Mouse Liver CYP2C39 Is a Novel Retinoic Acid 4-Hydroxylase

ITS DOWN-REGULATION OFFERS A MOLECULAR BASIS FOR LIVER RETINOID ACCUMULATION AND FIBROSIS IN ARYL HYDROCARBON RECEPTOR-NULL MICE*

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Livers of aryl hydrocarbon receptor (AHR)-null mice have high levels of retinoic acid (RA), retinol, and retinyl palmitate. Hepatic accumulation of RA in these mice may be responsible in part for the hepatic phenotype characterized by small liver size and fibrosis. The increased levels of hepatic RA may be due to decreased metabolism of RA to 4-hydroxyretinoic acid. To identify the P450 isoform(s) involved in RA metabolism, liver microsomes from AHR-null and wild-type mice were subjected to Western blotting and probed with antibodies to rat P450s that cross-react with murine forms. Signal intensity in Western blots probed with anti-rat CYP2C6 antibodies correlated with levels of RA 4-hydroxylation. Furthermore, this anti-rat CYP2C6 antibody inhibited RA 4-hydroxylation activity of wild-type mouse liver microsomes to the levels of AHR-null mouse liver. When used to screen a mouse liver cDNA expression library, this antibody exclusively recognized the murine P450 CYP2C39. Catalytic assays of five recombinant mouse CYP2Cs expressed inEscherichia coli revealed that only CYP2C39 was competent for RA 4-hydroxylation (Km = 812.3 nM and Vmax 47.85 (fmol/min/pmnl P450)). Real time reverse transcriptase-PCR used to assess the Cyp2C39 mRNA expression showed decreased levels (30%) of this transcript in AHR-null compared with wild-type liver, consistent with decreased protein levels observed by Western blot analysis using an antibody to a CYP2C39-specific peptide. These data show that CYP2C39 catalyzes RA catabolism and thus possibly controls RA levels in mouse liver. Down-regulation of Cyp2C39 is hypothesized to be responsible for the liver phenotype in the AHR-null mouse.

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), polychlorinated biphenyls, and polycyclic aromatic hydrocarbons are notorious environmental hazards causing acute and chronic toxicity (1). These chemicals exert their effects primarily by binding to the aryl hydrocarbon receptor (AHR), a ligand-dependent transcription factor (2). Ligand binding promotes dissociation of cytosolic AHR from heat shock protein 90 and dimerization with the aryl hydrocarbon receptor nuclear translocator. The AHR/aryl hydrocarbon receptor nuclear translocator heterodimer translocates to the nucleus, binds to upstream DNA regulatory elements, and activates expression of a large battery of target genes. These alterations in target gene expression underlie the toxic and carcinogenic effects of AHR ligands, as reported recently (3).

The carcinogenic effects of AHR ligands are inhibited by retinoids (4). AHR ligands have been shown to reduce tissue retinoid content and to induce preneoplastic lesions, such as squamous metaplasia. This lesion is also induced by retinoid deficiency and reversed by retinoids in culture and in vivo (5). Thus, squamous metaplasia results from both AHR ligand and retinoid deficiency. Given that AHR ligands can reduce tissue retinoid content, and that the AHR can influence gene expression even in the absence of exogenous ligand, it seemed likely that loss of the AHR might affect retinoid homeostasis. This was confirmed by our observation that livers from AHR-null mice exhibit a 3-fold increase in retinol esters, retinol and retinoic acid, relative to wild-type livers (6). Increased liver retinoids may cause liver fibrosis in the AHR-null mice through retinoic acid receptor-mediated induction of transglutaminase II, which in turn activates latent transforming growth factor-β (6, 7). Transforming growth factor-β causes decreased proliferation and elevated apoptosis, which could thus account for the small liver size and fibrosis (7). Indeed, liver fibrosis can be partially reversed upon feeding a vitamin A-deficient diet to the AHR-null mice.*

RA accumulation in the AHR-null mouse liver is associated with, and most likely caused by, a 65% decrease in RA metabolism (6). The primary pathway of RA metabolism and excretion in vertebrates starts with 4-hydroxylation (8). In liver tissue this activity is NADPH-dependent and is inhibited by carbon monoxide, suggesting that it may be catalyzed by a cytochrome P450. The AHR is known to regulate P450 genes, including Cyp1A1, Cyp1A2, and Cyp1B1 (9). These P450s are members of a superfamily of heme-containing enzymes that catalyze the oxidation of a large number of diverse substrates, including drugs, environmental toxins, carcinogens, endogenous steroids, and fatty acids (10). However, neither Cyp1A1 nor Cyp1A2, known to be regulated by the AHR status in mouse liver, metabolized RA (6). Conversely, mRNA levels of the only known murine hepatic RA-metabolizing P450, Cyp26 (i.e. CYP26A1 (11, 12)), were found to be similar in AHR wild-

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‡ The abbreviations used are: TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; RA, retinoic acid; AHR, aryl hydrocarbon receptor; RT, reverse transcriptase; HPLC, high pressure liquid chromatography; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

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type and null mice (6). Hence, the AHR appears to control the expression of an unidentified RA-metabolizing P450. This study was initiated to identify this P450 form.

### EXPERIMENTAL PROCEDURES

#### Materials—All trans-RA, hexane, isopropl alcohol, acetic acid, and ammonium acetate were purchased from Sigma. [11,12-3H]RA (specific activity, 51.2 Ci/mmol) was purchased from PerkinElmer Life Sciences. 4-Hydroxy- and 4-oxo-RA were a kind gift from Dr. Joseph L. Napoli (University of California at Berkeley). Acetonitrile and methanol were purchased from Burdick and Jackson (Muskegon, MI). All of the solvents used for HPLC were degassed through a 0.2-μm filter, and all of the reactions were carried out under a nitrogen stream.

**Animals, TCDD Treatment and Microsomes Preparation**—All animals used in this study were 20-week-old males and were maintained in a pathogen-free facility. TCDD was administered in single intraperitoneal injection at a dose of 80 μg/kg in corn oil. Mice were killed by CO2 asphyxiation 3 days after injection. Microsomes were prepared by differential centrifugation as described previously, and protein concentrations were determined by the dye-binding method (BCA kit, Pierce) using bovine serum albumin as a standard.

**Western Immunoblots**—Microsomal proteins (10 or 20 μg) were separated by SDS-PAGE on a 10% gel and transferred to nitrocellular membrane in TMB transfer buffer (25 mM Tris–Cl, pH 8.3, 192 mM glycine, 20% v/v methanol) at a constant 30 V overnight at 4 °C. Membranes were probed with antibodies directed against rat CYP2C6 (a generous gift from Dr. David Waxman), CYP2C11, and CYP2B1 (BD Gentest Corp., Woburn, MA), mouse CYP1A1 (a generous gift from Dr. Harry Gelboin) and mCYP26, and an antibody for mCYP2C39 raised against a CYP2C39-specific peptide, generated as described below. Binding of antibody was detected using horseradish peroxidase-conjugated secondary antibodies and the ECL method (Amer sham Biosciences). Relative band intensities (peak area) were measured by densitometry.

**Generation of Anti-CYPs Antibody**—Polyclonal anti-CYP26A1 antibody was raised by injection of a synthetic keyhole limpet hemocyanin-conjugated C-terminal peptide from mouse CYP26 (Ac-CVDNL-ParfittFQGSN-OH) into rabbits using a standard protocol (Quality Control Biochemicals, a division of BioSource International Inc., Camarillo, CA). A CYP2C39-specific peptide C-EEFSDRGSIPMV was synthesized by ResGen (a division of Invitrogen) and coupled to keyhole limpet hemocyanin through the terminal cysteine. Custom polyclonal antibodies specific for CYP2C39 were then produced by Covance Research Products Inc. (Denver, PA) as follows. At the start of production, a 1 mg/ml suspension of conjugated peptide was diluted 1:1 with Freund's complete adjuvant, and each of two rabbits received 500 μg (1 ml) following pre-bleed. After 4 weeks, rabbits were each boosted with 250 μg of peptide diluted 1:1 with Freund's incomplete adjuvant, followed after 2 weeks with a test bleed. Rabbits received 4 additional boosts at 4-week intervals, with production bleeds beginning after the third boost. Rabbits were terminated by exsanguination at completion of production. Antibodies used in this study were from the second production bleed.

**Enzymatic Assays and Determination of Kinetic Constants for RA 4-Hydroxylation by mCYP2C39 Isoform—RA metabolism in mouse liver microsomes was assayed as described previously (6). The N termini of the murine Cyp2C11 cDNAs were modified for optimal expression in Escherichia coli and expressed, partially purified and reconstituted with dilauroylphosphatidycholine and NDPH-cytochrome P450 oxidoreductase, as described previously (13, 14). Incubations containing a final concentration of 90 nM [3H]-labeled RA were initiated by the addition of NDPH and run for 1 h at 37 °C. For the determination of Km and Vmax values for RA 4-hydroxylation by mCYP2C39 isoform, recombinant and partially reconstituted mCYP2C39 isoform was incubated for 10 min at 37 °C, with a range of [3H]RA concentrations (22.5 to 720 nM); incubation time was within the linear range as determined from velocity versus time experiments. NDPH-minus controls were run side by side. Reactions were stopped by placing tubes in a dry ice/ethanol bath and processed as described below.

**HPLC**—Conversion of [11,12-3H]RA to more polar metabolites was analyzed on both reverse and straight phase columns. A Beckman model 110A pump was connected in series to a Knauer D-14163 spectrophotometer (Sonnetek, Inc., Upper Saddle River, NJ) and a Radio
dynamic radioactivity flow detector (150TR flow scintillation analyzer; Packard Instrument Co.). Samples were first analyzed on a Partisil 10 ODS-2 column (4.6 mm inside diameter × 25 cm; Whatman) fitted with a precolumn of Pollicular ODS (Whatman). Acetonitrile and 1% ammonium acetate in water (65:35 concentration) was used as mobile phase at a flow rate of 2.2 ml/min. Polar metabolites were further separated on a Zorbax-SIL (3 μm, 6.2 × 80 mm) column with 93.5:5:1.05 hexane/isopropl alcohol/methanol/acetic acid at a flow rate of 1 ml/min.

**Immunoinhibition—Liver microsomes (100 μg of protein) were pre-incubated for 30 min at room temperature with 0, 0.5, 1, and 2.5 mg of anti-rat CYP2C6 IgG of microsomes. Commercial rabbit IgG (Sigma) was used as a negative control. Each sample was then incubated at 37 °C in a shaking water bath for 20 min in 0.3 ml of 50 mM Tris–HCl, 150 mM KCl, 5 mM MgCl2, and an NADPH-regenerating system (2.5 units of glucose-6-phosphate dehydrogenase, 500 nM of NADP, and 0.5 μM of glucose 6-phosphate), pH 7.4, with 90 μM [3H]RA, according to Fiorella et al. (15). Reactions were stopped in a dry ice/methanol mixture before sample lyophilization. Each residue was extracted with 200 ml of methanol. After removal of particulates by centrifugation (3 min at maximum speed in a table top centrifuge), samples were stored at −70 °C until analyzed.

**RNA Extraction**—Total RNA was extracted from crushed, frozen mouse liver using TRIzol reagent (Invitrogen) according to the manufacturer's protocol. To ensure efficient removal of possible DNA contamination, RNA samples were further purified using RNeasy Mini Kit (Qiagen) according to the manufacturer's protocol for RNA clean up.

**cDNA Synthesis—**Purified total RNA (1 μg) was reverse-transcribed using SuperScript First-Strand Synthesis System for RT-PCR kit (Invitrogen), according to the manufacturer's protocol. Reactions were primed with oligo(dT)18 primers, and the total volume/reaction was 20 μl.

**PCR**—PCR was performed in a total volume of 50 μl using TaqMan PCR Core Reagent Kit (Applied Biosystems); 1 μl of cDNA sample was used as template. Both GAPDH and Cyp2C39 amplification primers were done in duplicate for each sample. The thermal cycling conditions included 2 min at 50 °C and 10 min at 95 °C, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min.

**Primers and Probes—**Primers and probe for Cyp2C39 mRNA were designed using the Primer Express (Applied Biosystems) computer program and then custom-synthesized by Applied Biosystems. Primers and probe nucleotide sequences for mCyp2C39 (GenBank TM accession number AF047726) are as follows: forward primer 5'-1067AGAGATTCTACAAACCTTGGCCCTATAGG-3'; reverse primer 5'-1177GATGTCAGTGACTTGGCGTCT1513-3'; and TaqMan probe 5'-FAM-1053A-ACATACCCCGTGACTTGGCTGATCATTAAAATT-3' (TAMRA). The boldface nucleotides in the TaqMan probe sequence represent the fully unique nucleotides for the Cyp2C39 isoform, which guarantee a specific amplification of this isoform over other very similar ones within the same subfamily. Both the primer pair set and probe for GAPDH were purchased from Applied Biosystems.

**Real Time RT-PCR—**Cyp2C39 expression in wild-type and AHR-null mouse liver samples was measured by real-time quantitative RT-PCR, based on TaqMan methodology, using the ABI PRISM 7700 Sequence Detection System (Applied Biosystems). This technique allows, by means of fluorescence emission, identification of the cycling point when PCR product is detectable (threshold cycle or Ct value). To normalize the amount of total RNA present in each reaction, we amplified the housekeeping gene GAPDH. Results are expressed as relative levels of Cyp2C39 mRNA, referred to wild-type samples (the calibrator), chosen to represent 1× expression of this gene. The AHR-null samples and the TCDD-treated AHR−/+ samples express a-fold Cyp2C39 mRNA relative to the calibrator. The amount of target, normalized to an endogenous reference (GAPDH) and relative to the calibrator is definite by the ΔΔCt method as described previously (Sequence Detective bulletin 2, Applied Biosystems). Specifically, the formula is as follows: target amount = 2−ΔΔCt, where ΔΔCt = (CtGAPDH − CtCyp2C39 sample) − (CtGAPDH sample) − (CtCyp2C39 calibrator − CtGAPDH calibrator). This method is based on the assumption that the target (Cyp2C39) and the reference (GAPDH) display equal amplification efficiencies. To verify this condition, we checked if ΔΔCt (the ratio of GAPDH variations according to template dilution) to this end, a standard curve composed of five different dilutions of wild-type mouse liver total RNA (1.25, 1, 0.75, 0.5, and 0.25 μg) was generated. The slope of this curve was −0.0832. (To ensure the appropriate amplification efficiency, the slope of the standard curve should be less than 0.1.)
Correlation between CYP2C Immunoreactive Protein Levels and RA Metabolism in Microsomes Prepared from AHR-null and Wild-type Mouse Liver—Initially, an approach that directly compared protein levels was taken to study modulation of P450 levels in wild-type and AHR-null mice. Due to a high degree of amino acid similarity, antibodies raised to P450s from one species often cross-react with those from other rodent species. Thus, probing Western immunoblots of AHR-null and wild-type mouse liver microsomes with a panel of antibodies raised against rat P450s could be used to identify an antibody that would cross-react with the RA-hydroxylating P450 and to highlight differences in this P450 between AHR-null and wild-type mice. The results of this screen are presented in Fig. 1. The intensity of signal on the blots was found to vary in the same rank order as RA 4-hydroxylase activity, using an anti-rat CYP2C6 antibody as probe (Fig. 1). Thus, proteins that were cross-reactive to the antibody to rat CYP2C6 were decreased in AHR-null livers relative to wild type (37% decrease was estimated by densitometry and radiometric quantification).

**RESULTS**

**Correlation between CYP2C Immunoreactive Protein Levels and RA Metabolism in Microsomes Prepared from AHR-null and Wild-type Mouse Liver**—Initially, an approach that directly compared protein levels was taken to study modulation of P450 levels in wild-type and AHR-null mice. Due to a high degree of amino acid similarity, antibodies raised to P450s from one species often cross-react with those from other rodent species. Thus, probing Western immunoblots of AHR-null and wild-type mouse liver microsomes with a panel of antibodies raised against rat P450s could be used to identify an antibody that would cross-react with the RA-hydroxylating P450 and to highlight differences in this P450 between AHR-null and wild-type mice. The results of this screen are presented in Fig. 1. The intensity of signal on the blots was found to vary in the same rank order as RA 4-hydroxylase activity, using an anti-rat CYP2C6 antibody as probe (Fig. 1). Thus, proteins that were cross-reactive to the antibody to rat CYP2C6 were decreased in AHR-null livers relative to wild type (37% decrease was estimated by densitometry and radiometric analysis).

Furthermore, they were not increased by in vivo pretreatment with TCDD. This validates the fact that, although RA metabolism was decreased in AHR-null mouse liver, this activity was not TCDD-responsive. The efficacy of TCDD treatment was confirmed using an anti-CYP1A1 antibody (Fig. 1). Thus, anti-rat CYP2C6 antibodies recognize a P450 form that follows the same trend as RA 4-hydroxylation in the AHR-null mouse. In contrast, the signal obtained with anti-rat CYP2C11 antibodies did not vary considerably between genotypes, indicating that only a subset of P450s is affected in the AHR-null mouse.

**RA Metabolism in Mouse Liver Microsomes by Anti-CYP2C6**

*F. Andreola, G. P. Hayhurst, G. Luo, S. S. Ferguson, F. J. Gonzalez, J. A. Goldstein, and L. M. De Luca,* unpublished data.
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Figure 3. mCyp2C39 mRNA expression level is reduced in AHR-null mouse liver but unaffected by TCDD treatment. Results of real time RT-PCR experiments are expressed as relative expression of Cyp2C39 mRNA, normalized to an endogenous reference (GAPDH) and relative to AHR wild-type samples (n = 7) (the calibrator) (ΔΔCt), chosen to represent 1× expression of this gene. The AHR-null mice/samples (n = 6) and the TCDD-treated AHR+/+ mice/samples (n = 3) expressed 0.304-fold* (95% confidence interval, 0.181–0.509) and 0.841-fold (95% confidence interval, 0.176–2.577) mCyp2C39 mRNA relative to the calibrator (refer under “Experimental Procedures” for further details), respectively. Each sample was assayed in duplicates in at least two independent experiments. * Statistically different from AHR-wt.

Figure 4. Reduced expression of CYP2C39 protein in AHR-null mice compared with their wild-type counterparts. A, partially purified recombinant CYP2C proteins (2C29, 2C37, 2C38, 2C39, and 2C40) were electrophoresed and transferred to nitrocellulose membranes. Membranes were immunoblotted with a rabbit anti-2C39 peptide antibody to demonstrate antibody specificity. B, the same antibody was used to probe membranes onto which AHR-null and wild-type liver microsomes had been transferred as indicated under “Experimental Procedures.” Denitometric analysis of the data showed a 63% decrease in CYP2C39 expression in the AHR-null mouse liver. (n = three mice per genotype.)

The evolutionary conservation of AHR expression and function and the noted phenotype of AHR-null mice (16–19) suggest that this receptor fulfills important physiological roles in vertebrates. Our previous work has identified a role for this transcription factor in retinoid homeostasis (6), since AHR-null mice showed elevated levels of RA and its precursor retinyl esters and retinol in their livers. This hormone is metabolized through 4-hydroxylation, the principal path of RA metabolism in vertebrates, and a reduction in the enzyme(s) responsible for this hydroxylation step would explain the observed RA accumulation (6). In humans, CYP2C8 appears to be the major hepatic RA 4-hydroxylase with CYP3A4 playing a minor role determining whether this enzyme is indeed capable of metabolizing RA, expressed, partially purified recombinant CYP2C39 was incubated in a reconstituted system with [3H]RA. A concentration of 90 nM RA was chosen for all incubations as this is in the same range as the reported physiological RA concentration in the liver (6). The specificity of the reaction was tested by assaying metabolite formation of the other highly homologous murine CYP2C isoforms (CYP2C29, -37, -38, -39, and -40). Of all the P450s tested, only CYP2C39 produced the major metabolites of RA (4-hydroxy- and 4-oxo-RA) at levels above that seen with the inactive P450 control (Table I), even though all P450 preparations were active in the metabolism of arachidonic acid (13). As a further test to ensure that lack of RA-hydroxylating activity by the murine CYP2Cs was not due to inactive P450, tolbutamide metabolism was assessed side-by-side with the RA incubations. Both CYP2C29 and 2C38 metabolized tolbutamide with turnover numbers of 6 and 1.1 nmol/mmol/min, respectively (not shown). Kinetic studies for RA oxidation were performed using the partially purified, recombinant mCYP2C39 isofrom. This isofrom exhibited high activity for the 4-hydroxylation of RA, with a V_max value of 47.85 fmol/min/pmoll of mCYP2C39 and a K_m of 812.29 nM. Background metabolite formation was assessed with an inactive recombinant human CYP2C19*5 allele (which contains a mutation in the heme-binding motif) (14). This allele has been found to be essentially inactive (<< 0.4% of wild-type CYP2C19) toward CYP2C19 substrates such as S-mephenytoin and tolbutamide (14). Background 4-hydroxy- and 4-oxo-RA formation was fairly high (–5% total radioactivity) in the absence of an active P450 (Table I). As no metabolites were observed in the absence of protein (not shown), this is most likely due to metabolism by contaminating bacterial proteins.

Cyp2C39 mRNA Is Dependent on AHR Status but Unresponsive to TCDD—Because of the unique activity of the bacterially expressed CYP2C39 in RA 4-hydroxylation, we tested whether AHR-null mice showed a decrease in Cyp2C39 mRNA compared with wild type. Fig. 3 shows a 70% decrease in Cyp2C39 mRNA as tested in real time RT-PCR experiments and specified in Fig. 3 legend. The difference was highly significant (95% confidence intervals). Interestingly, in vitro pretreatment with TCDD did not affect Cyp2C39 mRNA expression, thus suggesting that AHR status controls the expression of this RA 4-hydroxylating enzyme in an indirect manner. These data correlate well with the reported lack of induction by TCDD treatment of both anti-rat CYP2C6 immunoreactive protein(s) expression and in vitro RA metabolism in AHR wild-type mice.

Western Blot Analysis Using a CYP2C39-specific Antibody Verifies a Decrease CYP2C39 in the AHR-null Mouse Liver—Because CYP2C39 was the only cytochrome showing significant RA oxidizing activity of all the bacterially expressed isoforms tested (Fig. 2), and real time RT-PCR showed a reduction in the mRNA of Cyp2C39, we checked whether the observed lower RA 4-hydroxylation activity in the AHR-null mouse liver microsomes was also accompanied by a reduced CYP2C39 protein expression level by using a new specific polyclonal antibody for CYP2C39 raised to a specific CYP2C39 peptide. Fig. 4A shows the high specificity of an antibody for CYP2C39. This antibody recognized only the recombinant bacterially expressed CYP2C39, but it did not recognize any other structurally related murine CYP2C isoforms such as CYP2C29, CYP2C37, CYP2C38, and CYP2C40. Furthermore, Fig. 4B clearly shows a marked reduction in the CYP2C39 protein expression in the AHR-null liver. These data verify that CYP2C39 is the P450 that is specifically down-regulated in these livers.

DISCUSSION

The evolutionary conservation of AHR expression and function and the noted phenotype of AHR-null mice (16–19) suggest that this receptor fulfills important physiological roles in vertebrates. Our previous work has identified a role for this transcription factor in retinoid homeostasis (6), since AHR-null mice showed elevated levels of RA and its precursor retinyl esters and retinol in their livers. This hormone is metabolized through 4-hydroxylation, the principal path of RA metabolism in vertebrates, and a reduction in the enzyme(s) responsible for this hydroxylation step would explain the observed RA accumulation (6). In humans, CYP2C8 appears to be the major hepatic RA 4-hydroxylase with CYP3A4 playing a minor role...
CYP2C39 actively 4-hydroxylates RA ($K_m = 812$ nM, $V_{max} = 47.8$ fmol/min/pmol P450) with kinetic properties similar to hCYP2C8 in human liver. Furthermore, CYP2C39 protein was verified with a specific antibody to a RT-PCR and Western blot analysis, respectively. The decrease $\text{Cyp2C39}$ mRNA and protein levels as assessed by real-time AHR deficiency masks the possible RA induction of and/or AHR status does not appear to be a direct result of independent of TCDD and as such its regulation by retinoid

in the metabolism of RA and thus in the control of RA suggest that CYP2C39 is a physiologically important enzyme in the metabolism of RA and thus in the control of RA homeostasis.

These data also show a marked decrease (63%) in both Cyp2C39 mRNA and protein levels as assessed by real-time RT-PCR and Western blot analysis, respectively. The decrease in CYP2C39 protein was verified with a specific antibody to a CYP2C39 peptide. In addition, Cyp2C39 mRNA expression is independent of TCDD and as such its regulation by retinoid and/or AHR status does not appear to be a direct result of regulation at the promoter level. Conversely, it may be that AHR deficiency masks the possible RA induction of Cyp2C39 expression. Indeed, RA has a negative feedback role in controlling its own synthesis through inhibiting transcription of retinaldehyde dehydrogenase (23). The lack of TCDD induction of Cyp2C39 is consistent with our previous in vitro data showing lack of effect of this treatment on RA metabolism. Furthermore, rat Cyp2C6 immunoreactive protein also failed to respond to TCDD.

In conclusion, it is clear that mCYP2C39 represents a key component of the retinoid homeostatic mechanism. Moreover, although it remains to be determined how the AHR controls levels of CYP2C39, a transcriptional effect and consequent decrease in CYP2C39 protein level are consistent with the decreased RA metabolism in the AHR-null mouse liver.

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