Rat Dihydriodiol Dehydrogenase: Complexity of Gene Structure and Tissue-specific and Sexually Dimorphic Gene Expression

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ABSTRACT

Dihydriodiol dehydrogenase (DD; EC 1.3.1.20) catalyzes a novel pathway of polycyclic aromatic hydrocarbon (PAH) metabolism in which trans-dihydriodiol (proximate carcinogens) are oxidized to reactive o-quinones which are cytotoxic and genotoxic. In this study, the complementary DNA for rat liver DD was used to examine the structure and regulation of the DD gene. Southern analysis of rat genomic DNA confirmed that DD is a member of the multigene aldo-keto reductase superfamily. Conservative estimates indicate that the rat DD gene is at least 20-25 kilobases in length. Northern analysis showed that the rat liver transcript was 2.4 kilobases whereas the complementary DNA contains an open-reading frame of 966 nucleotides. Primer extension of male and female polyadenylated RNA indicated that the major transcription start sites are only 53 and 54 base pairs upstream from the translation start site, confirming that the RNA has a very long 3'-untranslated region. In male and female tissues, 2.4 kilobase transcripts predominate in liver, small intestine, and lung, which is consistent with a role for the enzyme in PAH metabolism. Transcripts were also detected in male (prostate)- and female (ovary, mammary gland, and uterus)-specific tissues. In the ovary, two transcripts were observed of 2.4 and 1.4 kilobases in length. Using bandedihydriodiol as a model substrate for PAH trans-dihydriodiol, highest levels of DD activity were observed in the liver and small intestine of both sexes. Enzyme activity is 2.5-fold higher in the female liver versus the male liver. This sexual dimorphism can be explained by increases in the DD mRNA and enzyme protein as measured by dot-blot and immunoprecipitation analyses, respectively. The latter measurements indicate that DD represents 1.0% of the soluble protein in female liver but is only 0.5% of the soluble protein in male liver. Hormonal ablation (ovariectomy and hypophysectomy) abolishes the sexual dimorphism observed in levels of DD mRNA, enzyme protein, and enzyme activity. Administration of estrogens to males is sufficient to establish the female pattern of gene expression. These data indicate that DD gene expression is hormonally regulated, that estrogens exert their effect at the level of the mRNA, and that aldo-keto reductases involved in PAH metabolism may have their expression regulated by female sex hormones.

INTRODUCTION

PAHs are human carcinogens which are activated by host metabolism to anti- and syn-diol-epoxides which exert mutagenic and carcinogenic effects (1). DD (EC 1.3.1.20) can suppress the formation of the epoxides since it will oxidize their anti-diol forms to generate superoxide anion and semiquinone radicals (2). Treatment of rat and human hepatoma cells with these PAH o-quinones results in cell death, and this has been attributed to either the production of superoxide anion or the depletion of glutathione (3). PAH o-quinones also have the potential to be genotoxic. Model studies with benzo[a]pyrene-7,8-dione, a product of the DD reaction, with calf-thymus DNA indicated that the level of adduct formation was comparable to that observed in reactions containing the anti-diol-epoxide of benzo[a]pyrene ([±]-anti-7,8,9,10-tetrahydrobenzo[a]pyrene] (4). The ability of PAH o-quinones to act as cytotoxins and genotoxins indicates that DD may initiate a new pathway of PAH activation.

In several species and tissues, DD copurifies with 3α- or 17β-HSD or aldehyde reductase (10-14). In rat liver, DD and 3α-HSD (EC 1.1.1.50) activities are catalyzed by the same enzyme (14). Additionally, this protein functions as an aromatic aldehyde, ketone, and quinone reductase (14), as a hydroxyprostaglandin dehydrogenase (15), and as a Y'-bile acid binder (16). The cDNA for rat liver DD has been cloned, sequenced, and overexpressed in Escherichia coli (17-20). Rat liver DD demonstrates high sequence similarity (>58%) with members of the aldo-keto reductase superfamily which are monomeric NADPH-dependent oxidoreductases (M, 34,000) with overlapping substrate specificity and catalyze the reduction of carboxyls on a variety of endogenous substrates (steroids and prostaglandins) and xenobiotics (21). Members of this family include bovine lung prostaglandin F synthase (22); the rat, bovine, and human aldose reductases (23-25); human aldehyde reductase (25); and human chlordecone reductase (26). Recently, the cDNA for a human bile acid binding protein has been cloned and expressed as a fusion protein (27). The thrombin-digested fusion protein oxidized benzenedihydriodiol and has 70% sequence identity at the amino acid level with rat liver DD. Thus both rat and human DD are related and are members of the aldo-keto reductase superfamily.

The contribution of DD to PAH metabolism and activation will depend upon the tissue-specific expression of this enzyme versus other enzymes that compete for trans-dihydriodiol proximate carcinogens. Even though DD is constitutively expressed and comprises 0.5% of the soluble protein in male rat liver, its expression is elevated in females. Earlier studies in which enzyme activity was measured following hormonal manipulation indicated that this sexual dimorphism was due to estrogens (28, 29). Furthermore, studies in immature hypophysectomized male and female rats indicated that both estrogens and growth hormone were able to increase enzyme activity (29). However, it was unclear from these data whether these increases could be explained by alterations in DD mRNA. Few studies have been performed with the available cDNAs for DD to examine gene structure or regulation.

In this paper, we use the cDNA for rat liver DD to probe the structure of the rat DD gene, to document tissue- and sex-specific gene expression, and to demonstrate that sexual dimorphic gene expression in the liver is regulated by estrogens at the level of the mRNA. The relevance of these observations to PAH metabolism/activation is discussed.
MATERIALS AND METHODS

Chemicals. Rat liver DD cDNA has been described (17). Restriction endonucleases were purchased from New England Bio-Labs (Beverly, MA); Bethesda Research Laboratories (Gaithersburg, MD); and Boehringer Mannheim (Indianapolis, IN). Avian myeloblastosis reverse transcriptase was purchased from New England Bio-Labs. Oligonucleotides were synthesized by the Oligonucleotide Synthesis Service, University of Pennsylvania, Philadelphia, Pennsylvania. Nick-translation and riboprobe kits were obtained from Promega (Madison, WI). Benzenedi-hydriodiol was synthesized from 1,4-cyclohexadiene (31). S-Adenosyl-L-methionine (p-toluene sulfonate salt) and 17β-estradiol-3-sulfate were products of Sigma Chemical Co. (St. Louis, MO). Porcine liver catechol-O-methyltransferase (5000 units/mg protein) was obtained from Calbiochem (San Diego, CA). [32P]CTP (specific activity, 3000 Ci/mmol), [35S]CTP (specific activity, 3000 Ci/mmol), and 5′-adenosyl-L-[methyl-3H]methionine (specific activity, 82–84 Ci/mmol) were obtained from Amersham (Arlington Heights, IL). NAD+ and NADP+ were obtained from Boehringer Mannheim.

Animals. All Sprague-Dawley rats were obtained from Charles River (Wilmington, MA). Male, female, and ovariectomized rats (180–200 g) were housed 7–10 days before use; adult male and female animals were hypophysectomized at 33 days of age (100 g) and were maintained 0–33 days post-surgery. The polyclonal monospecific rabbit anti-rat DD sera have been described (30). Goat anti-rabbit IgG horseradish peroxidase conjugate and 4-chloro-1-naphtholphosphorylase from Bio-Rad (Melville, NY). Benzene-dihydriodiol was synthesized from 1,4-cyclohexadiene (31). S-Adenosyl-L-methionine (p-toluene sulfonate salt) and 17β-estradiol-3-sulfate were products of Sigma Chemical Co. (St. Louis, MO). Porcine liver catechol-O-methyltransferase (5000 units/mg protein) was obtained from Calbiochem (San Diego, CA). [32P]CTP (specific activity, 3000 Ci/mmol), [35S]CTP (specific activity, 3000 Ci/mmol), and 5′-adenosyl-L-[methyl-3H]methionine (specific activity, 82–84 Ci/mmol) were obtained from Amersham (Arlington Heights, IL). NAD+ and NADP+ were obtained from Boehringer Mannheim.

Primer Extension Analysis. A [32P]-labeled oligonucleotide primer complementary to +17 to +34 of the coding region (1 × 106 cpm) was mixed with 10 μg of rat liver poly(A)+-RNA in 0.3 μl sodium acetate and precipitated with ethanol. The mixture was resuspended in 30 μl 1,4-piperazinediethanesulfonic acid (pH 6.4), denatured at 85°C for 10 min, and annealed for 12 h at 42°C. The RNA:deoxynucleotide hybrid was ethanol-precipitated and added to a reverse transcription reaction mixture (25 μl) containing 4 μl of 40 units avian myeloblastosis reverse transcriptase, 3.5 μl 4 μM dNTPs, 1.25 μl of 10X reverse transcriptase buffer (Promega). The reaction was allowed to proceed at 42°C for 90 min and was terminated by the addition of 1.0 μl of 0.5 μl EDTA and 1.0 μl ribonuclease A (1.0 mg/mL) and incubated for 30 min. The extended primer was isolated by adding 100 μl 2.5 M NH4OAc to the mixture which was then ethanol-precipitated and its size determined on a sequencing gel.

Extension Analysis. Genomic DNA was prepared from 1.0 g of frozen pulverized male rat liver (32), digested with restriction endonucleases, and electrophoresed on 0.8% agarose gels. Gels were denatured in 1.5 μ NaCl plus 0.5 μ NaOH for 50 min and then neutralized with 1.5 μ NaCl plus 0.5 μ Tris-HCl (pH 7.4) for 30 min. The DNA was transferred to nitrocellulose filters via capillary blotting in 10× SSPE (1.5 μ NaCl plus 0.15 μ NaH2PO4, pH 7.0). The filters were washed in 5× SSPE for 5 min, air-dried, and baked at 80°C for 2 h. Prehybridization was performed in 6× SSPE, 5× Denhardt’s, 0.5% SDS, and 50% formamide at 42°C. Filters were hybridized in the same buffer using [32P]-labeled riboprobe made from the following pGEM3 vectors: p3ot-HSD/DD-IIa (HindIII fragment) T7 (+129 to +250) SP6 cloned at the EcoRI site (the 5′-probe was synthesized from the vector using SP6 RNA polymerase); p3ct-HSD/DD-IIa (PstI fragment) SP6 (+334 to +511) T7 cloned at the PstI site (the anti-sense probe was synthesized with T7 RNA polymerase); and p3ct-HSD/DD-IIa (PstI fragment) SP6 (+334 to +511) T7 cloned at the PstI site (the anti-sense probe was synthesized with T7 RNA polymerase). Synthesis of the riboprobes was achieved using [32P]CTP and a riboprobe kit (Promega). Filters were subjected to low stringency washes once in 0.1× SSPE plus 0.1% SDS at 50°C and twice in 0.1× SSPE plus 0.1% SDS at 55°C; they were subjected to one high stringency wash in 0.1× SSPE plus 0.1% SDS at 68°C. Filters were subjected to autoradiography between the low and high stringency washes.

Southern Analysis. Total RNA (15 μg) and poly(A)+-RNA (2 μg) from each tissue were electrophoresed on 2% formaldehyde-1% agarose gels and electrotransferred to Nytran membranes. Northern blots were prehybridized and hybridized as described above and bands were detected using [32P]-labeled nick-translated probe corresponding to nucleotides +334 to +853 of the coding region. Filters were washed in 0.1× SSPE plus 0.1% SDS at 50–55°C for 45 min. Blots were stripped by boiling for 30 min and reprobed using a nick-translated IA probe to normalize for unequal loading and transfer. The use of the IA probe is preferred over a probe to β-actin because in subtraction hybridization experiments, IA was found to be unregulated by estrogen.
Fig. 3. Tissue distribution of 3α-HSD/DD mRNA by Northern analysis. RNA samples were electrophoresed on 2% formaldehyde-1% agarose gels and transferred to Nytran membranes. Blots were probed with a nick-translated [32P]-labeled cDNA probe corresponding to nucleotides +334 to +854 base pairs of the coding region: A, 15 μg total RNA per lane from the male tissue; B, 2 μg of poly(A)+-RNA per lane from the male tissue; C, 15 μg total RNA per lane from the female tissue; and D, 2 μg of poly(A)+-RNA per lane from the female tissue. The level of IA transcripts is also shown for the poly(A)+-RNA Northern to indicate the extent of equal loading and transfer of the RNA.

DIHYDRODIOL DEHYDROGENASE GENE STRUCTURE AND EXPRESSION

Total RNA

A

Heart
Kidney
Liver
Prostate
Seminal vesicle
Small intestine
Spleen
Testis

kb
9.5
7.5
4.4
2.4
1.4

B

Poly[A]+ RNA

Heart
Kidney
Liver
Prostate
Small intestine
Spleen
Testis

kb
9.5
7.5
4.4
2.4
1.4

C

Total RNA

Heart
Kidney
Liver
Prostate
Seminal vesicle
Small intestine
Spleen
Testis

kb
9.5
7.5
4.4
2.4
1.4

D

Poly[A]+ RNA

Heart
Kidney
Liver
Prostate
Seminal vesicle
Small intestine
Spleen
Testis

kb
9.5
7.5
4.4
2.4
1.4

IA

kb
1.7
1.7

gens, whereas β-actin was estrogen regulated (34). The resulting autoradiograms were analyzed using a UNISCAN computerized densitometer (Analtech, Newark, DE).

Dot-Blot Analysis. Increasing amounts of total RNA from each treatment group (0.2 to 20 μg) were applied in 17× standard saline citrate (1.5 M NaCl plus 0.15 M sodium citrate, pH 7.0) containing formamide in diethylpyrocarbonate-treated water to Nytran filters via vacuum application. Along the side of each unknown, increasing amounts of total RNA from female rat liver were immobilized for the construction of a standard curve. The filter was then prehybridized, probed with the [32P]-nick-translated cDNA probe (+334 to +853 of the coding region), and washed as described for Northern analysis. The resultant autoradiograms were scanned using the UNISCAN densitometer.
was constructed and the value of the unknown was computed from this curve. In every instance the correlation coefficient was greater than 0.995, indicating that RNA was quantitatively recovered. RNA values are reported relative to the female value of 1.0.

**Western Blot Analysis.** Cytosolic protein (50 μg/lane) from various tissues were separated by SDS-PAGE and electrotransferred to Nytran. Immunoblots were developed using rabbit anti-rat 3α-HSD/DD serum (as primary antibody) and goat anti-rabbit IgG-horseradish conjugate (as secondary antibody). Blots were developed using H2O2 and 4-chloro-1-naphthol as chromogen. Stained bands were quantified by densitometry and are reported relative to the band intensity observed for DD in rat liver cytosol.

**Enzyme Assay.** 3α-HSD and DD activities were measured in samples of rat liver cytosol spectrophotometrically using androsterone and benzenedihydriodiol as substrates, respectively (14). In all tissues, DD activity was measured using a radiochemical assay in which the oxidation of benzenedihydriodiol to catechol was linked to catechol-O-methyl transferase using [3H]-S-adenosyl-L-methionine as a methyl donor (35). For each sample, linearity with respect to time and protein concentration was established. Specific activities were determined as nmoles of guaiacol formed per min/mg protein. The radiochemical assay yields lower specific activities than the spectrophotometric assay and are related to differences in assay conditions (35).

**Preparation of Tissue Cytosols.** Rat tissue cytosols were prepared via differential centrifugation from 3 in 1 (w/v) homogenates (homogenization buffer, 50 mM Tris-HCl of pH 8.6 containing 250 mM sucrose, 1 mM EDTA, and 1 mM 2-mercaptoethanol) using crystallized bovine serum albumin (Intergen, Purchase, NY) as standard.

**Immunotitration of Rat Liver DD.** Aliquots (20–50 μL) of rat liver cytosol were incubated in 250 μL of 10 mM potassium phosphate buffer (pH 7.4) containing 0.15 mM KCl, 1 mM EDTA plus 1 mM 2-mercaptoethanol and increasing amounts of rabbit anti-rat DD sera 71535 and 71536 (B); DD activity was measured in tissue cytosols using benzenedihydriodiol in the radiochemical assay, and the mean ± SE for triplicate determinations is given (C).

**Protein Concentration.** Protein concentrations were determined by the method of Lowry et al. (36) using crystallized bovine serum albumin (Intergen, Purchase, NY) as standard.
RESULTS

Rat Liver Dihydrodiol Dehydrogenase Gene Structure. The fidelity of our cDNA for rat liver DD (17) was verified by its overexpression in Escherichia coli. The homogeneous recombinant enzyme was kinetically similar to the rat liver enzyme and functioned as both a DD and a 3α-HSD (20). To examine the organization of the DD gene, Southern analysis was performed using rat genomic DNA. Five restriction endonucleases which recognize different hexanucleotide sequences were used to digest genomic DNA, and filters were probed with riboprobes synthesized against the 5′ and 3′ regions of the cDNA as well as the open-reading frame. Post-hybridization washes were performed under low and high stringency conditions. Using the riboprobe for the open-reading frame and low stringency conditions, the banding pattern was complex, irrespective of the restriction endonuclease used. This banding pattern simplified with higher stringency washes suggesting that DD is a member of a multigene family. Using the riboprobes for the 5′ and 3′ regions of the cDNA, both low and high stringency washes produced similar banding patterns, suggesting that the genomic fragments detected reflect the restriction pattern for the DD gene (Fig. 1). Some of the fragments identified with the 5′ probe exceed 20 kilobases, and some of the fragments identified with the 3′ probe exceed 5 kilobases, indicating that the size of the rat DD gene is at least 20–25 kilobases in length and that the bulk of the gene lies 5′ upstream from the cDNA.

Northern analysis detects a transcript of 2.4 kilobases in rat liver, yet only 966 nucleotides constitute the open-reading frame (17). To locate the transcription start site, primer extension analysis was performed. Using rat liver poly(A)^+ RNA as a template for avian myeloblastosis reverse transcriptase and an end-labeled oligonucleotide complementary to +17 to +34 as primer, two major bands of 70 and 71 base pairs in length (90%), and two minor bands of 74 and 117 base pairs in length (10%) were detected on the resultant sequencing gel (Fig. 2). As a control, the primer was used to prime a dideoxy sequencing reaction using a PGM3 vector containing −129 to +250 base pairs of the cDNA clone. These data suggest that the DD gene has two major transcription start sites at 53 and 54 base pairs upstream from the translation start site and two minor sites 57 and 100 base pairs further upstream. Identical results were obtained irrespective of whether the poly(A)^+ RNA template was isolated from male or female rat liver. These results confirm that the bulk of the noncoding nucleotides (1,200 base pairs) in the mRNA appear to be located at the 3′-untranslated end.

Tissue and Sex-specific Expression. The metabolic activation of PAH to carcinogenic metabolites is organ and tissue specific (1). Levels of cytochrome P4501A1 (the predominant form of cytochrome P450 responsible for diol-epoxide formation) are highest in the liver and lower in the lung, small intestine, and kidney. Furthermore, the expression of cytochrome P4501A1 is sex dependent (37, 38). Since DD has been implicated in the further metabolism and/or activation of trans-dihydrodiol (proximate carcinogens), the tissue- and sex-specific expression of the DD gene was examined. Northern analysis of total RNA from male tissues detected a transcript (2.4 kilobases) only in liver and small intestine (Fig. 3). The same size transcript could be detected in lung, kidney, and spleen provided that poly(A)^+ RNA isolated and exposure times of more than 1 week were used. No transcript was detected in the male rat heart. Low levels of transcripts were detected in male-specific organs (e.g., prostate), but none were detected in the testis or seminal vesicle. Northern analysis of total RNA from female tissues detected a transcript of 2.4 kilobases only in liver and small intestine, consistent with the expression pattern observed in the male. The same size transcript could also be detected in the female lung when poly(A)^+ RNA was used, also consistent with the findings in the male. In contrast to the male, no transcripts were detected in female kidney and spleen, whereas transcripts were detected in female heart. These data indicate that certain tissues show sex-specific DD gene expression. Transcripts were also detected in the female-specific organs: ovary, mammary gland, and uterus. The ovary was characterized by the high level of two mRNA transcripts of 2.4 and 1.4 kilobases in length.

Transcript levels were also correlated to the level of immunoreactive protein (Figs. 4 and 5). In male tissues, liver, lung, and small intestine have the largest amounts of immunoreactive protein consistent with the transcript level. Some tissues have immunoreactive protein but no detectable transcripts, e.g., heart and seminal vesicle, while others have transcripts but no detectable immunoreactive protein, e.g., kidney. In female tissues, all tissues that possess transcripts have immunoreactive protein, and the correlation between mRNA and protein seems reasonable. One exception was the female kidney where no transcripts were detected but levels of immunoreactive protein were observed.
DD enzyme activity across tissues was also correlated with the levels of mRNA and immunoreactive protein. DD activity was measured directly using a radiochemical assay in which the oxidation of benzenedihydrodiol to catechol was linked to catechol-O-methyltransferase using [3H]-S-adenosyl-L-methionine as a methyl donor. In general, the level of enzyme activity follows mRNA levels. Notable exceptions are the small intestine, ovary, mammary gland, and uterus, where activity is lower than anticipated based on the detection of mRNA and immunoreactive protein. Sexual dimorphism in DD activity was clearest in the liver where the activity was 2.5-fold higher in the female.

**Regulation of DD Gene Expression.** Since the DD gene is 20–25 kilobases in length, identification of cis- and trans-acting factors that influence gene expression could be facilitated by studying the regulation of DD gene expression. The sexual dimorphism observed in rat liver DD activity has been documented previously (28, 29). We now report that Northern and dot-blot analysis indicate that females contain at least a 2-fold higher level of the transcript, suggesting that sexual dimorphism occurs at the level of the mRNA (Fig. 6). Immunotitration of enzyme activity confirms that DD represents 1% of the soluble protein in female rat liver cytosol and that in the male this value is reduced to 0.5% (Fig. 7).

Measurements of DD mRNA, enzyme protein, and enzyme activity were also performed in ovariectomized and Hypox animals (Fig. 7). Studies show that following hormonal ablation particularly hypophysectomy, the sexual dimorphism between males and females is abolished. After surgery, mRNA, enzyme protein, and enzyme activity are all lower in Hypox male and female rats than those observed in adult males. These changes are substantial since in the Hypox female, mRNA levels ultimately decrease by 10-fold, enzyme protein is reduced by 3-fold, and enzyme activity can be decreased by 3-fold relative to the adult female. In the Hypox male, similar changes are observed versus the adult male. Despite these dramatic changes, some evidence of the sexual dimorphism is retained at the earliest time points following surgery. Thus, at 12–13 days posthypophysectomy, the levels of DD mRNA and enzyme activity were still significantly higher in female versus male livers. By days 28–33 posthypophysectomy, any remaining differences were abolished. The length of time required to reach basal levels in the Hypox female indicates that factors other than pituitary hormones may influence DD gene expression.

To determine whether estrogens are involved in the establishment of the sexual dimorphism, 17β-estradiol-3-sulfate was administered to adult male rats. Within 2–3 days, DD mRNA increased 3-fold, enzyme protein doubled, and enzyme activity increased by 2-fold (Fig. 8).

**DISCUSSION**

DD plays an important role in the metabolism/activation of PAH trans-dihydrodiol (proximate carcinogens). The availability of the cDNA for rat liver DD permits the elucidation of gene structure and the mechanisms by which the expression of this gene is regulated.

DD cDNA has significant sequence identity with members of the aldo-keto reductase superfamily (17–19). Southern analysis using riboprobes complementary to the coding region supports the concept that the rat contains a number of related genes that encode for members of this superfamily. By contrast, Southern analysis using riboprobes complementary to the 5' and 3' regions of the cDNA under low and high stringency conditions indicate that the DD gene is at least 20–25 kilobases in length. This size is similar to that reported for the human aldose reductase gene which has been shown to be 21 kilobases in length and contains 9 introns (39).

Primer extension analysis indicates that the transcription start site is only a short distance upstream from the translation start site, therefore the transcript contains an extensive 3'-untranslated region (1.2 kb). The lengths of the 5' and 3'-untranslated regions of the transcript appear to be identical in male and female livers.
DD shows tissue-specific expression in that the highest levels of transcripts exist in liver and small intestine. This high level of expression may reflect the importance of DD in the metabolism of PAH. The liver is a major site of PAH metabolism in the rat, and it contains the largest amount of cytochrome P450A1 required to form diol-epoxides (37). The liver also transforms PAH to more polar metabolites and exports them to target tissues where they ultimately form DNA adducts (40). By contrast, the detection of DD transcripts by Northern analysis in most extrahepatic tissues required the isolation of poly(A)+-RNA and long exposure times, indicating their relative low abundance. In the rat ovary, two transcripts of 2.4 and 1.4 kilobases in length were detected. Recently, rat ovarian 20α-HSD has been partially sequenced and shown to contain significant sequence identity with 3α-HSD and other aldo-keto reductases (41). Additionally, rabbit ovary 20α-HSD has been sequenced and has 79% nucleotide identity with 3α-HSD, and the size of the 20α-HSD transcript is 1.2 kilobases (42). Thus, one of the transcripts detected with the DD cDNA probe in rat ovary may be 20α-HSD. In certain tissues, the correlation of DD mRNA with enzyme protein and enzyme activity does not hold. These inconsistencies are to be expected since our molecular probes may vary in their cross-reactivity with other members of the aldo-keto reductase superfamily. Thus, mRNA species and/or immunoreactive protein related to the aldo-keto-reductases may be detected which are not DD. It should be emphasized that aldose and aldehyde reductases have transcript sizes of 1.4 to 1.6 kilobases (25, 43) and that the most common transcript detected with our cDNA is 2.4 kilobases. Additionally, DD activity may be detected in some tissues in which our molecular probes may not cross-react with the transcript or protein responsible.

The sexual dimorphic expression of 3α-HSD/DD in rat liver (28, 29, 44) is characterized by a 2-fold elevation of enzyme activity in females. Understanding the molecular mechanisms responsible for the
high level of DD expression in females is important because of the ability of the enzyme to suppress the formation of PAH anti- and syn-diol epoxides. This paper indicates that estrogens alone are sufficient to establish the female pattern of DD expression in male rat liver. Estrogens achieve this effect at the level of the DD mRNA, but it is unclear from steady-state measurements whether this reflects an increase in gene transcription or an increase in the stability of the mRNA. Nuclear run-off assays will determine whether estrogens influence the rate of DD gene transcription but may require the identification of cells that contain estrogen-responsive DD activity. Estrogens achieve this effect at the level of the DD mRNA, but sufficient to establish the female pattern of DD expression in male rat liver. Estrogens achieve this effect at the level of the DD mRNA, but may require the identification of cells that contain estrogen-responsive DD activity. Identification of consensus sequences (e.g., estrogen responsive elements and/or anti-oxidant responsive elements) will provide additional clues to the identity of hormones, growth factors, and electrophiles that may regulate DD gene transcription and thereby influence tissue response to PAH chemical carcinogens.

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