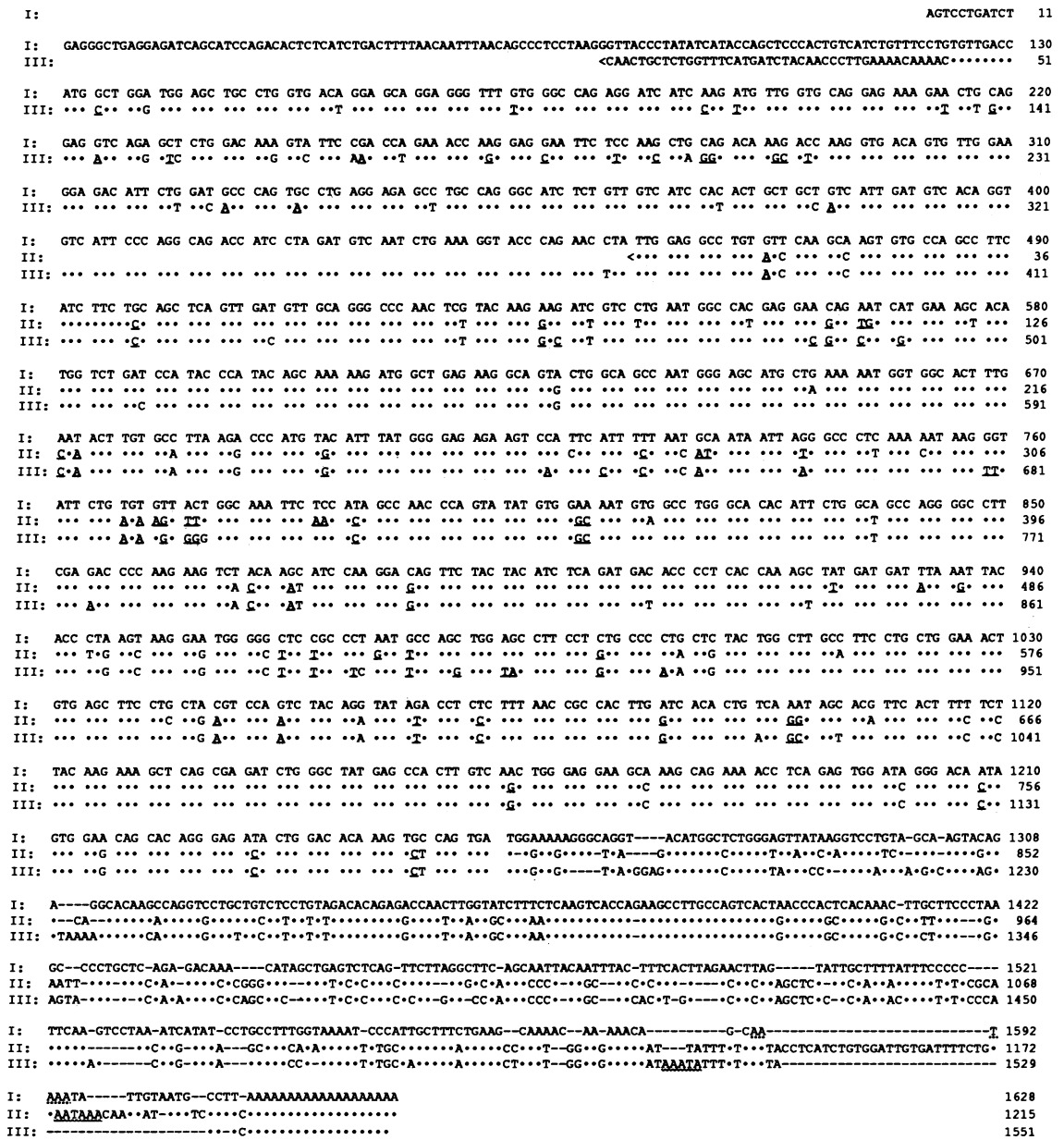

FIG. 1. Nucleotide sequences of mouse 3βHSD types I, II, and III shown aligned with the reading frame of 3βHSD I. Only those nucleotides in 3βHSD II and III that differ from 3βHSD I are given; identical nucleotides are represented by dots (•). Nucleotide substitutions that result in amino acid substitutions in the predicted amino acid sequence are underlined. Dashes in the 3′ untranslated regions indicate gaps introduced into the sequence to maximize alignment. Potential polyadenylylation signals are indicated with a dotted underline.

human placental 3βHSD; ref. 11) and diluted 1:2500, and immunoreactive proteins were detected with ^{125}I-labeled protein A (24).

Measurement of 3βHSD Enzyme Activity in Tissue Homogenates. Enzyme activity of 3βHSD was determined by measuring the conversion of [^{3}H]pregnenolone to [^{3}H]progesterone, [^{3}H]dehydroepiandrosterone (DHEA) to [^{3}H]androstenedione, and [^{3}H]dihydrotestosterone (DHT) to [^{3}H]androstanediol (25). Aliquots of tissue homogenates in 50 mM potassium phosphate-buffered saline, pH 7.4, were incubated at 37°C with the appropriate ^{3}H-labeled steroid substrate at a concentration of 2 μM and 0.5 mM NAD^+ (pregnenolone and DHEA) or NADH (DHT) in a total volume of 1 ml. Testicular and adrenal homogenates were incubated for 5 min; liver, kidney, and spleen homogenates were incubated for 30 min. Steroids were extracted, separated by thin-layer chromatography, and quantitated as described (25).

RESULTS

Isolation and Characterization of Mouse 3βHSD cDNA Clones. The mouse Leydig cell cDNA library was screened with a 1.2-kilobase (kb) *Eco*RI–*Sac* I fragment of a human placental 3βHSD cDNA clone (12). Two cDNA clones >1600 nucleotides in length were examined. The sequence of 3βHSD I shown in Fig. 1 is a composite of two clones, consisting of the entire 1608-nucleotide sequence of one cDNA and 20 nucleotides from the 3′ end of the second. The sequence contains a single 1122-nucleotide open reading frame encoding a protein of 373 amino acids. The predicted molecular weight, 42,059, is consistent with electrophoretic estimates of the molecular weight of the human placental 3βHSD protein (13, 26, 27), and the mouse testicular and mouse adrenal 3βHSD proteins (24). The ATG codon is flanked by the eukaryotic consensus sequence for translation initiation, including the invariant purine at −3 (28). The A+T-rich 3′ untranslated sequence contains a polyadenylylation signal (AATAAA) 14 nucleotides before the poly(A) tail. The nucleotide sequence of 3βHSD I shares 74% and 73% identity with the human placental 3βHSD and bovine ovarian 3βHSD nucleotide sequences, respectively (13, 29).

Using a 906-bp *Sac* I–*Bgl* II fragment of the Leydig cell 3βHSD I cDNA, a λUni-ZAP mouse liver cDNA library was screened for clones representing hepatic 3βHSD transcripts. Six clones were identified. Analysis by restriction mapping and sequencing demonstrated that the hepatic clones fell into two groups, 3βHSD II and 3βHSD III. To obtain cDNAs containing the entire open reading frame of 3βHSD II and III, a second mouse liver library, derived from adult male BALB/c mice, was screened using a 296-bp *Xba* I–*Eco*RI fragment of a 3βHSD I cDNA clone. One of the clones isolated was determined to be identical to the 3βHSD III obtained from the λUni-ZAP mouse liver library. This clone of 1518 bp contains the complete coding region, a large 3′ untranslated region, and 51 bp of the 5′ untranslated region. The sequence of 3βHSD III shown in Fig. 1 is a composite of two 3βHSD III clones, including the entire BALB/c clone and 33 bp from the 3′ end of the λUni-ZAP clone. The open reading frame is predicted to encode a protein with a molecular weight of 42,028. The sequence of the longest 3βHSD II clone isolated is shown in Fig. 1. It contains sequences corresponding to amino acids 109–373 of 3βHSD I and III and a complete 3′ untranslated sequence including a poly(A) tail. The coding regions 3βHSD I and III are 89.4% identical at the nucleotide level. The coding region of 3βHSD II is 90% and 94% identical to 3βHSD I and III, respectively. Within the 3′ noncoding region, 3βHSD I is 62% and 59% identical to 3βHSD II and III, respectively. Types II and III are 73% identical within this region. Within the 5′ untranslated region of 3βHSD III there is an 8-nucleotide stretch of complete identity with 3βHSD I immediately preceding the open reading frame. Further 5′ of this region, no identity between 3βHSD I and III is observed.

Expression of 3βHSD mRNAs in Steroidogenic and Nonsteroidogenic Tissues of Male and Female Mice. Total RNA from mouse adrenal gland, brain, spleen, kidney, liver, and gonads was examined by Northern analysis using the 906-bp *Sac* I–*Bgl* II fragment of the 3βHSD I cDNA (Fig. 1). This probe detected a 1.7-kb mRNA in samples from adrenal gland, ovary, testis, liver, and kidney (Fig. 2). No 3βHSD mRNA was detected in spleen or brain tissue (or lung, data not shown). Hybridization of the blot with a probe for β-actin demonstrated that the absence of 3βHSD mRNA in brain, spleen, and lung was not due to degradation of mRNA in these tissues (data not shown). In addition to the 1.7-kb mRNA, kidney contained a 1.9-kb mRNA that was not apparent in the other tissues examined. The levels of the 1.7-kb mRNA were much higher in adrenal gland and ovary than in testis, liver, and kidney. Note that the total amount of RNA applied to the lanes labeled ovary and adrenal gland was one-tenth of the amount applied to all other lanes (2 μg vs. 20 μg).

To examine the tissue-specific expression of the different types of 3βHSD among murine tissues, RNA isolated from gonads, liver, kidney, and adrenal glands of adult male and female mice was subjected to ribonuclease protection analysis using radiolabeled, antisense RNA probes transcribed from distinct regions of each 3βHSD form. The experiment depicted in Fig. 3 is representative of several ribonuclease protection experiments using the various probes either alone or in combination with RNA isolated from the tissues of three mice of each sex. A protected fragment of 104 nucleotides specific for the 3βHSD I probe was observed only in RNA from testis, ovary, and adrenal gland. No fragment of 104 nucleotides was detected in RNA from liver or kidney of either sex. Note that only 0.1 μg of RNA was used in the ovary and adrenal gland lanes instead of 10 μg as for other tissues. The 138-nucleotide protected fragment specific for 3βHSD II was detected only in liver and kidney RNA of both sexes (Fig. 3). The amount of 3βHSD II RNA in kidney was consistently greater than in liver. A 188-nucleotide protected fragment specific for 3βHSD III also was detected only in liver and kidney, but there was no apparent difference between the amount of 3βHSD III in liver and kidney. Neither 3βHSD II nor 3βHSD III was detected in adrenal or ovary RNA, even when 1.0 μg of total RNA from these tissues was examined (data not shown). These results demonstrate that expression of 3βHSD I is restricted to steroidogenic tissues, whereas 3βHSD II and III are found in the liver and kidney of both sexes.

Immunoblot Analysis of 3βHSD Protein. Tissue homogenates from male mice were subjected to SDS/PAGE and Western blotting using an antibody raised against human

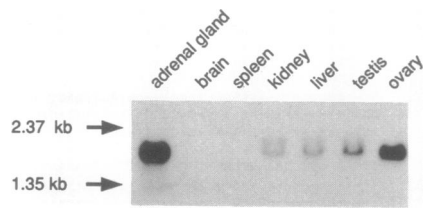

FIG. 2. Northern blot analysis of 3βHSD transcripts in steroidogenic and nonsteroidogenic tissues. The probe used was a 906-bp *Sac* I–*Bgl* II fragment of the clone 3βHSD I. All tissues were from male mice except for the ovary. Each lane contained 20 μg of total RNA except for the ovary and adrenal gland lanes, which contained 2 μg. The final washing of this blot was done in 15 mM NaCl/1.5 mM sodium citrate, pH 7/0.1% SDS at 60°C.

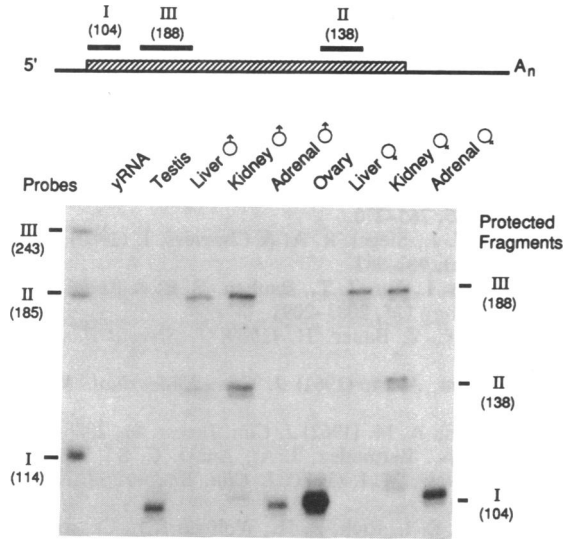

FIG. 3. Ribonuclease protection analysis of RNA from mouse gonads, liver, kidney, and adrenal gland with probes specific for 3βHSD I, II, and III. Diagram at the top shows the positions and lengths of the three probes (black bars; the number in parentheses is the length, in nucleotides, of the protected fragment produced by the probe). For the experiment depicted all three probes were added simultaneously. At left, the positions of the full-length probes are indicated. The positions of the specific protected fragments are indicated at right. The amount of RNA used in each lane was 10 μg except for the ovary and for the adrenal glands of both sexes, where 0.1 μg was used. yRNA, yeast RNA.

placental 3βHSD (11). The specificity of this antiserum for mouse Leydig cells and adrenal glands was validated previously (24). Fig. 4 illustrates a representative blot exposed for 24 hr. A single protein of 42 kDa was observed in Leydig cells, testis, adrenal gland, and kidney. Liver, however, exhibited a single immunoreactive protein of 45 kDa. No immunoreactive band was detected in lysates of spleen even after a 96-hr exposure. In addition, exposure of the blot for 96 hr did not detect an immunoreactive protein of 42 kDa in liver or a 45-kDa protein in any of the other tissues examined. Although the amount of total protein in the kidney sample was identical to the amount of protein applied from testis and liver and greater than 3-fold the amount from adrenal glands, the intensity of the kidney 42-kDa immunoreactive protein was very weak. These data demonstrate that the liver expresses a 3βHSD immunoreactive protein of a different size than is expressed in steroidogenic tissues. The data suggest that either the 3βHSD immunoreactive protein in the kidney is distinct from the one expressed in the steroidogenic tissues

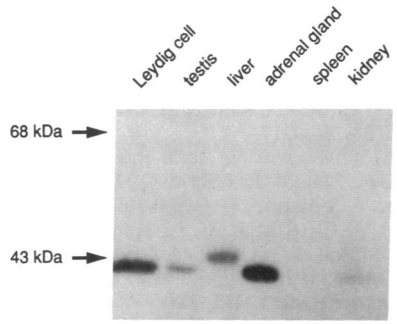

FIG. 4. Immunoblot analysis of 3βHSD protein from various mouse tissues. For the testis, liver, and kidney lanes, 100 μg of total protein was loaded on the gel; for spleen, 75 μg; and for adrenal gland, 30 μg. The Leydig cell lane contained the total protein from 5×10^4 Leydig cells. All tissues were from male mice.

Table 1. Specific activity of 3βHSD in mouse tissues

Tissue	Specific activity, pmol/min per μg of protein		
	Pregnenolone	DHEA	DHT
Testis	0.502 ± 0.031	0.651 ± 0.026	3.94 ± 0.39*
Adrenal	8.39 ± 0.69	9.09 ± 0.51	44.9 ± 3.0
Liver	0.097 ± 0.015	0.121 ± 0.013	0.666 ± 0.070*
Kidney	0.016 ± 0.002*	0.030 ± 0.001	0.187 ± 0.009
Spleen	<0.002	0.007 ± 0.001	0.016 ± 0.001

Values are means ± SEM for three mice or two mice (asterisks).

and the liver or that there is decreased translation or increased degradation of the protein.

3βHSD Enzymatic Activity. The 3βHSD enzyme activity was determined in tissue homogenates from a variety of male mouse tissues. Activity was measured using two 3β-hydroxy-Δ^5-steroids, pregnenolone and DHEA, and a 5α-reduced steroid, DHT, as substrates. Enzyme activity was determined in tissues from the same animal and, unless otherwise indicated, represents the mean from three different mice. Specific activity in adrenal gland was greater than 10-fold that observed in the testis, which was 5-fold greater than the activity observed in liver (Table 1). Specific activity in kidney was considerably lower than that in liver. 3βHSD activity in spleen was negligible. When 3βHSD enzyme activity is expressed as total activity per organ, a very different ratio of activities in the various tissues is observed (Table 2). The liver exhibits by far the greatest amount of total 3βHSD enzyme activity for all three of the substrates. Total 3βHSD activity was 5-fold greater in the liver than in testes and ≈10-fold greater than in the adrenal glands. Total enzyme activity in the kidneys was somewhat less than that observed in adrenal glands. 3βHSD enzyme activity in spleen was negligible. Comparison of 3βHSD enzyme activity among the three substrates indicates that activity with the 5α-reduced steroid as substrate was 5-fold greater than with the 3β-hydroxy-Δ^5-steroids as substrates. In all tissues examined, enzyme activity was slightly greater with DHEA as the substrate rather than pregnenolone.

DISCUSSION

The isolation of three distinct cDNAs from murine libraries demonstrates the presence of multiple forms of 3βHSD mRNA in murine tissues. The possibility of multiple 3βHSD genes in mammalian tissues was first suggested by clinical observations of 3βHSD-deficient patients who exhibited adrenal hyperplasia and male pseudohermaphroditism but who showed signs of virilization during puberty (6–10). In Southern blots of mouse genomic DNA with either of two distinct 3βHSD I probes, multiple restriction fragments were observed (P.A.B. and A.H.P., unpublished data), a finding consistent with the presence of multiple 3βHSD genes in the mouse. Similar results have been obtained in genomic Southern blots of human DNA (30). Nucleotide substitutions in the cDNAs reported here are scattered throughout the length of the sequence, suggesting that the different types of 3βHSD

Table 2. Activity of 3βHSD in mouse organs

Organ	Activity, nmol/min per total organ mass		
	Pregnenolone	DHEA	DHT
Testes*	9.08 ± 0.29	11.9 ± 0.3	68.6 ± 3.2*
Adrenals*	4.68 ± 0.68	5.31 ± 0.89	25.8 ± 4.1
Liver	44.8 ± 10.0	54.7 ± 9.1	301 ± 95*
Kidneys*	1.44 ± 0.30*	3.30 ± 0.13	18.1 ± 1.1
Spleen	<0.05	0.14 ± 0.02	0.36 ± 0.06

Values are means ± SEM for three mice or two mice (asterisks).
*Paired testes, adrenals, and kidneys.

mRNA are products of distinct genes rather than the result of alternative splicing mechanisms. Thus, the results of this study demonstrate the existence of multiple 3βHSD structural genes in the mouse and indicate that the various 3βHSD genes comprise a multigene family that is present both in humans and in rodents.

The ribonuclease protection experiments demonstrate that the three forms of 3βHSD exhibit marked tissue-specific expression in the mouse, with 3βHSD I being expressed in steroidogenic tissues and 3βHSD II and III being expressed in liver and kidney. The adrenal glands of both sexes and the ovary appear to be the richest sources of 3βHSD I, having 10- to 100-fold more 3βHSD I mRNA per unit of total RNA than the testis (Figs. 2 and 3). It must be taken into consideration, however, that Leydig cells, which are the only testicular cells to express 3βHSD, represent <5% of all cells in the mouse testis (31). Our results also indicate that 3βHSD II is more abundant in the kidney than in the liver and that 3βHSD III is equally abundant in both tissues (Fig. 3). Whether it is 3βHSD II or III or an as yet unidentified form of 3βHSD that is responsible for the 1.9-kb band observed in kidney RNA cannot be determined from the results of these experiments. Although we have not isolated a unique mouse kidney 3βHSD cDNA, several additional observations suggest that the kidney may express a gene distinct from the ones expressed in the liver, gonads, and adrenal glands. For instance, the amount of 3βHSD mRNA in the male kidney is approximately equal to that in the testis based on equal amounts of total RNA, yet the amount of antigenic protein and the amount of enzyme activity are considerably less (Fig. 4; Tables 1 and 2). The male kidney also exhibits very low enzyme activity with the 3β-hydroxy-Δ^5-steroids, pregnenolone and DHEA, as substrates. These data suggest that the complement of 3βHSD mRNA and protein expressed in the kidney is different from that present in the liver or steroidogenic tissues.

While this manuscript was in preparation two reports appeared providing evidence for more than one 3βHSD gene in the rat. Zhao *et al.* reported the isolation of a rat liver-specific 3βHSD cDNA (32) and two types of 3βHSD cDNAs isolated from a rat ovarian library that were distinct from the rat liver 3βHSD cDNA (33). Both type I and type II cDNAs were expressed in rat ovaries, testes, and adrenal glands. It is premature to make comparisons between the different rat and mouse 3βHSD gene products.

The high capacity of the liver for the conversion of 3β-hydroxy-Δ^5-steroids to 3-keto-Δ^4-steroids suggests that the liver could play an important role in overall steroid hormone production. Furthermore, hepatic expression of at least two 3βHSD gene products that are distinct from the gonadal and adrenal gene products may explain the clinical manifestations of 3βHSD enzyme deficiency (6–10). A distinct liver protein that can convert 3β-hydroxy-Δ^5-steroids to 3-keto-Δ^4-steroids, especially DHEA, which is produced in large amounts by the adrenal gland in patients exhibiting 3βHSD deficiency, could account for various degrees of virilization in male and female patients. The difference in the predicted molecular weight of 3βHSD III and the molecular weight of the immunoreactive protein identified in liver homogenates by immunoblot analysis suggests that the 3βHSD protein(s) may undergo posttranslational modification in the liver. Future studies should determine the importance of peripheral conversion of 3β-hydroxy-Δ^5-steroids to 3-keto-Δ^4-steroids in the mouse and the human.

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1. Milewich, L., Shaw, C. B. & Sontheimer, R. D. (1988) *Ann. N.Y. Acad. Sci.* **548**, 66–89.
2. Devine, P. L., Kelly, N. S. & Adams, J. B. (1986) *J. Steroid Biochem.* **25**, 265–270.
3. Weidenfeld, J., Siegel, R. A. & Chowers, I. (1980) *J. Steroid Biochem.* **13**, 961–963.
4. Jung-Testas, I., Hu, Z. T., Baulieu, E. E. & Robel, P. (1989) *Endocrinology* **125**, 2083–2091.
5. Bauer, H. C. & Bauer, H. (1989) *J. Steroid Biochem.* **33**, 643–646.
6. Bongiovanni, A. M. (1961) *J. Clin. Endocrinol. Metab.* **21**, 860–862.
7. Bongiovanni, A. M. (1962) *J. Clin. Invest.* **41**, 2086–2092.
8. Parks, G. A., Bermudez, J. A., Anast, C. S., Bongiovanni, A. M. & New, M. I. (1971) *J. Clin. Endocrinol. Metab.* **33**, 269–278.
9. Rosenfield, R. I., Rich, B. H., Wolfsdorf, J., Cassorla, F. S., Parks, J. S., Bongiovanni, A. M., Wu, C. H. & Shackelton, C. H. L. (1980) *J. Clin. Endocrinol. Metab.* **51**, 345–353.
10. Pang, S., Levine, L. S., Stoner, E., Opitz, J. M., Pollack, M. S., Dupont, B. & New, M. I. (1983) *J. Clin. Endocrinol. Metab.* **56**, 808–818.
11. Doody, K. M., Carr, B. R., Rainey, W. E., Byrd, W., Murry, B. A., Strickler, R. C., Thomas, J. L. & Mason, J. I. (1990) *Endocrinology* **126**, 2487–2492.
12. Lorence, M. C., Murray, B. A., Trant, J. M. & Mason, J. I. (1990) *Endocrinology* **126**, 2493–2498.
13. Luu-The, L., Lachance, Y., Labrie, C., Leblanc, G., Thomas, J. L., Strickler, R. C. & Labrie, F. (1989) *Mol. Endocrinol.* **3**, 1310–1312.
14. Quinn, P. G. & Payne, A. H. (1984) *J. Biol. Chem.* **259**, 4130–4135.
15. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **162**, 156–159.
16. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), 2nd Ed.
17. Feinberg, A. & Vogelstein, B. (1984) *Anal. Biochem.* **137**, 266–267.
18. Hales, D. B. & Payne, A. H. (1989) *Endocrinology* **124**, 2099–2104.
19. Tabor, S. & Richardson, C. C. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 4767–4771.
20. Lim, H. M. & Pene, J. J. (1988) *Gene Anal. Techniq.* **5**, 32–39.
21. Henikoff, S. (1984) *Gene* **28**, 351–359.
22. Gilman, M. (1990) in *Current Protocols in Molecular Biology*, eds. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (Wiley Interscience, New York), Vol. 1, pp. 4.7.1–4.7.8.
23. Perkins, L. M. & Payne, A. H. (1988) *Endocrinology* **123**, 2675–2681.
24. Nolan, C. J. & Payne, A. H. (1990) *Mol. Endocrinol.* **4**, 1459–1464.
25. Stalvey, J. R. D. & Payne, A. H. (1984) *Endocrinology* **115**, 1500–1505.
26. Thomas, J. L., Berko, E. A., Faustino, A., Myers, R. P. & Strickler, R. C. (1988) *J. Steroid Biochem.* **31**, 785–793.
27. Thomas, J. L., Myers, R. P. & Strickler, R. C. (1989) *J. Steroid Biochem.* **33**, 209–217.
28. Kozak, M. (1987) *Nucleic Acids Res.* **15**, 8125–8148.
29. Zhao, H.-F., Simard, J., Labrie, C., Breton, N., Rhéaume, E., Luu-The, V. & Labrie, F. (1989) *FEBS Lett.* **259**, 153–157.
30. Lorence, M. C., Corbin, C. J., Kamimura, N., Mahendroo, M. S. & Mason, J. I. (1990) *Mol. Endocrinol.* **4**, 1850–1855.
31. Mori, H., Shimizu, D., Fukunishi, R. & Christensen, A. K. (1982) *Anat. Rec.* **204**, 333–339.
32. Zhao, H.-F., Rhéaume, E., Trudel, C., Couët, J., Labrie, F. & Simard, J. (1990) *Endocrinology* **127**, 3237–3239.
33. Zhao, H.-F., Labrie, C., Simard, J., de Launoit, Y., Trudel, C., Martel, C., Rhéaume, E., Dupont, E., Luu-The, V., Pelletier, G. & Labrie, F. (1991) *J. Biol. Chem.* **266**, 583–593.