0090-9556/03/3107-908–915$7.00
DRUG METABOLISM AND DISPOSITION
Vol. 31, No. 7
1058/1071404
DMD 31:908–915, 2003
Printed in U.S.A.

NUCLEAR RECEPTOR, PREGNANE X RECEPTOR, IS REQUIRED FOR INDUCTION OF UDP-GLUCURONOSYLTRANSFERASES IN MOUSE LIVER BY PREGNENOLONE-16α-CARBONITRILE

CHUAN CHEN, JEFF L. STAUDINGER,[1] AND CURTIS D. KLAASSEN

Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, Kansas City, Kansas

(Received January 7, 2003; accepted March 25, 2003)

This article is available online at http://dmd.aspetjournals.org

ABSTRACT:

The aim of this study was to determine the role of pregnane X receptor (PXR) in the induction of UDP-glucuronosyltransferases (UGTs) by pregnenolone-16α-carbonitrile (PCN). Four- to six-month-old male wild-type and PXR-null mice received control or PCN-treated (1500 ppm) diet for 21 days. On day 22, livers were taken to prepare microsomes and total RNA to determine UGT activity and mRNA levels, respectively. In wild-type mice, PCN treatment significantly increased UGT activities toward bilirubin, 1-naphthol, chloramphenicol, thyroxine, and triiodothyronine. On control diet, the UGT activities toward the above substrates (except for 1-naphthol) in the PXR-null mice were significantly higher than those of wild-type mice. However, UGT activities in PXR-null mice were not increased by PCN. In agreement with the above findings, mRNA levels of mouse *Ugt1a1* and *Ugt1a9*, which are involved in the glucuronidation of bilirubin and phenolic compounds, were increased about 100% in wild-type mice following PCN treatment, whereas the expression of *Ugt1a2*, *1a6*, and *2b5* was not affected. In contrast, PCN treatment had no effect on the mRNA levels of these *UGT*s in PXR-null mice. Taken together, these results indicate that PCN treatment induces glucuronidation in mouse liver, and that PXR regulates constitutive and PCN-inducible expression of some UGTs.

Glucuronidation is a phase II biotransformation pathway that plays a major role in the metabolism and elimination of hydrophobic compounds, including environmental pollutants, drugs, steroid hormones, bilirubin, and thyroid hormones [thyroxine (T_4[2]) and triiodothyronine (T_3)] (Burchell et al., 1998). This reaction is catalyzed by UDP-glucuronosyltransferases (UGTs), which are located in endoplasmic reticulum of liver and other tissues. Molecular cloning identified two families of UGTs, *UGT1* and *UGT2* (Mackenzie et al., 1997). *UGT1* family members are encoded by *UGT1* gene locus, which can potentially generate up to 12 isozymes with unique amino-terminal sequences but an identical carboxyl-terminal sequence by a mechanism of exon sharing (Ritter et al., 1992; Emi et al., 1995). In contrast, *UGT2* family members appear to be encoded by independent genes.

Treatment of rats with prototypical microsomal enzyme inducers (MEIs), such as 3-methylcholanthrene (3-MC), polychlorinated biphenyls, phenobarbital (PB), pregnenolone-16α-carbonitrile (PCN), and clofibrate results in differential induction of UGT activities in liver. 3-MC and other cytochrome P450 1A enzyme inducers increase the glucuronidation of planar chemicals, such as 1-naphthol and 4-nitrophenol. PB and other CYP2B enzyme inducers tend to induce the glucuronidation of bulky chemicals, such as chloramphenicol and morphine (Bock et al., 1973). PCN and other CYP3A enzyme inducers induce UGT activities toward digitoxigenin-monodigitoxoside and bilirubin, whereas clofibrate and other CYP4A enzyme inducers increase the glucuronidation of bilirubin but not digitoxigenin-monodigitoxoside (Watkins et al., 1982; Watkins and Klaassen, 1982). The differential induction of UGTs in rats was the subject of intense research for many years, contributing greatly to the characterization of UGT isozymes.

The recent discovery of several receptors within the cell that function as ligand-activated transcription factors has shed significant light on the molecular mechanisms for the up-regulation of many phase I and phase II biotransformation enzymes following treatment with MEIs. For instance, the cytosolic aryl hydrocarbon receptor is involved in the induction of CYP1A1 by polycyclic aromatic hydrocarbons (Whitlock et al., 1996), whereas three members of the nuclear receptor family, the constitutive androstane receptor (CAR), the pregnane X receptor (PXR), and the peroxisome proliferator-activated receptor α mediate the induction of CYP2B (Honkakoski et al., 1998), CYP3A (Kliewer et al., 1998), and CYP4A (Muerhoff et al., 1992) by xenobiotics, respectively. Generally, the transcriptional regulation via CAR, PXR, and peroxisome proliferator-activated receptor α involves the formation of heterodimers between these receptors and 9-*cis* retinoic acid receptor, binding of the heterodimer to response elements

This work was supported by National Institutes of Health Grant ES-08156 to Curtis D. Klaassen.

[1] Current address: Department of Pharmacology and Toxicology, University of Kansas, Lawrence, KS 66045.

[2] Abbreviations used are: T_4, thyroxine; T_3, triiodothyronine; UGTs, UDP-glucuronosyltransferases; MEIs, microsomal enzyme inducers; 3-MC, 3-methylcholanthrene; PB, phenobarbital; PCN, pregnenolone-16α-carbonitrile; CAR, constitutive androstane receptor; PXR, pregnane X receptor; RXR, 9-*cis* retinoic acid receptor; GA, glucuronic acid; CHAPS, 3-[(3-chlolamidopropyl) dimethylammonio]-1-propanesulfonic acid; TSH, thyroid stimulating hormone; UGTs, UDP-glucuronosyltransferases.

Address correspondence to: Curtis D. Klaassen, Ph.D., Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, 3901 Rainbow Boulevard, Kansas City, KS 66160. E-mail: cklaasse@kumc.edu

in the regulatory region of target genes, and ligand-dependent *trans*-activation of gene expression.

The involvement of the aforementioned receptors in the induction of the UGTs by MEIs is not well characterized in comparison to cytochromes P450, in spite of some recent progress, such as the recent characterization of a PB response enhancer module in human *UGT1A1* gene that can be activated by PB in the presence of CAR (Sugatani et al., 2001). Although the induction of hepatic UGT activities by the potent PXR ligand PCN in rats is well documented in the literature (Watkins et al., 1982; Watkins and Klaassen, 1982), few studies have been conducted to determine the effects of PCN on mRNA levels of *UGT*s in rodents and other species, or the role of PXR in the regulation of UGTs, until recently. In a study that was aimed at exploring the regulatory roles of PXR and CAR in different phases of xenobiotic metabolism, Maglich et al. (2002) showed that PCN increases mRNA levels of mouse *Ugt1a1* in a PXR-dependent manner. However, it remains unclear whether other mouse UGTs are similarly up-regulated by PCN and whether increases in levels of gene transcripts are coupled with corresponding changes in levels of protein and enzyme activity.

The present study was conducted to address these important issues by determining the effects of PCN on microsomal glucuronidation of five representative aglycones (i.e., bilirubin, 1-naphthol, chloramphenicol, T_4, and T_3) and mRNA levels of *UGT* genes in liver of wild-type and PXR-null mice. According to the nomenclature of mouse UGTs (Mackenzie et al., 1997), cDNA sequence information for five mouse *UGT* genes is available. Four of them belong to the *UGT1* family, namely mouse *Ugt1a1*, *1a2*, *1a6*, and *1a9*, and the other one is *Ugt2b5*. Consequently, gene-specific probe sets for these UGTs were generated to measure mRNA levels using QuantiGene signal amplification assay. Up-regulation of *UGT* gene transcripts by PCN was related to inducibility of hepatic glucuronidation activities. To our knowledge, this is the first comprehensive study on the inducibility of the entire collection of cloned mouse UGTs by PCN and possible involvement of PXR in UGT induction.

Materials and Methods

Materials. UDP-glucuronic acid (UDP-GA), PCN, T_4, T_3, 1-naphthol, chloramphenicol, 1-[1-^{14}C]-naphthol, and 3-[(3-chlolamidopropyl) dimethylammonio]-1-propanesulfonic acid (CHAPS) were purchased from Sigma-Aldrich (St. Louis, MO). Bilirubin and ethyl anthranilate were purchased from Aldrich Chemical (Milwaukee, WI). [^{125} I]-T_4, [^{125}I]-T_3, and D-threo-[dichloroacetyl-1, 2-^{14}C]-chloramphenicol were obtained from PerkinElmer Life Sciences (Boston, MA). All other reagents used were acquired from the above suppliers and were of reagent grade or better.

Animals and Treatment. Wild-type and PXR-null mice as described by Staudinger et al. (2001a) were bred in the laboratory animal facilities of the University of Kansas Medical Center. Animals were housed in polypropylene cages (less than four animals per cage) on corn-cob bedding. Animal room temperature was maintained at approximately 21°C with a 12-h light cycle. Four- to six-month-old male mice were fed either control or PCN-treated (1,500 ppm) diet for 21 days. Animals had free access to feed and water. Feed consumption and body weights were recorded every 2 days. On day 22, mice were decapitated. Livers were immediately removed and snap-frozen in liquid nitrogen. Tissue samples were stored at −80°C until assayed.

Preparation of Microsomes. Microsomes were prepared by ultracentrifugation (100,000*g* for 60 min) of the postmitochondrial supernatant (10,000*g* for 20 min) from a 10% liver homogenate (w/v), prepared in 50 mM Tris-HCl (pH 7.4) containing 150 mM KCl as described by Lu and Levin (1972). The microsomal fraction was washed by homogenization in 10 mM EDTA (pH 7.4) containing 150 mM KCl. After ultracentrifugation, the resulting pellets were covered with a small volume of 250 mM sucrose and stored at −80°C.

UGT Activity toward Bilirubin. The method of Heirwegh et al. (1972) was followed to determine UGT activity toward bilirubin. The microsomal pellets were suspended in 0.25 M sucrose (0.4 g eq. wet weight of liver/ml). The resulting preparation was further diluted 1:1 with digitonin solution (5.4 mg/ml) and agitated for at least 20 min at 4°C before use. Reaction mixtures contained 200 μl of 0.5 M triethanolamine-HCl buffer (pH 7.7), 40 μl of 125 mM $MgCl_2$, 200 μl of bilirubin-albumin mixture (0.25 mg/ml), 200 μl of digitonin-activated microsomal preparation, and 20 μl of UDP-GA (77 mM). After incubation at 37°C for 15 min, reactions were stopped by adding 2 ml of glycine-HCl buffer (pH 2.7). The tubes were placed in a water bath at 25°C. Ethyl anthranilate diazo reagent (1 ml) was added, and diazo-coupling was allowed to proceed at 25°C for 30 min. The reaction was terminated by an 8-min incubation with 0.5 ml of freshly prepared ascorbic acid (100 mg/ml). Tubes were placed on ice to cool. The contents of each tube were then shaken vigorously (30 times) with 2 ml of 2-pentanone/*n*-butyl acetate (17:3, v/v). The tubes were placed in −20°C freezer for a few min. The contents of each tube were then vortexed thoroughly. Following centrifugation (1,000*g* for 5 min), absorbance of pigment in the resulting organic phases was measured at 546 nm using the extraction solvent as a blank reference.

UGT Activities toward 1-Naphthol and Chloramphenicol. UGT activities toward 1-naphthol and chloramphenicol were assayed in both native and activated microsomal preparation according to the method of Hazelton et al. (1985) with minor modification. Briefly, the microsomal pellets were suspended in 0.25 M sucrose (1 g eq. wet weight of liver/ml). A portion of this suspension was diluted 1:1 with 0.25 M sucrose, and the resulting preparation was termed "native" microsomes. A second portion of the suspension was diluted with an equal volume of 0.25 M sucrose containing 16 mM CHAPS. This preparation was termed "activated" microsomes. Both of the diluted microsomal preparations were agitated for at least 20 min at 4°C. The reaction mixture for both assays was 0.2 M Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 2.2 mM saccharic acid-1,4-lactone, 1-naphthol (0.5 mM, 0.04 μCi), or chloramphenicol (2.0 mM, 0.4 μCi), and microsomal preparation (0.1 mg and 0.25 mg of protein for 1-naphthol and chloramphenicol assay, respectively) in a final volume of 0.5 ml. Reactions were initiated by the addition of 4 mM UDP-GA. Blank controls received water and 0.25 M sucrose with or without CHAPS instead of UDP-GA and microsomal preparation. After incubation at 37°C for 15 min (1-naphthol) or 20 min (chloramphenicol), reactions were stopped by the addition of ice-cold ethanol (1-naphthol) or water (chloramphenicol). The latter was then heated in boiling water for 45 s. The parent compounds were separated from the glucuronidated products by extraction with chloroform (1-naphthol) and isoamyl acetate (chloramphenicol). After extraction, radioactivity in an aliquot of the aqueous phase was determined with a liquid scintillation counter.

UGT Activities toward T_4 and T_3. UGT activities toward T_4 and T_3 were determined as described by Hood and Klaassen (2000). Reaction mixture (final volume, 150 μl) was made up of 75 mM Tris-HCl, 7.5 mM $MgCl_2$, 30 mM UDP-GA, 1 μM T_4 or T_3 (approximately 100,000 cpm), and 0.1 mM propylthiouracil (to inhibit outer-ring deiodinase activity). Reactions were started by adding 50 μl of protein (final concentration of 250 μg/ml for T_4 and 81 μg/ml for T_3) and incubated at 37°C for 60 min. The reactions were stopped by adding 200 μl of ice-cold methanol. ^{125}I-T_4-glucuronide or ^{125}I-T_3-glucuronide was separated from unconjugated T_4 or T_3 with a Sephadex LH-20 column (1-ml bed volume) (Amersham Biosciences Inc., Piscataway, NJ). The amount of ^{125}I-T_4 or ^{125}I-T_3 in the eluates was quantified using a Packard gamma counting system (PerkinElmer Life Sciences, Boston, MA).

Isolation of Total RNA. Total RNA was isolated using RNAzol Bee reagent (Tel-Test Inc., Friendswood, TX) according to the manufacturer's instruction and resuspended in diethyl pyrocarbonate-treated water. The concentration and purity of total RNA in each sample were assessed by measuring absorbance at 260 and 280 nm. The integrity of RNA samples was verified by checking the integrity of 18S and 28S rRNA separated by formaldehyde-agarose gel electrophoresis.

Design of Specific Oligonucleotide Probe Sets for the Analysis of Mouse UGT mRNA Levels Using QuantiGene Signal Amplification Assay. The nomenclature for mouse UGTs as described by Mackenzie et al. (1997) was followed in this study. The GenBank accession numbers for the mouse UGTs examined, namely *Ugt1a1*, *1a2*, *1a6*, *1a9*, and *2b5*, are listed in Table 1, despite the existence of multiple GenBank accession numbers for the same or portions of the same coding sequence for most of these genes. For *UGT1A* family members, cDNA sequences were subjected to multiple alignment

TABLE 1

List of oligonucleotide probes generated for the analysis of mRNA levels of mouse UGTs using QuantiGene signal amplification assay.

UGT Gene	GenBank No.[a]	Function[b]	Sequence	Target
Ugt1a1	L02333	CE	atactcagccagtggcttccaTTTTTctcttggaaagaaagt	129–149
		CE	ttcgtgccccttctgctgTTTTTctcttggaaagaaagt	172–189
		CE	acagggaacttcctcagagtgtaaTTTTTctcttggaaagaaagt	240–263
		CE	gtgcagcaggtgggagcaTTTTTctcttggaaagaaagt	400–417
		LE	ggaccgaacacacacaacagaaTTTTTaggcataggacccgtgtct	65–86
		LE	cccagcgtgggatgcatagTTTTTaggcataggacccgtgtct	87–105
		LE	tccatagggaacactaacagcctTTTTTaggcataggacccgtgtct	106–128
		LE	gagctgctgaataactccaagcTTTTTaggcataggacccgtgtct	150–171
		LE	gcttcaggtgctatgaccacaacTTTTTaggcataggacccgtgtct	190–212
		LE	aatgatccttcttttatgtgtattgagTTTTTaggcataggacccgtgtct	213–239
		LE	ccctttgactttcatatatattttgaTTTTTaggcataggacccgtgtct	350–376
		LE	gaggccataaactcggcattTTTTTaggcataggacccgtgtct	418–437
		BL	cacattttccttctggaatggc	264–285
		BL	ccaagttccaccaaagtagctgt	286–308
		BL	aatcttgattaaaggcagtccgt	309–331
		BL	ccacgcgcagcagaaaag	332–349
		BL	gccagctaggagcatactggaat	377–399
Ugt1a2	D87866	CE	ccctgagattcctcgaagggTTTTTctcttggaaagaaagt	23–42
		CE	ccttcagcccagggcaggTTTTTctcttggaaagaaagt	63–80
		CE	tttcttgctgatattcttccttggTTTTTctcttggaaagaaagt	251–274
		LE	gcacacatagtcccgtgtccatTTTTTaggcataggacccgtgtct	1–22
		LE	gggtaacaccagcacttttgcaTTTTTaggcataggacccgtgtct	81–102
		LE	gcacaacatccctcatgctcagTTTTTaggcataggacccgtgtct	121–142
		LE	caggaccacagtctggtgacctTTTTTaggcataggacccgtgtct	159–180
		LE	ggtcttctcaatgtcgctcagtaTTTTTaggcataggacccgtgtct	275–297
		LE	ccttcacaaaatgttgtgttttaaaTTTTTaggcataggacccgtgtct	298–322
		BL	gcacacagcaggagcagcag	43–62
		BL	ccactggctgccctccat	103–120
		BL	cgggcgtggagctccc	143–158
		BL	tgtgcacagtcacctctgaagc	181–202
		BL	gggtgaagaagtcttctcctttga	203–226
		BL	tatatggaaaggcataggttttga	227–250
Ugt1a6	U16818	CE	ggaagctgagggtggcagagtTTTTTctcttggaaagaaagt	523–543
		CE	atggctgggtctgtgaacagaTTTTTctcttggaaagaaagt	566–586
		CE	acagggcttgggctttgacTTTTTctcttggaaagaaagt	676–694
		CE	catgaccggcctgggatattTTTTTctcttggaaagaaagt	910–929
		LE	ccttcaggaggctctggcagtTTTTTaggcataggacccgtgtct	502–522
		LE	gcatcaaacttgttctccctgaTTTTTaggcataggacccgtgtct	544–565
		LE	ccaggatcacaccacagggcTTTTTaggcataggacccgtgtct	587–606
		LE	agagggcaggttgagatactcagTTTTTaggcataggacccgtgtct	607–629
		LE	caagcatgtgttccagagagcaTTTTTaggcataggacccgtgtct	654–675
		LE	gtgtagaatctgggcacataggacTTTTTaggcataggacccgtgtct	695–718
		LE	acgtcatgtggtctgagaatttgTTTTTaggcataggacccgtgtct	719–741
		LE	actttgaatacagacaataatataggtagttTTTTTaggcataggacccgtgtct	783–813
		LE	gaggtctgaggcaatgatttcatTTTTTaggcataggacccgtgtct	814–836
		LE	gccacagagaattctggtgtaaggTTTTTaggcataggacccgtgtct	862–885
		LE	cgaacacaaaatcgtaccgtaacaTTTTTaggcataggacccgtgtct	886–909
		BL	tgggaaacctctgaagaggtagac	630–653
		BL	gccagccgttggggaa	742–757
		BL	ttccaagatgttaacaatgaagttg	758–782
		BL	aaggtagggacacatctctcttgag	837–861
Ugt1a9	L27122	CE	ccagcagcctgcctgccTTTTTctcttggaaagaaagt	42–58
		CE	attcagtgatttccccagctgTTTTTctcttggaaagaaagt	163–183
		CE	tctgtccaggtcttccagagtgtTTTTTctcttggaaagaaagt	212–234
		CE	ccattgagtgtaagataaatacttgaactcTTTTTctcttggaaagaaagt	235–264
		LE	tgggccaagccagaggcTTTTTaggcataggacccgtgtct	25–41
		LE	tgaatgagtttctccacaaccatcTTTTTaggcataggacccgtgtct	96–119
		LE	ccacaacctcatgccctctgTTTTTaggcataggacccgtgtct	120–139
		LE	ccaactcacctctgggatgactaTTTTTaggcataggacccgtgtct	140–162
		LE	gaacgtatactgtgttctggagttttTTTTTaggcataggacccgtgtct	265–290
		LE	tgtcgttaaacaaactcctgcagTTTTTaggcataggacccgtgtct	339–361
		LE	ctgcttcaagtactccactaacttctTTTTTaggcataggacccgtgtct	362–387
		BL	ttccatccatgggcacca	59–76
		BL	tgcatggtgaaccagtggc	77–95
		BL	gagaaattgagtaagtcttcactgtaca	184–211
		BL	tctggctgaaccagtcaagaaa	291–312
		BL	tgtgaaaatgttagttcaaagaaacc	313–338
Ugt2b5	X06358	CE	gcagcagcagagcagaaatccTTTTTctcttggaaagaaagt	26–46
		CE	aaggtctcagaacagtgacttcatgTTTTTctcttggaaagaaagt	163–187
		CE	aaacttaaggccaggtgatttttTTTTTctcttggaaagaaagt	212–234
		LE	aacactttcccacatttcacagaTTTTTaggcataggacccgtgtct	70–92
		LE	gaattccatcggccacaccTTTTTaggcataggacccgtgtct	93–111
		LE	gtattatttttatgttcatccaatgactTTTTTaggcataggacccgtgtct	112–139
		LE	tgggatcaagaacatagtaagctgTTTTTaggcataggacccgtgtct	188–211
		LE	tcataagtccacacatccacaaacTTTTTaggcataggacccgtgtct	288–311
		BL	tctgaagcagcaacttatctgca	47–69
		BL	gcctctctgtaccagttcatcca	140–162
		BL	tttactgacagatgtaggaaaagtttc	235–261
		BL	ttaatgaaaaaattttccagattgtc	262–287

[a] UGT sequence information is available on the UDP-glucuronosyltransferase website: http://www.unisa.edu.au/pharm_medsci/Gluc_trans/.
[b] Function of the oligonucleotide probe in the QuantiGene assay: CE, capture extender; LE, label extender; BL, blocker probe.

analysis (CLUSTAL W; available at http://www.ddbj.nig.ac.jp) and only the 5′-variable regions were used as the target sequences for probe design. For mouse *Ugt2b5*, the entire cDNA sequence was used for probe design. These target sequences were analyzed by ProbeDesigner software version 2.0 (Bayer Corp.-Diagnostics Div., Tarrytown, NY) to generate three groups of oligonucleotide probes (i.e., capture extender, label extender, and blocker probe). Their functions in the assay were described previously (Hartley and Klaassen, 2000). All probes were designed with a T_m of approximately 63°C to allow hybridization under constant conditions (i.e., 53°C). To ensure minimal cross-reactivity with other mouse sequences, each candidate probe was submitted to the National Center for Biotechnological Information for nucleotide comparison by the basic local alignment search tool (BLASTn). Oligonucleotides with a high degree of similarity (>80%) to other mouse gene transcripts were eliminated from the design. Detailed information about the final probe sets is listed in Table 1. Each probe were synthesized on a 50-nmol synthesis scale by Operon Technologies (Alameda, CA) and obtained desalted and lyophilized. Probes were diluted in 0.5 ml of 1× Tris EDTA buffer (pH 8.0) and stored at −20°C.

QuantiGene Signal Amplification Assay. The application of QuantiGene signal amplification assay to the measurement of mRNA levels of drug-metabolizing enzymes was extensively described and validated by Hartley and Klaassen (2000). Briefly, capture extenders, label extenders, and blocker probes for each specific UGT were combined and diluted to 50, 200, and 100 fmol/μl, respectively, in the lysis buffer. All reagents for analysis (i.e., lysis buffer, capture hybridization buffer, amplifier/label probe buffer, and substrate solution) were supplied in the assay kit (Bayer Diagnostics). Total RNA (10 μg) was added to each well of a 96-well plate along with 50 μl of capture hybridization buffer and 50 μl of each diluted probe set. RNA was allowed to hybridize for at least 16 h at 53°C. Plates were then rinsed twice with wash buffer (400 μl). Amplifier molecules (100 μl) diluted in amplifier/label probe buffer (1:1,000) were added to each well, and plates were incubated at 46°C for 60 min. Plates were rinsed again with wash buffer followed by the addition of 100 μl of label probe (1:1,000 in amplifier/label buffer) to each well. After incubation at 46°C for 60 min, plates were rinsed twice with wash buffer. Alkaline phosphatase-mediated luminescence was triggered by the addition of a dioxetane substrate solution (100 μl/well). The enzymatic reaction was allowed to proceed for 30 min at 37°C, and luminescence was measured with the Quantiplex 320 bDNA Luminometer (Bayer Diagnostics) interfaced with Quantiplex Data Management Software version 5.02 (Bayer Diagnostics) for analysis of luminescence from 96-well plates.

Statistical Analysis. Results were expressed as means ± standard error of means (S.E.). Treatment groups consisted of 6 to 8 animals. Difference between groups was analyzed using analysis of variance followed by Newman-Keuls test. p values less than 0.05 were considered statistically significant.

Results

PCN treatment in rodents results in an increase in liver mass (Japundzic et al., 1974). This effect is absent in PXR-null mice, suggesting that the nuclear receptor is required for PCN-induced hepatomegaly (Staudinger et al., 2001b). In the present study, liver-to-body-weight ratio in wild-type mice was significantly increased following 21-day PCN treatment. PCN treatment did not increase liver mass in the null mice (Fig. 1). These data indicate the absence of functional PXR in the null mice. However, a significant increase in liver-to-body-weight ratio was observed for PXR-null mice in comparison to control wild-type mice but was not reported in an earlier study (Staudinger et al., 2001b). The basis for this difference is unclear.

Effect of PCN Treatment on Hepatic UGT Activities. Hepatic microsomal glucuronidation of five different aglycones (i.e., bilirubin, 1-naphthol, chloramphenicol, T_4, and T_3) was measured to determine the effects of PCN on UGT activities. As shown in Fig. 2, a 2.1-fold increase in hepatic UGT activity toward bilirubin was seen in wild-type mice following PCN treatment. PCN treatment, however, had no effect on bilirubin UGT activity in PXR-null mice, although the basal

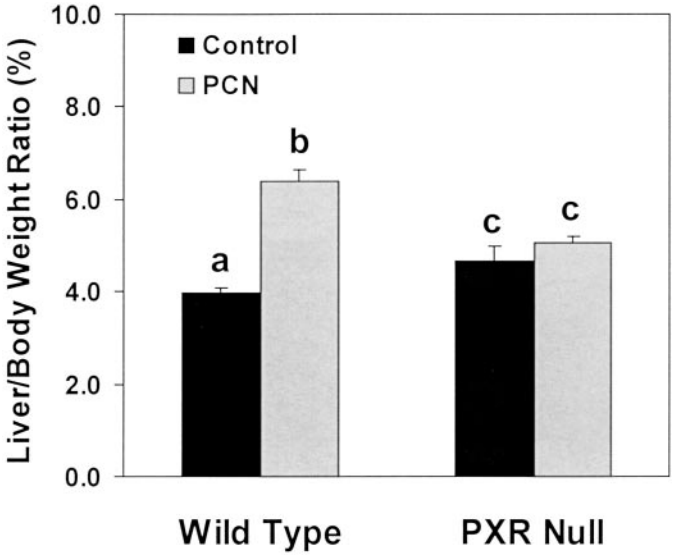

FIG. 1. *Effect of PCN treatment on liver-to-body weight ratio in wild-type and PXR-null mice.*

Values are expressed as the mean ± S.E. ($n = 6–8$). Bars not sharing at least one letter are statistically different from each other ($p < 0.05$).

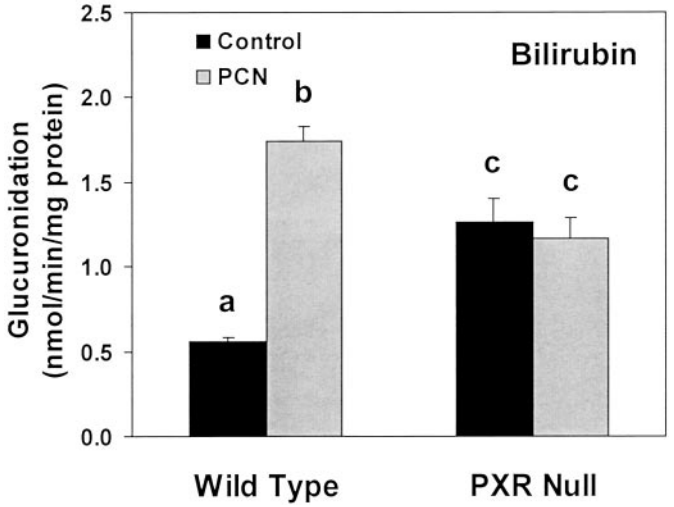

FIG. 2. *Effect of PCN treatment on hepatic UDP-glucuronosyltransferase activity toward bilirubin in wild-type and PXR-null mice.*

Values are expressed as the mean ± S.E. ($n = 6–8$). Bars not sharing at least one letter are statistically different from each other ($p < 0.05$).

activity in the null mice was about 125% higher than that of wild-type mice.

The glucuronidation of 1-naphthol and chloramphenicol was measured using native and detergent-treated microsomal preparations as described previously (Hazelton et al., 1985). UGT activity to 1-naphthol was increased by 360 to 500% by the detergent treatment, whereas that to chloramphenicol was increased by only 45% (Fig. 3). Nevertheless, PCN increased the UGT activities to 1-naphthol and chloramphenicol by about 40 and 60%, respectively, in both native and activated microsomal preparations. In contrast, 1-naphthol and chloramphenicol UGT activities in PXR-null mice were not increased by PCN. Furthermore, basal levels of UGT activity to chloramphenicol, but not 1-naphthol, in the null mice were significantly higher than those of wild-type mice.

Glucuronidation of T_4 and T_3 results in inactivation of their bio-

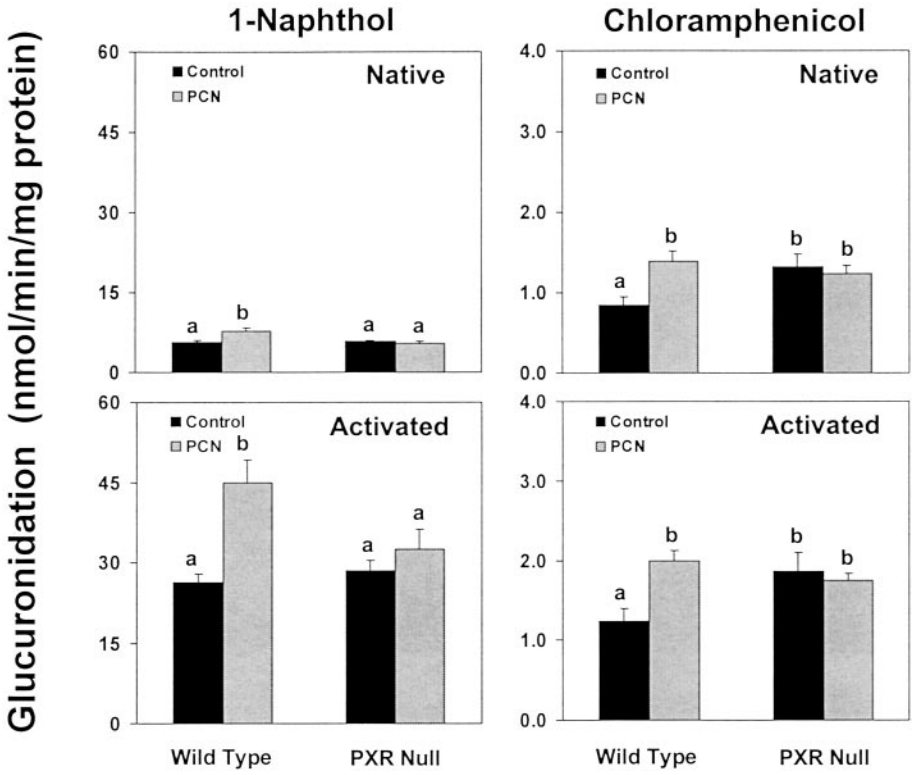

FIG. 3. *Effect of PCN treatment on hepatic UDP-glucuronosyltransferase activities toward 1-naphthol and chloramphenicol in wild-type and PXR-null mice.*

Enzyme activities were measured in both native and detergent-treated (activated) microsomes as described under *Material and Methods*. Values are expressed as the mean ± S.E. ($n = 6-8$). Bars not sharing at least one letter are statistically different from each other ($p < 0.05$).

logical activities and facilitates their elimination from the body. In wild-type mice, PCN increased T_4 and T_3 glucuronidation by 80 and 40%, respectively (Fig. 4). However, no difference in glucuronidation of thyroid hormones was observed between groups of control and PCN-treated PXR-null mice. UGT activity toward T_4 and T_3 of PXR-null mice on control diet was about 35 and 30% higher than that of wild-type controls, respectively.

Effect of PCN Treatment on Hepatic mRNA Levels of UGTs. Despite the across-the-board increase in hepatic glucuronidation of a range of aglycones shown above, little information on the role of PXR in the regulation of a particular mouse UGT isozyme can be deduced from these results. Therefore, oligonucleotide probe sets specific to the known mouse *UGT* genes were designed and used to determine the effects of PCN on mRNA levels of these genes using the QuantiGene signal amplification assay.

In wild-type mice, PCN increased the hepatic mRNA levels of *Ugt1a1* and *1a9* by 190 and 80%, respectively, but had no effect on those of *Ugt1a2* and *1a6* (Fig. 5). In PXR-null mice, the mRNA levels of these four *UGT1* family members were not significantly increased by PCN treatment. Furthermore, *Ugt1a1* mRNA levels of the null mice on control diet were 100% higher than those of wild-type controls. Therefore, the PXR-dependent increase in *Ugt1a1* and *1a9* gene transcripts and the higher basal levels of *Ugt1a1* message seen in the null mice as compared with the wild-type mice are in accordance with the effects of PCN on hepatic UGT activities as well as the higher constitutive glucuronidation activities seen in the null mice. The value of relative light unit detected using the *Ugt1a2* probeset was very low (less than 1); thus, suggesting that this gene is not as highly expressed in liver as the other members of *UGT1* family examined.

As shown in Fig. 6, *Ugt2b5* mRNA levels in liver of both wild-type and PXR-null mice were not affected by PCN treatment. Also, no difference in the basal levels of *Ugt2b5* gene transcript was observed between wild-type and null mice.

Discussion

PCN belongs to a group of steroids known as "catatoxic steroids", which are named after their ability to afford protection against various types of intoxication by accelerating the elimination of harmful chemicals from the body (Kourounakis et al., 1977). Treatment of rodents with PCN results in the induction of drug metabolism enzymes and transport systems including CYP3A (Kliewer et al., 1998), UGTs (Watkins and Klaassen, 1982; Hazelton and Klaassen, 1988), sulfotransferases (Liu and Klaassen, 1996), organic anion transporting polypeptide 2 (Guo et al., 2002), and multidrug resistance protein 2 (Johnson and Klaassen, 2002). The involvement of the nuclear receptor PXR in the up-regulation of CYP3A, Oatp2, and sulfotransferase by PCN treatment was recently established (Staudinger et al., 2001a; Sonoda et al., 2002). Along the same line, the data presented herein showed that hepatic UGT activities to the five representative aglycones as well as the steady-state mRNA levels of *Ugt1a1* and *1a9* were increased in a PXR-dependent manner, suggesting that induction of the major phase II biotransformation family of enzymes, namely the UGTs, is also under control by PXR. Thus, these findings, along with reports by others (Kliewer et al., 1998; Staudinger et al., 2001a; Sonoda et al., 2002) support the notion that PXR is an important component of the body's adaptive defense mechanism against toxic substances.

In the present study, basal levels of hepatic UGT activities toward bilirubin, chloramphenicol, T_4, and T_3 were significantly higher in PXR-null mice than wild-type mice. This is accompanied by about a doubling in basal levels of *Ugt1a1* gene transcripts in null mice as

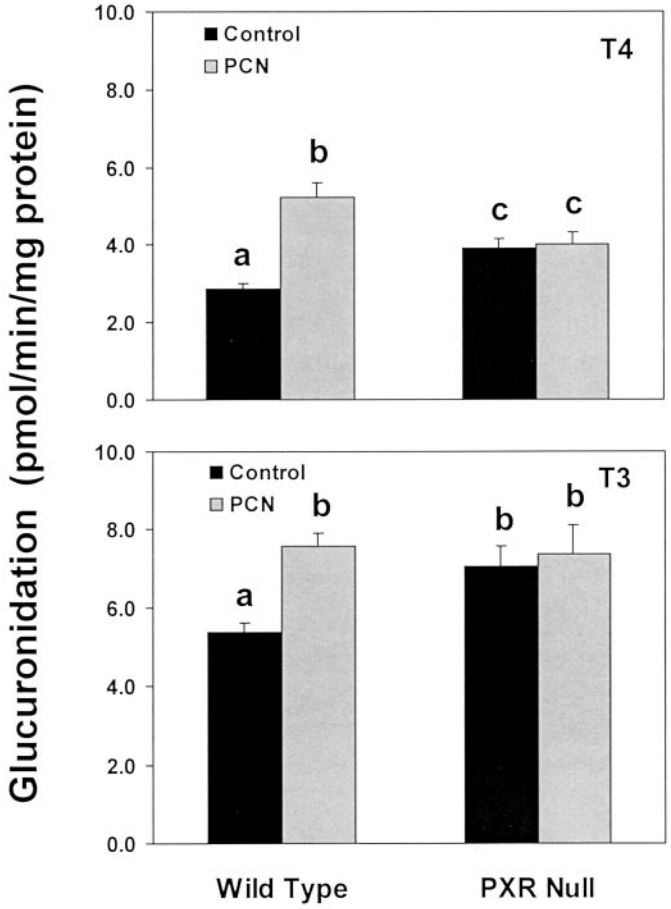

FIG. 4. *Effect of PCN treatment hepatic UDP-glucuronosyltransferase activities toward thyroxine (T_4) and triiodothyronine (T_3) in wild-type and PXR-null mice.*

Values are expressed as the mean ± S.E. ($n = 6–8$). Bars not sharing at least one letter are statistically different from each other ($p < 0.05$).

compared with wild types, but the difference is marginally insignificant ($p = 0.072$). Similarly, Staudinger et al. (2001a,b) reported a slight but statistically significant increase in *Cyp3a11* mRNA levels and activity as indicated by testosterone 6β-hydroxylation activity and zoxazolamine resistance in the same line of PXR-null mice. In contrast, reports by Xie et al. (2000) showed that ablation of PXR function has no effect on the basal levels of *Cyp3a11* gene transcription and CYP3A activity in another independently developed PXR-null mouse model. Despite the lack of insight into the basis for the discrepancy between the two lines of PXR-null mice, the findings of the present study, together with those of Staudinger et al. (2001a,b), support possible involvement of PXR in the control of constitutive expression of biotransformation enzymes such as UGTs and CYP3A.

The induction of hepatic UGT activities to bilirubin, 1-naphthol, chloramphenicol, T_4, and T_3 by PCN treatment, seen in the present study does not agree with the findings of a previous study (Viollon-Abadie et al., 1999), which showed no induction of UGT activities toward bilirubin, *p*-nitrophenol, androsterone, T_4, and T_3, in OF-1 mice treated with PCN p.o. for 14 days. The results of the present study, however, agree with the dose-dependent increase in hepatic glucuronidation of chloramphenicol, 1-naphthol, and T_4 in B6C3F1 mice given PCN-treated diets for 21 days (Hood et al., 2003). Furthermore, Maglich et al. (2002) observed a PXR-dependent induction of UGT1A1 in the same line of wild-type and PXR-null mice following PCN treatment (two i.p. injections within 24 h). In the present study, the estimated daily dose of PCN is 125–175 mg/kg (average body weight of the animals, 30 g; daily food intake, 2.5–3.5 g per animal). This dose range of PCN is close to that used in the other three previous studies (about 100 mg of PCN/kg). Although the dosage and duration of PCN treatment in the current study and the study by Allen et al. (submitted) are similar to those in the study of Viollon-Abadie et al. (1999), the results of the two studies that used either B6C3F1 mice or PXR-null and wild-type mice differ drastically from those of the study that used OF-1 mice. In contrast, a PXR-dependent induction of UGT(s) was observed in the same line of PXR-null and wild-type mice treated either acutely (Maglich et al., 2002) or subchronically with PCN (the present study). Therefore, it seems that a strain difference in the control of UGT induction following PCN treatment may exist in mice. This conclusion is analogous to the difference in induction of phenol UGT activities observed in C57BL/6 and DBA/2 mice, which are responsive and nonresponsive to 3-MC-type inducers, respectively (Bock et al., 1982). Further studies are necessary to elucidate possible mechanisms underlying this strain difference in UGT induction by PCN.

Generally, UGT1 isozymes are categorized into the following two groups based on substrate preferences: bilirubin UGT and 3-MC inducible phenol UGT. UGT1A1 is the most important glucuronosyltransferase for bilirubin glucuronidation in human and rats, whereas human UGT1A6 and 1A9 as well as rat UGT1A6 and 1A7 are the major transferases for phenol glucuronidation (Burchell et al., 1998). Human UGT1A1 is inducible by PB. In contrast, bilirubin glucuronidation in rats is not readily inducible by PB but can be greatly induced by the hypolipidemic agent clofibrate as well as PCN (Bock et al., 1973; Watkins et al., 1982; Watkins and Klaassen, 1982). In the present study, PCN treatment increased hepatic UGT activities to bilirubin, and the phenolic compound 1-naphthol in mice. The increases in UGT activities were accompanied by parallel changes in mRNA levels of *Ugt1a1* and *Ugt1a9* but not *Ugt1a2*, *1a6*, and *2b5*. These findings agree with the predicted function of mouse UGT1A1 as bilirubin UGT and mouse UGT1A9 as phenol UGT, based on their high sequence similarity to rat and human bilirubin and phenol UGT isoform(s) (Kong et al., 1993). However, an in vitro enzyme expression study, so far, has not been conducted to confirm the substrate specificity of these two isozymes. Interestingly, mouse UGT1A6, a known phenol UGT (Lamb et al., 1994), was not induced by PCN in the present study. Therefore, it seems that the two phenol UGTs in mice, namely UGT1A6 and UGT1A9, are differentially regulated by PCN. The existence of distinct promoters for the multiple first exons encoding the variable regions of *UGT1* family members is the underlying mechanism for the differential induction of these isozymes.

Hepatic microsomal glucuronidation of chloramphenicol is not impaired in Gunn rats, which lack UGT1 enzyme activities due to a genetic mutation in the common region of UGT1 (Watkins and Klaassen, 1982). These findings suggest that the glucuronidation of chloramphenicol is catalyzed by UGTs other than those belonging to the *UGT1* family. In the present study, hepatic UGT activity to chloramphenicol was induced by PCN in wild-type mice but not in the null mice. However, PCN treatment had no effect on mRNA levels of *Ugt2b5*, a mouse UGT outside the *UGT1* family (Kimura and Owens, 1987). Thus, the identity of the UGT isozyme(s) involved in chloramphenicol glucuronidation remains unclear. The most likely explanation for this observation is that at least one other *UGT2* family member is involved in the glucuronidation of chloramphenicol, and is inducible by PCN via the PXR regulatory pathway.

Glucuronidation of thyroid hormones and subsequent excretion of the conjugates into bile serve as the major route for the elimination of T_4 and T_3. In rats, UGT activity to T_4 is increased following treatment

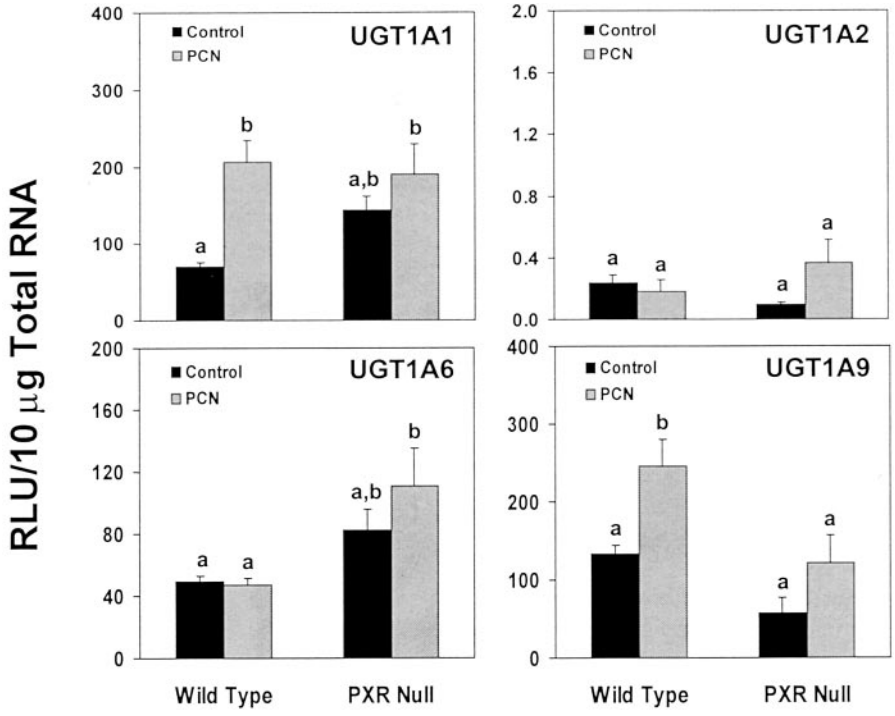

FIG. 5. *Effect of PCN treatment on mRNA levels of mouse* UGT1 *family members, namely, Ugt1a1, 1a2, 1a6, and 1a9 in liver of wild-type and PXR-null mice.*

Values are expressed as the mean ± S.E. ($n = 6–8$). Bars with different letters are statistically different from each other ($p < 0.05$).

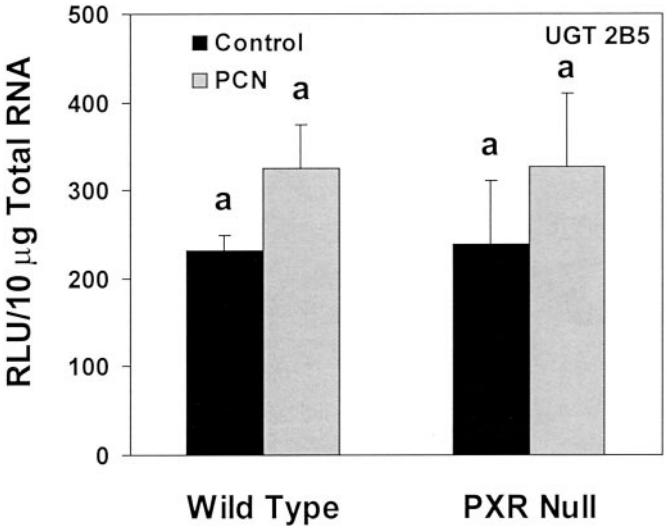

FIG. 6. *Effect of PCN treatment on mRNA levels of mouse Ugt2b5 in liver of wild-type and PXR-null mice.*

Values are expressed as the mean ± S.E. ($n = 6–8$). Bars with different letters are statistically different from each other ($p < 0.05$).

with PCN, PB, 3-MC, and polychlorinated biphenyl, among which only PCN and PB produced an increase in T_3-UGT activity (Barter and Klaassen, 1992; Liu et al., 1995; Hood and Klaassen, 2000). Although all of these compounds decrease serum T_4 concentrations, only PCN and PB are associated with increase in circulating concentrations of thyroid stimulating hormone (TSH) (Barter and Klaassen, 1994; Liu et al., 1995), which regulates thyroid hormone synthesis. Increased serum TSH concentrations are of concern because sustained increases in TSH have been associated with chemicals such as propylthiouracil and potassium percholorate that induce thyroid cancer in lab animals (Kanno et al., 1990; Hood et al., 1999). Thus, it would be of interest to determine which UGT isozyme(s) catalyze(s) the glucuronidation of thyroid hormones, and how xenobiotics induce UGT activities toward thyroid hormones. With such information, a screening assay for potential thyroid tumor promoters could be developed. More than one UGT isozyme is thought to be responsible for glucuronidation of thyroid hormones in liver. In rats, T_4 glucuronidation is catalyzed by both bilirubin UGT and 3-MC-inducible phenol UGT, whereas androsterone-metabolizing UGT2B2 may be the isozyme for T_3 glucuronidation (Visser et al., 1993). In humans, convincing results showed that UGT1A1 and 1A9 catalyze T_4 glucuronidation, whereas studies on the role of UGTs in T_3 metabolism remain inconclusive (Findlay et al., 2000). In contrast, little is known about the identity of UGTs responsible for the glucuronidation of thyroid hormones in mice. The present study shows for the first time that treatment of mice with PCN increased hepatic UGT activities toward T_4 and T_3 in a PXR-dependent manner, which is correlated with changes in mRNA levels of mouse *Ugt1a1* and *Ugt1a9*. These results suggest that thyroid hormones may be glucuronidated in mice by bilirubin and phenol UGTs, which are regulated by the ligand-activated transcription factor PXR. It should be noted that our results cannot exclude the possibility that thyroid hormones are glucuronidated by some unknown PCN-inducible UGTs. Future studies are needed to determine whether induction of thyroid hormone glucuronidation by PCN in mice would be associated with disturbed thyroid homeostasis, as seen in rats.

In conclusion, the present study provides evidence supporting the involvement of PXR in the constitutive expression and induction of UGTs by PCN. In light of the major role of hepatic glucuronidation played in the metabolism of a wide range of endogenous and xenobiotic compounds, these findings further emphasize the pivotal role of PXR in the control of biotransformation processes.

References

Barter RA and Klaassen CD (1992) Rat liver microsomal UDP-glucuronosyltransferase activity toward thyroxine: characterization, induction and form specificity. *Toxicol Appl Pharmacol* **115:**261–267.

Barter RA and Klaassen CD (1994) Reduction of thyroid hormone levels and alteration of thyroid function by four representative UDP-glucuronosyltransferase inducers in rats. *Toxicol Appl Pharmacol* **128:**9–17.

Bock KW, Frohling W, Remmer H, and Rexer B (1973) Effects of phenobarbital and 3-methylcholanthrene on substrate specificity of rat liver microsomal UDP-glucuronyltransferase. *Biochim Biophys Acta* **327:**46–56.

Bock KW, Lilienblum W, and Pfeil H (1982) Functional heterogeneity of UDP-glucuronosyltransferase activities in C57BL/6 and DBA/2 mice. *Biochem Pharmacol* **31:** 1273–1277.

Burchell B, Brierley CH, Monaghan G, and Clarke DJ (1998) The structure and function of the UDP-glucuronosyltransferase gene family. *Adv Pharmacol* **42:**335–338.

Emi Y, Ikushiro S, and Iyanagi T (1995) Drug-responsive and tissue-specific alternative expression of multiple first exons in rat UDP-glucuronosyltransferase family 1 (UGT1) gene complex. *J Biochem (Tokyo*) **117:**392–399.

Findlay KA, Kaptein E, Visser TJ, and Burchell B (2000) Characterization of the uridine diphosphate-glucuronosyltransferase-catalyzing thyroid hormone glucuronidation in man. *J Clin Endocrinol Metab* **85:**2879–2883.

Guo GL, Staudinger J, Ogura K, and Klaassen CD (2002) Induction of rat organic anion transporting polypeptide 2 by pregnenolone-16alpha -carbonitrile is via interaction with pregnane X receptor. *Mol Pharmacol* **61:**832–839.

Hartley DP and Klaassen CD (2000) Detection of chemical-induced differential expression of rat hepatic cytochrome P450 mRNA transcripts using branched DNA signal amplification technology. *Drug Metab Dispos* **28:**608–616.

Hazelton GA, Hjelle JJ, and Klaassen CD (1985) Effects of butylated hydroxyanisole on hepatic glucuronidation capacity in mice. *Toxicol Appl Pharmacol* **78:**280–290.

Hazelton GA and Klaassen CD (1988) UDP-glucuronosyltransferase activity toward digitoxigenin-monodigitoxoside. Differences in activation and induction properties in rat and mouse liver. *Drug Metab Dispos* **16:**30–36.

Heirwegh KP, Van De Vijver M, and Fevery J (1972) Assay and properties of digitonin-activated bilirubin uridine-diphosphate glucuronyltransferase from rat liver. *Biochem J* **129:**605–618.

Honkakoski P, Zelko I, Sueyoshi T, and Negishi M (1998) The nuclear orphan receptor CAR-retinoid X receptor heterodimer activates the phenobarbital-responsive enhancer module of the CYP2B gene. *Mol Cell Biol* **18:**5652–5658.

Hood A, Allen ML, Liu Y-P, Liu J, and Klaassen CD (2003) Induction of T_4 UDP-GT activity, serum thyroid stimulating hormone, and thyroid follicular cell proliferation in mice treated with microsomal enzyme inducers. *Toxicol Appl Pharmacol* **188:**6–13.

Hood A and Klaassen CD (2000) Differential effects of microsomal enzyme inducers on in vitro thyroxine (T_4) and triiodothyronine (T_3) glucuronidation. *Toxicol Sci* **55:**78–84.

Hood A, Liu J, and Klaassen CD (1999) Effects of phenobarbital, pregnenolone-16alpha-carbonitrile and propylthiouracil on thyroid follicular cell proliferation. *Toxicol Sci* **50:**45–53.

Japundzic MM, Garg BD, Kovac K, and Japundzic IP (1974) Effect of pregnenolone-16-alpha-carbonitrile on mitotic activity in the intact and regenerating rat liver. *Experientia* **30:**562–563.

Johnson DR and Klaassen CD (2002) Regulation of rat multidrug resistance protein 2 by classes of prototypical microsomal enzyme inducers that activate distinct transcription pathways. *Toxicol Sci* **67:**182–189.

Kanno J, Matsuoka C, Furuta K, Onodera H, Miyajima H, Maekawa A, and Hayashi Y (1990) Tumor promoting effect of goitrogens on the rat thyroid. *Toxicol Pathol* **18:**239–246.

Kimura T and Owens IS (1987) Mouse UDP glucuronosyltransferase. cDNA and complete amino acid sequence and regulation. *Eur J Biochem* **168:**515–521.

Kliewer SA, Moore JT, Wade L, Staudinger JL, Watson MA, Jones SA, McKee DD, Oliver BB, Willson TM, Zetterstrom RH, et al. (1998) An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway. *Cell* **92:**73–82.

Kong AN, Ma M, Tao D, and Yang L (1993) Molecular cloning of two cDNAs encoding the mouse bilirubin/phenol family of UDP-glucuronosyltransferases (mUGTBr/p). *Pharm Res* **10:**461–465.

Kourounakis P, Selye H, and Tache Y (1977) Catatoxic steroids. *Adv Steroid Biochem Pharmacol* **6:**35–57.

Lamb JG, Straub P, and Tukey RH (1994) Cloning and characterization of cDNAs encoding mouse Ugt1.6 and rabbit UGT1.6: differential induction by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Biochemistry* **33:**10513–10520.

Liu J, Liu Y, Barter RA, and Klaassen CD (1995) Alteration of thyroid homeostasis by UDP-glucuronosyltransferase inducers in rats: a dose-response study. *J Pharmacol Exp Ther* **273:**977–985.

Liu L and Klaassen CD (1996) Regulation of hepatic sulfotransferases by steroidal chemicals in rats. *Drug Metab Dispos* **24:**854–858.

Lu AY and Levin W (1972) Partial purification of cytochromes P-450 and P-448 from rat liver microsomes. *Biochem Biophys Res Commun* **46:**1334–1339.

Mackenzie PI, Owens IS, Burchell B, Bock KW, Bairoch A, Belanger A, Fournel-Gigleux S, Green M, Hum DW, Iyanagi T, et al. (1997) The UDP glycosyltransferase gene superfamily: recommended nomenclature update based on evolutionary divergence. *Pharmacogenetics* **7:**255–269.

Maglich JM, Stoltz CM, Goodwin B, Hawkins-Brown D, Moore JT, and Kliewer SA (2002) Nuclear pregnane X receptor and constitutive androstane receptor regulate overlapping but distinct sets of genes involved in xenobiotic detoxification. *Mol Pharmacol* **62:**638–646.

Muerhoff AS, Griffin KJ, and Johnson EF (1992) The peroxisome proliferator-activated receptor mediates the induction of CYP4A6, a cytochrome P450 fatty acid omega-hydroxylase, by clofibric acid. *J Biol Chem* **267:**19051–19053.

Ritter JK, Chen F, Sheen YY, Tran HM, Kimura S, Yeatman MT, and Owens IS (1992) A novel complex locus UGT1 encodes human bilirubin, phenol and other UDP-glucuronosyltransferase isozymes with identical carboxyl termini. *J Biol Chem* **267:**3257–3261.

Sonoda J, Xie W, Rosenfeld JM, Barwick JL, Guzelian PS, and Evans RM (2002) Regulation of a xenobiotic sulfonation cascade by nuclear pregnane X receptor (PXR). *Proc Natl Acad Sci USA* **99:**13801–13806.

Staudinger J, Liu Y, Madan A, Habeebu S, and Klaassen CD (2001b) Coordinate regulation of xenobiotic and bile acid homeostasis by pregnane X receptor. *Drug Metab Dispos* **29:**1467–1472.

Staudinger JL, Goodwin B, Jones SA, Hawkins-Brown D, MacKenzie KI, LaTour A, Liu Y, Klaassen CD, Brown KK, Reinhard J et al. (2001a) The nuclear receptor PXR is a lithocholic acid sensor that protects against liver toxicity. *Proc Natl Acad Sci USA* **98:**3369–3374.

Sugatani J, Kojima H, Ueda A, Kakizaki S, Yoshinari K, Gong QH, Owens IS, Negishi M, and Sueyoshi T (2001) The phenobarbital response enhancer module in the human bilirubin UDP-glucuronosyltransferase UGT1A1 gene and regulation by the nuclear receptor CAR. *Hepatology* **33:**1232–1238.

Viollon-Abadie C, Lassere D, Debruyne E, Nicod L, Carmichael N, and Richert L (1999) Phenobarbital, beta-naphthoflavone, clofibrate, and pregnenolone-16alpha-carbonitrile do not affect hepatic thyroid hormone UDP-glucuronosyl transferase activity and thyroid gland function in mice. *Toxicol Appl Pharmacol* **155:**1–12.

Visser TJ, Kaptein E, van Toor H, van Raaij JA, van den Berg KJ, Joe CT, van Engelen JG, and Brouwer A (1993) Glucuronidation of thyroid hormone in rat liver: effects of in vivo treatment with microsomal enzyme inducers and in vitro assay conditions. *Endocrinology* **133:**2177–2186.

Watkins JB, Gregus Z, Thompson TN, and Klaassen CD (1982) Induction studies on the functional heterogeneity of rat liver UDP-glucuronosyltransferases. *Toxicol Appl Pharmacol* **64:**439–446.

Watkins JB and Klaassen CD (1982) Induction of UDP-glucuronosyltransferase activities in Gunn, heterozygous and Wistar rat livers by pregnenolone-16 alpha- carbonitrile. *Drug Metab Dispos* **10:**590–594.

Whitlock JP Jr, Okino ST, Dong L, Ko HP, Clarke-Katzenberg R, Ma Q, and Li H (1996) Cytochromes P450 5: induction of cytochrome P4501A1: a model for analyzing mammalian gene transcription. *FASEB J* **10:**809–818.

Xie W, Barwick JL, Downes M, Blumberg B, Simon CM, Nelson MC, Neuschwander T, Brunt EM, Guzelian PS, and Evans RM (2000) Humanized xenobiotic response in mice expressing nuclear receptor SXR. *Nature (Lond)* **406:**435–439.