0026-895X/02/6203-638–646$7.00
MOLECULAR PHARMACOLOGY
Vol. 62, No. 3
1646/1002911
Mol Pharmacol 62:638–646, 2002
Printed in U.S.A.

Nuclear Pregnane X Receptor and Constitutive Androstane Receptor Regulate Overlapping but Distinct Sets of Genes Involved in Xenobiotic Detoxification

JODI M. MAGLICH, CATHERINE M. STOLTZ, BRYAN GOODWIN, DIANE HAWKINS-BROWN, JOHN T. MOORE, and STEVEN A. KLIEWER

Nuclear Receptor Discovery Research (J.M.M., C.M.S., B.G., J.T.M., S.A.K.) and Bioscience Support (D.H.-B.), GlaxoSmithKline, Research Triangle Park, North Carolina

Received February 1, 2001; accepted May 20, 2001

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

ABSTRACT

The nuclear pregnane X receptor (PXR) and constitutive androstane receptor (CAR) play central roles in protecting the body against environmental chemicals (xenobiotics). PXR and CAR are activated by a wide range of xenobiotics and regulate cytochrome P450 and other genes whose products are involved in the detoxification of these chemicals. In this report, we have used receptor-selective agonists together with receptor-null mice to identify PXR and CAR target genes in the liver and small intestine. Our results demonstrate that PXR and CAR regulate overlapping but distinct sets of genes involved in all phases of xenobiotic metabolism, including oxidative metabolism, conjugation, and transport. Among the murine genes regulated by PXR were those encoding PXR and CAR. We provide evidence that PXR regulates a similar program of genes involved in xenobiotic metabolism in human liver. Among the genes regulated by PXR in primary human hepatocytes were the aryl hydrocarbon receptor and its target genes *CYP1A1* and *CYP1A2*. These findings underscore the importance of these two nuclear receptors in defending the body against a broad array of potentially harmful xenobiotics.

The body must protect itself against myriad xenobiotics, including those ingested in the diet or absorbed through the skin or lungs. Two members of the nuclear receptor family of ligand-activated transcription factors, termed the pregnane X receptor (PXR; NR1I2) and constitutive androstane receptor (CAR; NR1I4), are expressed in the liver, intestine, lung, and other tissues, where they have important roles in detecting xenobiotics and stimulating genes encoding cytochrome P450 enzymes (P450s) and other proteins involved in their detoxification and elimination from the body (Waxman, 1999; Honkakoski and Negishi, 2000). Mice lacking either of these receptors are hypersensitive to treatment with various xenobiotics, including the anesthetic tribromoethanol and the muscle relaxant zoxazolamine (Wei et al., 2000; Xie et al., 2000a).

PXR is activated by a structurally diverse collection of xenobiotics, including both prescription drugs (e.g., macrocyclic antibiotics, antimycotics, glucocorticoids) and herbs (e.g., St. John's wort) (Bertilsson et al., 1998; Blumberg et al., 1998; Kliewer et al., 1998; Lehmann et al., 1998; Moore et al., 2000a). PXR was originally shown to regulate the expression of CYP3A isozymes by binding as a heterodimer with the 9-*cis* retinoic acid receptor (NR2B1) to xenobiotic response elements located in the regulatory regions of these genes (Bertilsson et al., 1998; Blumberg et al., 1998; Kliewer et al., 1998; Lehmann et al., 1998). PXR is now known to regulate the expression of several additional genes encoding proteins involved in xenobiotic metabolism, including multidrug resistance protein 1 (MDR1) (Geick et al., 2001; Synold et al., 2001), multidrug resistance-associated protein 2 (MRP2) (Dussault et al., 2001; Kast et al., 2002), and organic anion transporter polypeptide 2 (Staudinger et al., 2001). PXR ligands, including the synthetic steroid pregnenolone 16α-carbonitrile (PCN) and the macrocyclic antibiotic rifampicin, have been shown to regulate other classes of genes, including those encoding glutathione *S*-transferases (GSTs), UDP-glucuronosyltransferases (UGTs), and sulfotransferases (SULTs) (Madhu and Klaassen, 1991; Liu and Klaassen, 1996; Dunn et al., 1999; Runge-Morris et al., 1999; Falkner et al., 2001), which suggests a broad role for PXR in detoxification of xenobiotics. Although PXR evolved to protect the body, its activation by drugs represents the basis for a common

J.M.M. and C.M.S. contributed equally to this work.

ABBREVIATIONS: PXR, pregnane X receptor; CAR, constitutive androstane receptor; P450, cytochrome P450; MDR, multidrug resistance protein; MRP, multidrug resistance-associated protein; PCN, pregnenolone 16α-carbonitrile; GST, glutathione *S*-transferase; UGT, UDP glucuronosyltransferase; SULT, sulfotransferase; PB, phenobarbital; TCPOBOP, 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene; DMSO, dimethyl sulfoxide; RTQ-PCR, real time quantitative polymerase chain reaction; AhR, aryl hydrocarbon receptor.

class of potentially life-threatening drug interactions in which one drug accelerates the metabolism of another (Moore and Kliewer, 2000). Thus, understanding the processes regulated by PXR has important pharmaceutical ramifications.

CAR seems to be activated by a more restricted set of chemicals, including phenobarbital (PB) and the planar hydrocarbon 1,4-bis[2-(3,5-dichloropyridyloxy)] benzene (TCPOBOP) (Sueyoshi et al., 1999; Moore et al., 2000b; Tzameli et al., 2000). Like PXR, CAR regulates the expression of target genes by binding to xenobiotic response elements as a heterodimer with 9-*cis* retinoic acid receptor. CAR was originally demonstrated to regulate *CYP2B* gene expression (Honkakoski et al., 1998). CAR has since been shown to regulate a number of genes involved in a variety of biological processes (Sugatani et al., 2001; Kast et al., 2002; Ueda et al., 2002). Recent studies have also demonstrated overlap in the genes regulated by PXR and CAR. For instance, PXR can regulate *CYP2B* genes and CAR can regulate *CYP3A* genes (Xie et al., 2000b; Goodwin et al., 2001; Smirlis et al., 2001; Wei et al., 2002). This cross talk provides a mechanism for amplifying the body's detoxification response to a broad range of chemicals.

In the current study, we have exploited PXR- and CAR-selective ligands as well as PXR- and CAR-null mice to examine systematically whether PXR and CAR regulate genes involved in the different phases of xenobiotic metabolism in murine liver and small intestine and in human hepatocytes.

Materials and Methods

Chemicals. PCN was purchased from Biomol Research Labs (Plymouth Meeting, PA), PB and rifampicin were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO), and TCPOBOP was purchased from Maybridge plc (Tintagel, England).

Human Hepatocytes. Human hepatocytes were purchased from BioWhittaker, Inc. (Walkersville, MD). They were maintained in Williams' Medium E (Invitrogen, Carlsbad, CA) supplemented with 100 nM dexamethasone (Invitrogen), 2 mM L-glutamine (Invitrogen) and insulin-transferrin-selenium (Invitrogen) and were treated with either rifampicin (10 μM) or PB (1 mM). Rifampicin was added to the culture medium as a 1000× stock in DMSO; PB was added directly into the medium. Control cultures received DMSO alone. Cells were harvested after 48 h.

Experimental Animals and Protocols. All procedures performed were in compliance with the Animal Welfare Act and Unites States Department of Agriculture regulations and were approved by the GlaxoSmithKline Institutional Animal Care and Use Committee. CAR-null mice were generated by Deltagen, Inc. (Redwood City, CA) by homologous recombination using a targeting vector that deletes nucleotides 38 to 159 of the CAR open reading frame. This targeting event removes the first zinc finger of the DNA binding domain and results in a frame shift. PXR-null mice were generated as described previously (Staudinger et al., 2001). Mice were maintained on standard laboratory chow and were allowed food and water ad libitum. Six- to eight- week old PXR-null, CAR-null, and wild-type mice (three per group) were treated for 28 h with either vehicle (corn oil plus 5% DMSO), PCN (100 mg/kg), or TCPOBOP (0.3 mg/kg) in corn oil plus 5% DMSO. Two intraperitoneal injections were administered at 0 h and 24 h and the mice were sacrificed 4 h after the last injection. Livers and epithelial cells derived by scraping the small intestine were frozen in liquid nitrogen.

RNA Preparation and Real Time Quantitative PCR Analysis. Total RNA from mouse tissues or human hepatocytes were isolated using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. Real-time quantitative PCR (RTQ-PCR) was performed using an ABI PRISM 7700 Sequence Detection System instrument and software (Applied Biosystems, Inc., Foster City, CA). RNA samples were diluted to 100 μg/ml and treated with 40 U/ml RNA-free deoxyribonuclease I for 30 min at 37° C followed by inactivation at 75° C for 5 min. Samples were quantitated by spectrophotometry and diluted to a concentration of 10 ng/μl. Samples were then assayed in duplicate or triplicate 25-μl reactions using 25 ng of RNA per reaction. Gene-specific primers were used at 7.5 or 23 pmol per reaction and the gene-specific probe was used at 5 pmol per reaction. Primers and probes were designed using Primer Express Version 2.0.0 (Applied Biosystems) and synthesized by Keystone Laboratories (Camarillo, CA). All primers and probes were entered into the NCBI Blast program to ensure specificity. Fold induction values were calculated by subtracting the mean threshold cycle number for each treatment group from the mean threshold cycle number for the vehicle group and raising 2 to the power of this difference. The expression levels of selected mouse genes (Table 1) and human genes (Table 2) were compared between vehicle and chemical treatment. For animal studies, the average of each treatment group (3 animals per group) was used.

Northern Blot Analysis. Total RNA (10 μg) was resolved on a 1% agarose/2.2 M formaldehyde denaturing gel and transferred to a nylon membrane (Hybond N+; Amersham Bioscience, Piscataway, NJ). Blots were hybridized with ^{32}P-labeled cDNAs corresponding to mALAS1 (bases 599-1037 of the published cDNA; GenBank accession number M63245), mouse UGT1a1 (bases 561–845 of the published cDNA; GenBank accession number L27122), human CYP1A1 (bases 82–1620 of the published cDNA; GenBank accession number BC023019), human CYP1A2 (bases 65–1612 of the published cDNA; GenBank accession number NM_000761), or human UGT1A1 (bases 75–766 of the published cDNA; Genbank accession number NM000463). The blots were subsequently reprobed with a radiolabeled β-actin cDNA (BD Clontech Laboratories Inc., Palo Alto, CA). The intensity of signals was quantified using ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

Results

Wild-type or PXR-null mice were treated with the PXR-selective ligand PCN or vehicle alone. In parallel, wild-type or CAR-null mice were treated with the CAR-selective ligand TCPOBOP. RNA was prepared from the livers and small intestines of the mice, and the expression of 36 candidate genes involved in xenobiotic metabolism was evaluated by RTQ-PCR. The complete list of mouse genes that were analyzed is shown in Table 1. Data for genes whose expression was changed ≥1.5-fold in one or both tissues are shown in Table 3.

Genes Encoding Phase I (Oxidative Metabolism) Enzymes. As expected, *Cyp3a11* was induced by PCN treatment in both liver and small intestine (Table 3, line 6). No regulation of *Cyp3a11* was observed in PXR-null mice. *Cyp3a11* expression was also induced by TCPOBOP in the liver but not the small intestine of wild-type mice (Table 3, line 6). This induction was not seen in the CAR-null mice. These data demonstrate that both PXR and CAR regulate *Cyp3a11* expression in vivo. Expression of *Cyp2b10*, the prototypical CAR target gene, was dramatically induced in the liver and modestly induced in the small intestine of wild-type mice treated with TCPOBOP (Table 3, line 5). These effects were not seen in CAR-null mice. PCN also induced *Cyp2b10* expression in the liver and small intestine, albeit to a much lesser extent than TCPOBOP (Table 3, line 5), indicating that CAR has more robust effects on *Cyp2b10* expression than PXR.

TABLE 1
Mouse primer-probe sets and gene abbreviations

Gene	UniGene	GenBank	Forward Primer	Reverse Primer	Probe
Aminolevulinic acid synthase 1	Alas1	M63245	GATGCCAGGCTGTGAAATTTACT	CTGTTGCGAATCCCTTGGAT	TGATTCCGGGAACCATGCCTCCAT
Aldehyde dehydrogenase 1A1	Aldh1a1	S75713	GCTAGCTACAATGGAGGCACTCA	GCAGCCTCCTAAATCCGACA	TATGCATTGGCAAAGACTTTCCCACCA
Aldehyde dehydrogenase 1A7	Aldh1a7	U96401	TGCTGGCTACAATGGAATCG	TGCTGATCTCTACATCCAACAGGT	TGCATGAGCAAAGACTTTCCCAGCATT
Aryl hydrocarbon receptor	Ahr	D38417	CATCTATCTGTGTCCTCCTTCAAGC	CCGCACTTGCTCACGGA	TGCGCTGTTAGACAGCCATTTTCTCATG
Cytochrome P450 1a1	Cyp1a1	NM_009992	GCTTGAGTGAGAAGGTCACTCTCTT	CGATCGGCCAATGGTCTCT	TTTGGGCAAGCGAAAGTGCATCG
Cytochrome P450 2a4	Cyp2a4	J03549	CAGCCAACGTTATGGTCCTGTA	GTCCGCACAGCACCACAA	TCACCATCTATCTGGGATCTCGCCGA
Cytochrome P450 2b10	Cyp2b10	NM_009998	GGGAAAGCGCATTTGTCTTG	ATGGACGTGAAGAAAAGGAACAAT	TGAAAGCATTGCCCGCAGCG
Cytochrome P450 2e1	Cyp2e1	NM_021282	TTCGGGCCAGTGTTCACA	GACAGCCTTGTAGCCATGCA	CACCTGGGTCAGAGGCGCATCG
Cytochrome P450 2j5	Cyp2j5	U62294	CAGGCGGAGAT TGACAGAGTT	GCATGGACTCTCGGTCAGACA	TTGGCCACAAGAGGCAGGTGAGC
Cytochrome P450 2j6	Cyp2j6	U62295	GCTGCTACCGGCTCCTTGT	ACTGCAGCCACCAACAGAGTC	ACGATCTGGGCAGCGCTCCATC
Cytochrome P450 3a11	Cyp3a11	X60452	GATTAAGAATGTGCTAGTGAAGGAATGT	ATTATCCCCACTGGGCCAAA	TCTGTCTTCACAAACCGGCGGGA
20-α-Hydroxysteroid dehydrogenase	20a-HSD	AB059565	TGATTGCCCTTCGCTACCA	CTGATTCTCTCCTCATTGAAACTCTTG	TGGACCGTGGGATTGTGGCCCTA
Flavin containing monooxygenase 1	Fmo1	U87456	GATCTGCGGGCCATGCT	GGGTACATGGGCCAAAGAAGA	CTGACTGACCCACGACTGGCTCTGAG
Flavin containing monooxygenase 5	Fmo5	U90535	CAATATCCAGCCTCTGGTCTTCA	GGAGTACAGGGCCCCAGTAGTA	TGACCCCAGGCTGGCATTACGG
Glutathione S-transferase α 1 (Ya)	Gsta1	M19255	CGTTACTTGCCTGCCTTTGAA	AGAACTTCCAGTAGGTGGATGTCC	CCAAGACTACCTTGTGGGCAACAGGC
Glutathione S-transferase μ 1	Gstm1	J04632	GTACCGTATGTTTGAGCCCAAGT	AGCGGGCCAGGAAGTCC	CCTGGACGCCTTCCCAAACCTGA
Glutathione S-transferase μ 2	Gstm2	NM_008183	TCTGAGTTTCTGGGCAAGCA	GGACATCATAAACAAGAAAATCCACAT	CCATGGTTTGCAGGGAACAAGGTCAC
Glutathione S-transferase θ 1	Gstt1	X98055	TTGTTGGGCCCCACATCT	CCACCACCTACAGGATGCATC	CCGACTTGGTGGCCATCACAGAGC
Glycine *N*-methyltransferase	Gnmt	D89664	CCAGACTGCAAAGGTGACCA	GGGCCGCACCATGCT	AGCGAGCACCGGCTGGCACTA
Metallothionein-1	Mt1	S62785	TGCTCCACCGGCGG	TTTGCAGACACAGCCCTGG	CCCGTGGGCTGCTCCAAATGTG
Mdr1a	Abcb1a	M33581	CCCCCGAGATTGACAGCTAC	ACTCCACTAAATTGCACATTTCCTTC	CACGCAAGGCCTAAAGCCGAATATGTT
Mdr1b	Abcb1b	NM_011075	TGGCAAAGCCGGAGAGATC	GCTTATATCCTGTCTCAGCATGGAT	ACCAAAGCGAGTCCGATACATGGTTTTCA
Mdr2	Abcb4	J03398	CGTGACATTTAATGAAGTCGTGTTC	CTCAGCCCCTGAAGCACTG	CTATCCCACCCGGGCCAACGTG
Mrp1	Abcc1a	AF022908	AAGCAGCCTGTACGGATTGTGT	CCAACTGGGAACTTCCCTTAGG	TGCCCCTCCCAAAGATCCCAGC
Mrp2	Abcc2	NM_013806	GCTGGGAGAAATGGAGAATGTC	GACTGCTGAGGGACGTAGGCTA	TGGGCATATCACCATCAAGGGCTCC
Mrp3	Abcc3	AK006128	CCTTGGCCATCCCATAGAAG	AGTAGCTGAATTTGATTCTCCAGTCA	TGCCTCCGGGCCGCATG
PXR	NR1I2	AF031814	TCCAGCGCAGCGTGGTA	GCAGGATATGGCCGACTACAC	TTTGCCCTCACCCTGAAGGCCTACA
CAR	NR1I3	AF009327	CAGGGTTCCAGTACGAGTTTTG	AGGCTCCTGGAGATGCAGTC	AGTCGATCCTCCACTTCCATAAAAACCTGAA
Organic anion transporting peptide 2 (Oatp2)	Slc21a5	NM_030687	TGCTGACTGCAACACAAAGTGT	AGCTGACATGTATGATAGACCATTGTC	CTGCTTAACGAACACATGGGACCCAGTG
Organic cation transporter 1 (Oct1)	Slc22a1	AF010259	GCGGCCTTTATCATCCTTGTC	CAGATTTGATGCCGCTATTGG	TTGACCGCATTGGCCGCATCT
P450 oxidoreductase	Por	D17571	CCTTCGATGCCAAGAATCCA	CTCAGTGCCTTGGTTCAGCTT	TCCTGGCTGCTGTCACCACG
Thiosulfate sulfurtransferase	Tst	U35741	CACGCAAAGAGTACCAGGAGC	TCCCGGCATTCCTCTATATCA	CGTGCCTGGCGCGTCCTTCT
Amine N-sulfotransferase	Sultn	AF026073	TTTGGAGCATTGCTGAGCAT	GGGATATGTGGAGATCAAAATGTCA	TCCCAAGTAGAGTCATTTGAAGCCCGG
Sulfotransferase 1A1	Sult1a1	L02331	GACAATGGAGCAACTGCAGAAC	CCAGACTTTGGGTACGTGCTG	CAGCCTGGCCTGATGATGTGCTCA
Sulfotransferase 2B1	Sult2b1	AF026072	GACCACATCAAGGGCTGGA	CTGCAGCTCCTCATAGGTGATAAA	CCGGATGCAGAACCAAGAGAACTTCCT
Thioether S-methyltransferase	Temt	M88694	TGCGTGCTGACCTTCCTG	GGCTGCCCGGTAGGTATCTAT	CCATGGAGTGTGCCTGCCCTGA
UDP-glucuronosyltransferase 1A1	Ugt1a1	L02333	TTTCAGTGACAGCCCCACAGT	CACGGCAGTCTGAGAATCCA	CGCCAGCTGACGGCTTTCTCCC
UDP-glucuronosyltransferase 2B5	Ugt2b5	X06358	ACTTTAGGACACAATACCAGAGTGTACAA	AAGGCTTTGGTTTTTGGATGAC	TGGCTCCCCCAGAATGACCTCCTT

TABLE 2
Human primer-probe sets and gene abbreviations

Gene	UniGene	GenBank	Forward Primer	Reverse Primer	Probe
Aminolevulinic acid synthase 1	ALAS1	NM_000688	TGATGCCAGGCTGTGAGATTTA	GCTGTTTCGAATCCCTTGGA	TCTGATTCTGGGAACCATGCCTCCA
Aldehyde dehydrogenase 1A1	ALDH1A1	M31984	CCGCAAGACAGGCTTTTCAG	AGTCGCCCCCTCTCGG	TGGATCTCCGTGGCGTACTATGGATGC
Aryl hydrocarbon receptor	AHR	D16354	CGGCTGGGCACCATGA	CTGGGATTGGCTTTACTGTTTTCT	TCACCTACGCCAGTCGCAAGCG
Cytochrome P450 1A1	CYP1A1	X02612	CCTCTGATTGGGCACATGCT	CTGCTGGCTCATCCTTGACA	CCCTGGGAAAGAACCCGCACCT
Cytochrome P450 1A2	CYP1A2	NM_000761	AGCTTCTCCTGGCCTCTGC	GGACTTTTCAGGCCTTTGGG	ATCTTCTGCCTGGTATTCTGGGTGCTCA
Cytochrome P450 2A6	CYP2A6	X13930	AAGCGGATTTGTCTTGGTGAA	TGGAGGATGGTGGTGAAGAAG	TCGCCCGAGCGGAATTGTTCC
Cytochrome P450 2B6	CYP2B6	NM_000767	AAGCGGATTTGTCTTGGTGAA	TGGAGGATGGTGGTGAAGAAG	TCGCCCGAGCGGAATTGTTCC
Cytochrome P450 1B1	CYP1B1	U03688	TCAACCAGTGGTCTGTGAATCAT	CCAAGAATCGAGCTGGATCAA	CCCAGTGAAGTGGCCTAACCCGGAG
Cytochrome P450 3A4	CYP3A4	NM_017460	CAGGAGGAAATTGATGCAGTTTT	GTCAAGATACTCCATCTGTAGCACAGT	CCCAATAAGGCACCACCCACCTATGA
Glutathione S-transferase A2	GSTA2	M16594	AAATGATCCTTCTTCTGCCCTTT	TTTGTTTTCTCTTGGATCAAGGC	CTCAACCTGAGGAACAAGATGCCAAGCT
MDR1	ABCB1	M14758	GTCCCAGGAGCCCATCCT	CCCGGCTGTTGTCTCCAT	TGACTGCAGCATTGCTGAGAACATTGC
PXR	NR1I2	AF061056	CGAGCTCCGCAGCATCA	TGTATGTCCTGGATGCGCA	TGCTCAGCACACCCAGCGGCT
CAR	NR1I3	NM_005122	CACATGGGCACCATGTTTGA	AAGGGCTGGTGATGGATGAA	TTTGTGCAGTTTAGGCCTCCAGCTCATCT
P450 oxidoreductase	POR	AF258341	CAGTCACCACCAACCGGAAG	TGTCCAATTCCAGGTGCATG	AACCAGGGAACCGAGCGCCAC
Sulfotransferase 1A1	SULT1A1	NM_001055	GTGTCACCGAGCTCCCATCT	AGTCTCCATCCCTGAGGGAATC	CATGCGGGTGCCCTTCCTTGA
Sulfotransferase 2B1	SULT2B1	NM_004605	GGACTTCCTCAAAGGCGAAGT	ATCCGAAGCCAGCCCTTAAT	TTTGGCTCCTGGTTCGACCA

TABLE 3
Effects of PCN and TCPOBOP on expression of genes in wild type and receptor-null mice. Data represent fold activation relative to treatment with vehicle alone. The standard deviation among the samples used for each experiment is shown in parentheses. No changes were observed for genes listed in Table 1 but not in this table.

	Liver		Small Intestine		Liver		Small Intestine	
Gene	$PXR^{+/+}$	$PXR^{-/-}$	$PXR^{+/+}$	$PXR^{-/-}$	$CAR^{+/+}$	$CAR^{-/-}$	$CAR^{+/+}$	$CAR^{-/-}$
1 Aldh1a1	2.1 (0.01)	N.C.	2.6 (0.08)	N.C.	1.9 ± 0.2	−1.5 ± 0.2	N.C.	N.C.
2 Aldh1a7	1.6 (0.11)	N.C.	1.7 (0.04)	N.C.	1.9 ± 0.04	N.C.	N.C.	N.C.
3 Cyp1a1	N.C.	N.C.	−15 (0.01)	1.9 (0.04)	2.3 ± 0.3	N.C.	N.C.	−2.9 (2.1)
4 Cyp2a4	N.C.	N.C.	N.E.	N.E.	3.4 (0.70)	N.C.	N.E.	N.E.
5 Cyp2b10	12 (0.14)	1.8 (0.68)	1.8 (0.13)	N.C.	110 (0.04)	N.C.	4.8 (0.10)	N.C.
6 Cyp3a11	3.5 (0.08)	N.C.	3.5 (0.10)	N.C.	2.0 (0.1)	N.C.	N.C.	N.C.
7 Gsta1	16 (0.5)	−1.7 (1.0)	4.3 (0.17)	1.6 (0.19)	15 (0.40)	N.C.	N.C.	N.C.
8 Gstm1	2.2 (0.07)	N.C.	8.9 (0.09)	1.6 (0.11)	2.4 (0.30)	N.C.	1.9 (0.20)	−2.1 (0.16)
9 Gstm2	2.2 (0.23)	N.C.	3.4 (0.02)	N.C.	2.8 (0.14)	2.0 (0.23)	N.C.	N.C.
10 Gstt1	N.C.	N.C.	N.C.	N.C.	−2.5 (0.80)	N.C.	1.8 (0.70)	N.C.
11 Sultn	N.C.	N.C.	N.C.	N.C.	3.2 (0.02)	N.C.	N.C.	N.C.
12 Ugt1a1	2.8 (0.11)	N.C.	1.6 (0.17)	N.C.	N.C.	N.C.	N.C.	N.C.
13 Mdr1a	3.2 (0.35)	−1.6 (0.09)	2.3 (0.16)	N.C.	1.7 (0.01)	1.7 (0.17)	1.7 (0.06)	N.C.
14 Mdr1b	1.8 (0.02)	N.C.	1.8 (0.37)	N.C.	N.C.	N.C.	N.E.	N.E.
15 Mrp1	N.C.	N.C.	N.C.	N.C.	2.3 (0.2)	N.C.	N.C.	N.C.
16 Mrp2	N.C.	N.C.	1.6 (0.06)	N.C.	2.0 (0.01)	N.C.	N.C.	N.C.
17 Mrp3	3.0 (0.29)	N.C.	N.C.	N.C.	1.9 (0.06)	N.C.	N.C.	N.C.
18 Oatp2	9.2 (0.14)	N.C.	N.E.	N.E.	N.C.	N.C.	N.C.	N.C.
19 Alas1	2.9 (0.27)	1.7 (0.18)	3.4 (0.14)	N.C.	2.1 (0.01)	3.2 (0.04)	N.C.	N.C.
20 AhR	N.C.	N.C.	N.C.	N.C.	1.9 (0.50)	N.C.	N.C.	N.C.
21 Por	2.8 (0.44)	N.C.	2.0 (0.07)	1.6 (0.14)	3.1 (0.11)	1.9 (0.11)	N.C.	−1.5 (0.16)
22 CAR	2.6 (0.37)	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
23 PXR	2.7 (0.26)	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.

N.C., no change; N.E., no expression detected.

We examined whether PXR and CAR regulate other P450s. TCPOBOP had a modest inductive effect on *Cyp1a1* in liver and no effect in small intestine in wild-type mice (Table 3, line 3). Notably, TCPOBOP decreased *Cyp1a1* expression in the small intestine of CAR-null mice, suggesting that this chemical may have CAR-independent effects on gene expression. PCN did not affect *Cyp1a1* expression in liver but strongly suppressed its expression in small intestine in a PXR-dependent manner (Table 3, line 3). Similarly, PXR has previously been shown to repress expression of *Cyp7a1* (Staudinger et al., 2001). Thus, PXR can either stimulate or suppress gene expression. *Cyp1a1* is known to be highly inducible by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and polycyclic aromatic hydrocarbon carcinogens. This induction is mediated by the aryl hydrocarbon receptor (AhR), a member of the helix-loop-helix/PER/ARNT/SIM (periodicity/AhR nuclear translocator/single-minded) family of transcription factors (Honkakoski and Negishi, 2000). *Ahr* expression was modestly induced by TCPOBOP in liver in a CAR-dependent manner (Table 3, line 20), suggesting that CAR may regulate *Cyp1a1* indirectly though effects on AhR.

As reported previously (Smith et al., 1993; Wei et al., 2002), TCPOBOP induced the expression of *Cyp2a4* in the liver of wild-type mice (Table 3, line 4). Consistent with the results of others, this effect was not seen in CAR-null mice (Wei et al., 2002). TCPOBOP did not affect *Cyp2a4* expression in the small intestine. PCN had no effect on *Cyp2a4* in either tissue. CYP2A4 hydroxylates a variety of steroid hormones, including androgens and estrogens, at the 15 position, and also metabolizes xenobiotics (Fernandez-Salguero and Gonzalez, 1995). The regulation of *Cyp2a4* by CAR but not PXR suggests that these two receptors may have distinct roles in metabolizing endogenous steroids.

P450s require a heme prosthetic group to oxidize substrates. Aminolevulinic acid synthase 1 (ALAS1) catalyzes the first and rate-limiting step in heme biosynthesis and is up-regulated in response to various xenobiotics, including the CAR activator PB (May et al., 1995). *Alas1* expression was increased 2.9- and 3.4-fold in the liver and small intestine, respectively, of wild-type mice treated with PCN (Table 3, line 19). This induction was reduced to 1.7-fold in the liver and was completely absent in the small intestine of PXR-null mice, indicating a role for PXR in the induction of *Alas1* expression. The regulation of *Alas1* by TCPOBOP was notable in two respects (Table 3, line 19). First *Alas1* was induced by TCPOBOP in liver but not small intestine. Second, the induction of *Alas1* expression was not diminished in the CAR-null mice, indicating that the effect of TCPOBOP on *Alas1* expression does not require CAR. Similar data were obtained via Northern blot analysis (Figure 1). PCN treatment increased liver *Alas1* expression 4.2-fold in wild-type mice and 2.1-fold in PXR-null mice, whereas TCPOBOP increased *Alas1* expression by 2-fold in the wild-type mice and 3.6-fold in the CAR-null mice. The basis for TCPOBOP-mediated induction of *Alas1* remains unclear. A similar, CAR-independent induction of *Alas1* by PB was recently reported (Ueda et al., 2002). PXR and CAR also regulated the expression of the gene encoding P450 oxidoreductase, which is an essential component of the P450 monooxygenase complex and binds FMN, FAD, and NADPH cofactors. PCN induced *Por* in the liver and intestine (Table 3, line 21); these effects were either reduced or eliminated in PXR-null mice. TCPOBOP up-regulated *Por* in liver via a mechanism that was partially dependent on CAR (Table 3, line 21). Similar results have been reported using PB (Ueda et al., 2002).

Aldehyde dehydrogenases are known to be induced in response to PB in rodents, including the ALDH1A7 isozyme in mice (Vasiliou et al., 2000). PCN treatment induced the expression of *Aldh1a7* in mouse liver and small intestine in a PXR-dependent fashion (Table 3, line 2). PCN also induced the expression of the gene encoding ALDH1A1 (Table 3, line 1), a closely related protein involved in retinoic acid metabolism (Vasiliou et al., 2000). TCPOBOP induced the expression of *Aldh1a1* and *Aldh1a7* in the liver in a CAR-dependent manner but did not increase their expression in the small intestine (Table 3, lines 1 and 2).

Genes Encoding Phase II (Conjugation) Enzymes. We examined the expression of a number of genes encoding different classes of phase II enzymes including GSTs, UGTs, and SULTs (Mulder and Jakoby, 1990). GSTs catalyze the conjugation of glutathione to electrophilic substrates, which are often the products of phase I metabolism. These substrates include xenobiotics. Thus, GST conjugation is a major pathway for the detoxification of a variety of different substances. The mammalian GSTs are divided into several families of cytosolic enzymes including GSTs α (GSTA), μ (GSTM), and, θ (GSTT). Several GST genes were regulated by PXR and/or CAR including *Gsta1*, *Gstm1*, *Gstm2*, and *Gstt1* (Table 3, lines 7–10). *Gsta1* was markedly induced by PCN (16-fold) and TCPOBOP (15-fold) in liver (Table 3, line 7). This induction was absent in PXR-null and CAR-null mice. A more modest induction of *Gsta1* (4.3-fold) was observed in the small intestine of PCN-treated mice; in con-

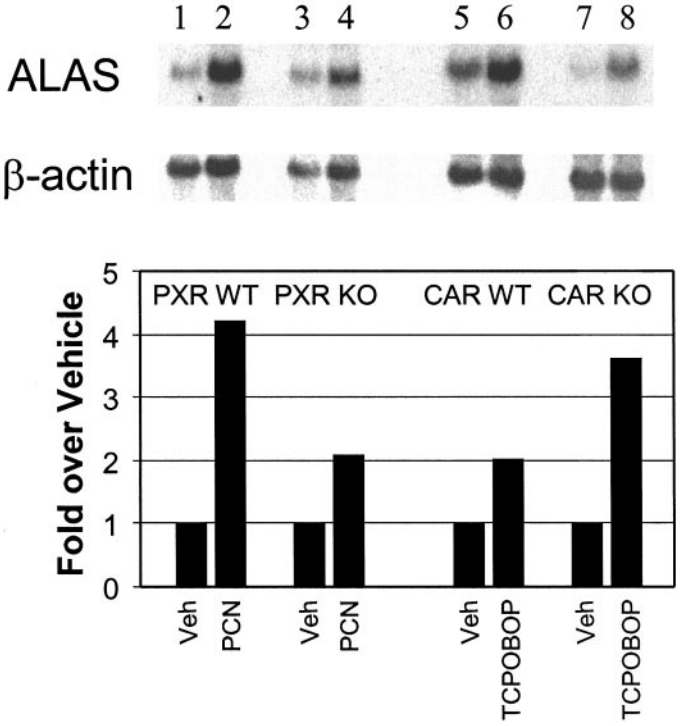

Fig. 1. Induction of *Alas1* expression in mouse liver. Total RNA was prepared from the livers of three wild-type and PXR-null mice treated with vehicle or PCN and three wild-type and CAR-null mice treated with vehicle or TCPOBOP. RNA samples were pooled and Northern blot analysis performed with a probe for *Alas1*. Relative mRNA abundance (plotted as fold relative to vehicle treatment) is shown at the bottom.

trast, no induction of *Gsta1* was observed in the small intestine of TCPOBOP-treated animals (Table 3, line 7). PCN induced the expression of the *Gstm1* and *Gstm2* isoforms in the liver and small intestine in a PXR-dependent manner (Table 3, lines 8 and 9). Whereas TCPOBOP induced expression of both *Gstm1* and *Gstm2* in the liver, only *Gstm1* was induced in the small intestine (Table 3, lines 8 and 9). No induction of *Gstm1* was seen in the livers of TCPOBOP-treated CAR-null mice; in fact, TCPOBOP decreased *Gstm1* expression in these animals (Table 3, line 8). However, *Gstm2* was still induced by TCPOBOP in the livers of CAR-null animals. Taken together, these data reveal a complex pattern of GST regulation by PCN and TCPOBOP in the liver and small intestine of mice, including PXR- and CAR-dependent and independent effects.

We examined whether PXR and CAR regulated other genes whose products are involved in conjugative metabolism of xenobiotics. The SULTs and UGTs represent families of enzymes that increase the solubility of many xenobiotics and lipophilic hormones by catalyzing their conjugation to sulfate or glucuronyl groups, respectively (Mulder and Jakoby, 1990). TCPOBOP induced expression of the sulfotransferase gene *Sultn* in liver but not small intestine (Table 3, line 11); this regulation was lost in CAR-null mice. PCN had no effect on *Sultn* in either tissue. TCPOBOP did not regulate *Ugt1a1* $\geq$1.5-fold in the liver as assessed by RTQ-PCR. This was unexpected because a previous study showed that PB induces UGT1A1 in human hepatocytes via CAR (Sugatani et al., 2001). This apparent disparity may reflect either differences in the chemicals used or the species studied. Conversely, PCN up-regulated *Ugt1a1* 2.8-fold in liver in a PXR-dependent manner but had a lesser effect (1.6-fold) in the small intestine (Table 3, line 12). Similar data were obtained by Northern blot analysis (Figure 2). PCN treatment increased liver *Ugt1a1* expression by only 1.7-fold in the wild-type mouse but had little or no effect in the PXR knock-out strain. TCPOBOP had only very modest effects on *Ugt1a1* expression in wild-type and CAR-null mice (~1.5- and 1.2-fold, respectively) as measured by Northern blot analysis. Overall, the data demonstrate that PXR and CAR regulate a program of genes whose products conjugate xenobiotics.

Genes Encoding Transporters. We examined the expression of several proteins that transport xenobiotics and other lipophilic substances in enterohepatic tissues including MDR1a and MDR1b, which function as broad-specificity efflux pumps in the intestine and apical transporters in the liver, and MRP2 and MRP3 (Stieger and Meier, 1998). *MDR1* has been shown to be regulated by PXR in human intestinal cell lines (Geick et al., 2001; Synold et al., 2001); *MRP2* is regulated by CAR and PXR in vitro (Dussault et al., 2001; Kast et al., 2002); *MRP3* is regulated by both CAR and PXR activators (Ogawa et al., 2000; Schrenk et al., 2001). Both *Mdr1a* and *Mdr1b* were up-regulated by PCN in the liver and intestine in a PXR-dependent manner (Table 3, lines 13 and 14). *Mdr1a* was regulated 1.7-fold by TCPOBOP in the liver and intestine of wild-type mice (Table 3, line 13). TCPOBOP-mediated regulation of *Mdr1a* was absent in the small intestine of CAR-null mice but was intact in the livers of TCPOBOP-treated CAR-null mice. TCPOBOP did not regulate *Mdr1b* expression (Table 3, line 14). Expression of MRP2, a basolateral protein that transports glutathione and sulfate conjugates of xenobiotics and various natural compounds, was modestly increased in the small intestine of PCN-treated mice and the liver of TCPOBOP-treated mice (Table 3, line 16). These effects were absent in the corresponding receptor-null mice. Expression of MRP3, which mediates the sinusoidal efflux of organic anions, was induced 3.0-fold and 1.9-fold in the livers of PCN and TCPOBOP-treated mice, respectively (Table 3, line 17). These effects were not observed in the PXR- and CAR-null mice. Thus, PXR and CAR regulate distinct but overlapping sets of genes encoding xenobiotic transporters in the liver and small intestine.

Finally, we examined whether PXR and CAR regulate their own and each other's expression. Interestingly, PCN induced CAR and PXR mRNA by 2.6- and 2.7-fold, respectively (Table 3, lines 22, 23). In contrast, TCPOBOP did not regulate PXR and CAR expression. These data demonstrate that PXR autoregulates its expression and reveal another level of cross-talk between PXR and CAR, namely that PXR regulates CAR expression. Interestingly, this relationship is not reciprocal; CAR does not seem to regulate PXR expression.

Human Hepatocytes. We next examined whether PXR and CAR activators regulated a similar program of genes in human hepatocytes. Primary cultures of human hepatocytes derived from two different donors were treated with either rifampicin or PB, and the expression of fourteen genes involved in xenobiotic metabolism was evaluated by RTQ-PCR. Rifampicin is a selective ligand for human PXR; PB activates both human CAR and human PXR (Lehmann et al., 1998; Pascussi et al., 2000). No selective activators of human CAR have been reported to date. The complete list of human genes analyzed is shown in Table 2.

As expected, rifampicin and PB treatment resulted in the

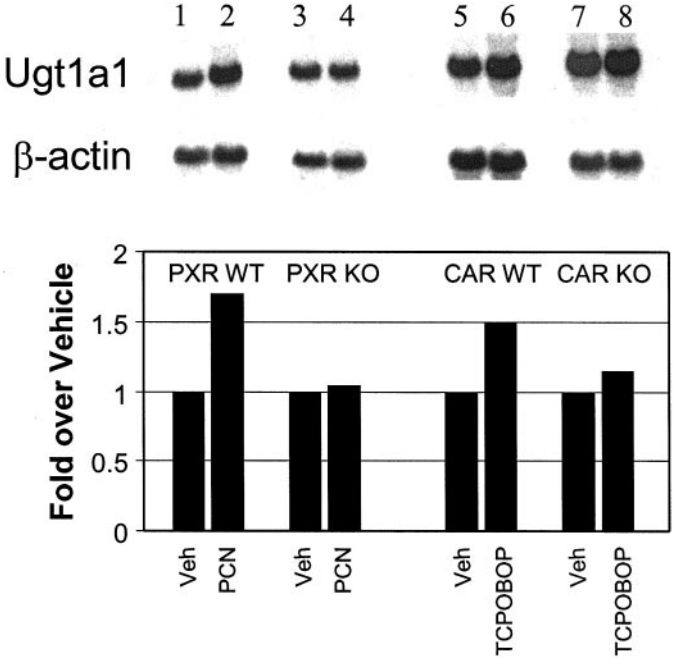

Fig. 2. Induction of *Ugt1a1* expression in mouse liver. Total RNA was prepared from the livers of three wild-type and PXR-null mice treated with vehicle or PCN and three wild-type and CAR-null mice treated with vehicle or TCPOBOP. RNA samples were pooled and Northern blot analysis performed with a probe for *Ugt1a1*. Relative mRNA abundance (plotted as fold relative to vehicle treatment) is shown at the bottom.

TABLE 4
Effects of rifampicin (RIF) and PB on expression of genes in primary cultures of human hepatocytes derived from two donors. Data represent fold activation relative to treatment with vehicle alone.

	Donor 1		Donor 2	
Gene	RIF	PB	RIF	PB
1 ALDH1A1	2.7	3.2	1.6	2.1
2 CYP1A1	27	1.3	15	5.9
3 CYP1A2	26	1.7	3.2	1.8
4 CYP1B1	1.6	N.C.	1.7	N.C.
5 CYP2A6	N.C.	1.7	2.5	5.2
6 CYP2B6	4.9	9.5	8.4	4.5
7 CYP3A4	5.7	4.9	4.6	2.9
8 GSTA2	23	18	4.7	2.1
9 MDR1	7.5	13	3.0	3.4
10 SULT1A1	3.0	3.6	N.C.	N.C.
11 ALAS1	9.8	9.2	5.5	9.6
12 AhR	2.6	3.8	2.1	1.9
13 POR	N.C.	1.5	2.2	2.9
14 CAR	N.C.	N.C.	N.C.	N.C.
15 PXR	N.C.	N.C.	1.5	2.0

N.C., no change.

induction of *CYP3A4* and *CYP2B6* in both sets of cultured hepatocytes (Table 4, lines 6 and 7). It is not clear why rifampicin was a more efficacious inducer of CYP2B6 in one set of cultured hepatocytes and PB a more efficacious inducer in the other. Rifampicin treatment also resulted in a marked induction of *CYP1A1* and *CYP1A2* expression and a much more modest effect on *CYP1B1* (Table 4, lines 2–4). Interestingly, PB had a much less pronounced effect on the regulation of these genes than rifampicin. The reason for these differences between rifampicin and PB is not known. Changes in *CYP1A1* and *CYP1A2* mRNA levels were independently analyzed by Northern blot analysis (Figure 3). In agreement with the RTQ-PCR data, *CYP1A1* mRNA levels were induced 24-fold by rifampicin in one set of hepatocytes (donor-1) but not by phenobarbital (Figure 3A). In a second set of hepatocytes, *CYP1A1* mRNA was induced 7.6-fold by rifampicin and 3.2-fold by phenobarbital (Figure 3A). These data are in good agreement with those derived from RTQ-PCR (compare Figure 3 with Table 4, line 2). *CYP1A2* mRNA levels were induced 7.8-fold by rifampicin and less than 2-fold by phenobarbital in one set of hepatocytes (donor-1; Figure 3B). In the second set of hepatocytes, *CYP1A2* mRNA levels were induced 3.2-fold by rifampicin and 1.4-fold by phenobarbital (Figure 3B). Again, the results observed between the RTQ-PCR and Northern blotting results were in general agreement (compare Figure 3 with Table 4, line 3).

The gene encoding AhR, which regulates *CYP1A1*, *CYP1A2*, and *CYP1B1*, was also induced by rifampicin and PB (Table 4, line 12). This may account in part for the induction of AhR target genes. Interestingly, *AhR* expression seems to be up-regulated by PXR and possibly CAR in human hepatocytes, whereas *AhR* was not stimulated by PXR in mouse liver (Table 3, line 20). Thus, there seem to be important species differences in the regulation of *AhR* by PXR. PB induced expression of *CYP2A6* in both sets of human hepatocyte cultures, whereas rifampicin had only a modest effect in one set of human hepatocytes (Table 4, line 5). *ALAS1* was strongly up-regulated and P450 reductase more modestly regulated by rifampicin and PB in human hepatocytes (Table 4, lines 11 and 13). Finally, both rifampicin and PB induced expression of *ALDH1A1* (Table 4, line 1). Taken together, these data indicate that PXR and possibly CAR regulate a number of genes involved in oxidative metabolism of xenobiotics in human hepatocytes.

We also examined the effects of rifampicin and PB on the expression of genes whose products are involved in the conjugation and transport of xenobiotics. Both chemicals had marked effects on the expression of *GSTA2* (Table 4, line 8) and *MDR1* (Table 4, line 9). *SULT1A1* was induced by rifampicin and phenobarbital in hepatocytes derived from one donor but not the other (Table 4, line 10). The reason for this difference is not known. We were unable to detect *UGT1A1* mRNA by RTQ-PCR despite attempts with four different sets of primers and probes. However, Northern blot analysis demonstrated that *UGT1A1* was induced by phenobarbital in both donors (Figure 4). These data are in agreement with those previously reported (Sugatani et al., 2001). This effect may be mediated through CAR because rifampicin had little or no effect on *UGT1A1* expression (Figure 4). Taken together, our data support a role for PXR and perhaps CAR in the regulation of genes involved in different phases of xenobiotic metabolism in human hepatoctyes.

Discussion

It is well established that PXR and CAR regulate the expression of P450 family members, including CYP2B, CYP2C, CYP2H, and CYP3A isozymes, in various species (Waxman, 1999; Honkakoski and Negishi, 2000). In this report, we have exploited receptor-selective agonists to identify additional genes regulated by PXR in murine liver and intestine and in primary cultures of human hepatocytes. Our

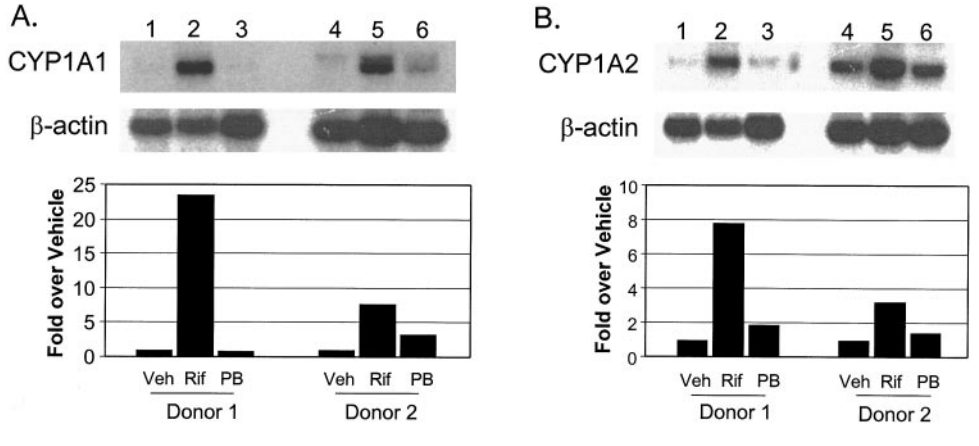

Fig. 3. Induction of *CYP1A1* and *CYP1A2* expression in primary cultures of human hepatocytes. Northern blot analysis of *CYP1A1* (A) and *CYP1A2* (B) mRNA levels was performed with total RNA (10 μg) prepared from human hepatocytes (2 donors) treated for 48 h with vehicle alone (0.1% DMSO; lanes 1 and 4), rifampicin (10 μM; lanes 2 and 5), or PB (1 mM; lanes 3 and 6). Relative mRNA abundance (plotted as fold relative to vehicle treatment) is shown at the bottom.

results demonstrate that PXR and CAR coordinately regulate programs of genes involved in all phases of xenobiotic metabolism, including oxidative metabolism, conjugation, and transport in mouse liver and small intestine. Moreover, our data provide evidence that PXR has a similarly broad regulatory role in human hepatocytes. Many of the genes regulated by PXR and/or CAR are also implicated in the metabolism and excretion of bilirubin and bile acids and other steroids. Because both CAR and PXR can be regulated by endogenous substances, such as steroids (Forman et al., 1998; Kliewer et al., 1998; Moore et al., 2000b; Staudinger et al., 2001; Xie et al., 2001), these nuclear receptors may also be important components in the body's defenses against chemicals that are produced during periods of stress or disease.

Although there is a high degree of overlap in the genes regulated by PXR and CAR, there are many genes that are differentially regulated by these two receptors in mice. Two interesting trends emerged in our mouse studies. First, PXR regulated a number of genes in small intestine that were not regulated by CAR, including *Aldh1a1*, *Aldh1a7*, *Cyp3a11*, *Gsta1*, *Gstm2*, *Mdr1b*, and *Mrp2* (Table 3). Second, CAR stimulated the expression of several genes in liver that were not regulated by PXR, including *Cyp1a1*, *Cyp2a4*, *Sultn*, *Mrp1*, and *Mrp2* (Table 3). These data suggest that PXR and CAR have overlapping but distinct biological functions and, furthermore, suggest that PXR and CAR may play more dominant roles in xenobiotic metabolism in small intestine and liver, respectively.

Among the genes regulated by PXR in murine liver were those encoding PXR and CAR (Table 3, lines 22 and 23). These data demonstrate that PXR is under autoregulation and reveal an additional level of cross-talk between PXR and CAR. This cross talk is asymmetric in that CAR did not regulate *Pxr* expression. PXR also weakly regulated its own expression in human hepatocytes derived from one of the two donors but did not seem to regulate *CAR* (Table 4, lines 14 and 15). Thus, PXR may be weakly autoregulatory in the human liver.

We note that several genes, including *Alas1*, *Gstm2*, and *Por*, seem to be regulated by TCPOBOP in a CAR-independent fashion (Table 3, lines 9, 19, 21). Similar CAR-independent effects on *Alas1* expression in liver were recently described for PB (Ueda et al., 2002). It also seems that there may be a PXR-independent component to PCN regulation of *Alas1* in mouse liver (Table 3, line 19). These data suggest that these chemicals mediate at least some of their effects through other signaling pathways. Moreover, they underscore the importance of using genetic models such as receptor-null mice to validate the pharmacologic data derived with receptor-selective ligands.

Our data indicate that PXR regulates a similar program of genes involved in the solubilization and excretion of xenobiotics in mouse and human hepatocytes. However, there seem to be species-specific effects. One of the most interesting of these differences relates to *AhR* and its target genes *CYP1A1 and CYP1A2*. In human hepatocytes, activation of PXR by rifampicin results in a modest induction of AhR and a marked induction of its target genes *CYP1A1* and *CYP1A2* (Table 4, lines 2, 3, and 12). These data raise the intriguing possibility that in human liver, PXR activation effectively primes hepatocytes to respond to challenges by other classes of xenobiotics such as aryl hydrocarbons that might otherwise escape detection by PXR. Thus, there seems to be cross-talk between different structural classes of xenobiotic-sensing transcription factors. We do not know at this point whether PXR regulates *AhR*, *CYP1A1*, and *CYP1A2* directly or indirectly. In contrast, activation of PXR by PCN in mouse liver did not induce either *AhR* or *Cyp1a1* (Table 3, lines 3 and 20). Expression of both *AhR* and *Cyp1a1* was induced modestly in mouse liver by activation of CAR (Table 3, lines 3 and 20).

In summary, we have shown that PXR and CAR regulate overlapping but distinct programs of genes involved in the detoxification of xenobiotics and endogenous lipophilic chemicals in mice. Moreover, we show that PXR regulates a similar program of genes in human hepatocytes. Our data provide evidence for a broad role for these nuclear receptors in defending the body against potentially harmful chemicals.

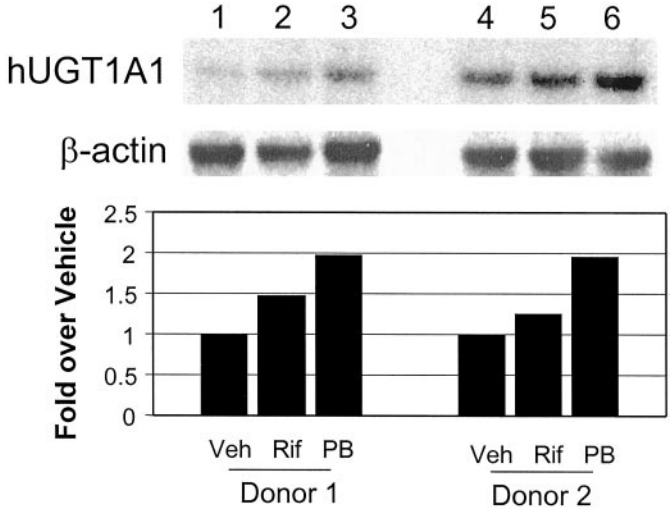

Fig. 4. Induction of *UGT1A1* expression in primary cultures of human hepatocytes. Northern blot analysis of *UGT1A1* mRNA levels was performed with total RNA (10 μg) prepared from human hepatocytes (2 donors) treated for 48 h with vehicle alone (0.1% DMSO; lanes 1 and 4), rifampicin (10 μM; lanes 2 and 5), or PB (1 mM; lanes 3 and 6). Relative mRNA abundance (plotted as fold relative to vehicle treatment) is shown at the bottom.

Acknowledgments

We are indebted to Mary Cameron, Jennifer Dew, Joe Watson, and Jo Sledge for their technical assistance and Dr. Curtis Klaasen for comments on the manuscript.

References

Bertilsson G, Heidrich J, Svensson K, Asman M, Jendeberg L, Sydow-Backman M, Ohlsson R, Postlind H, Blomquist P, et al. (1998) Identification of a human nuclear receptor defines a new signaling pathway for CYP3A induction. *Proc Natl Acad Sci USA* **95:**12208–12213.

Blumberg B, Sabbagh W Jr, Juguilon H, Bolado J Jr, van Meter CM, Ong ES, and Evans RM (1998) SXR, a novel steroid and xenobiotic-sensing nuclear receptor. *Genes Dev* **12:**3195–3205**.**

Dunn RT 2nd, Gleason BA, Hartley DP, and Klaassen CD (1999) Postnatal ontogeny and hormonal regulation of sulfotransferase SULT1B1 in male and female rats. *J Pharmacol Exp Ther* **290:**319–324.

Dussault I, Lin M, Hollister K, Wang EH, Synold TW, and Forman BM (2001) Peptide mimetic HIV protease inhibitors are ligands for the orphan receptor SXR. *J Biol Chem* **276:**33309–33312.

Falkner KC, Pinaire JA, Xiao GH, Geoghegan TE, and Prough RA (2001) Regulation of the rat glutathione S-transferase A2 gene by glucocorticoids: involvement of both the glucocorticoid and pregnane X receptors. *Mol Pharmacol* **60:**611–619.

Fernandez-Salguero P and Gonzalez FJ (1995) The CYP2A gene subfamily: species differences, regulation, catalytic activities and role in chemical carcinogenesis. *Pharmacogenetics* **5:**S123–8.

Forman BM, Tzameli I, Choi HS, Chen J, Simha D, Seol W, Evans RM, and Moore DD (1998) Androstane metabolites bind to and deactivate the nuclear receptor CAR-β. *Nature (Lond)* **395:**612–615.
Geick A, Eichelbaum M, and Burk O (2001) Nuclear receptor response elements mediate induction of intestinal MDR1 by rifampin. *J Biol Chem* **276:**14581–14587.
Goodwin B, Moore LB, Stoltz CM, McKee DD, and Kliewer SA (2001) Regulation of the human *CYP2B6* gene by the nuclear pregnane X receptor. *Mol Pharmacol* **60:**427–431.
Honkakoski P and Negishi M (2000) Regulation of cytochrome P450 (CYP) genes by nuclear receptors. *Biochem J* **347:**321–337.
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.
Kast HR, Goodwin B, Tarr PT, Jones SA, Anisfeld AM, Stoltz CM, Tontonoz P, Kliewer S, Willson TM, and Edwards PA (2002) Regulation of multidrug resistance-associated protein 2 (ABCC2) by the nuclear receptors pregnane X receptor, farnesoid X-activated receptor and constitutive androstane receptor. *J Biol Chem* **277:**2908–2915.
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.
Lehmann JM, McKee DD, Watson MA, Willson TM, Moore JT, and Kliewer SA (1998) The human orphan nuclear receptor PXR is activated by compounds that regulate *CYP3A4* gene expression and cause drug interactions. *J Clin Invest* **102:**1016–1023.
Liu L and Klaassen CD (1996) Regulation of hepatic sulfotransferases by steroidal chemicals in rats. *Drug Metab Dispos* **24:**854–858.
Madhu C and Klaassen CD (1991) Protective effect of pregnenolone-16α-carbonitrile on acetaminophen-induced hepatotoxicity in hamsters. *Toxicol Appl Pharmacol* **109:**305–313.
May BK, Dogra SC, Sadlon TJ, Bhasker CR, Cox TC, and Bottomley SS (1995) Molecular regulation of heme biosynthesis in higher vertebrates. *Prog Nucleic Acid Res Mol Biol* **51:**1–51.
Moore JT and Kliewer SA (2000) Use of the nuclear receptor PXR to predict drug interactions. *Toxicology* **153:**1–10.
Moore LB, Goodwin B, Jones SA, Wisely GB, Serabjit-Singh CJ, Willson TM, Collins JL, and Kliewer SA (2000a) St. John's wort induces hepatic drug metabolism through activation of the pregnane X receptor. *Proc Natl Acad Sci USA* **97:**7500–7502.
Moore LB, Parks DJ, Jones SA, Bledsoe RK, Consler TG, Stimmel JB, Goodwin B, Liddle C, Blanchard SG, Willson TM, et al. (2000b) Orphan nuclear receptors constitutive androstane receptor and pregnane X receptor share xenobiotic and steroid ligands. *J Biol Chem* **275:**15122–7.
Mulder GJ and Jakoby WB (1990) in *Conjugation Reactions in Drug Metabolism* (Mulder GJ ed) pp 107–161, Taylor and Francis, Ltd., London.
Ogawa K, Suzuki H, Hirohashi T, Ishikawa T, Meier PJ, Hirose K, Akizawa T, Yoshioka M, and Sugiyama Y (2000) Characterization of inducible nature of MRP3 in rat liver. *Am J Physiol* **278:**G438–G446.
Pascussi JM, Gerbal-Chaloin S, Fabre JM, Maurel P, and Vilarem MJ (2000) Dexamethasone enhances constitutive androstane receptor expression in human hepatocytes: consequences on cytochrome P450 gene regulation. *Mol Pharmacol* **58:** 1441–1450.
Runge-Morris M, Wu W, and Kocarek TA (1999) Regulation of rat hepatic hydroxysteroid sulfotransferase (SULT2–40/41) gene expression by glucocorticoids: evidence for a dual mechanism of transcriptional control. *Mol Pharmacol* **56:**1198–1206**.**
Schrenk D, Baus PR, Ermel N, Klein C, Vorderstemann B, and Kauffmann HM (2001) Up-regulation of transporters of the MRP family by drugs and toxins. *Toxicol Lett* **120:**51–57.
Smirlis D, Muangmoonchai R, Edwards M, Phillips IR, and Shephard EA (2001) Orphan receptor promiscuity in the induction of cytochromes P450 by xenobiotics. *J Biol Chem* **276:**12822–12826.
Smith G, Henderson CJ, Parker MG, White R, Bars RG, and Wolf CR (1993) 1,4-Bis[2-(3, 5-dichloropyridyloxy)]benzene, an extremely potent modulator of mouse hepatic cytochrome P-450 gene expression. *Biochem J* **289:**807–813.
Staudinger JL, Goodwin B, Jones SA, Hawkins-Brown D, MacKenzie KI, LaTour A, Liu Y, Klaassen CD, Brown KK, Reinhard J, et al. (2001) The nuclear receptor PXR is a lithocholic acid sensor that protects against liver toxicity. *Proc Natl Acad Sci USA* **98:**3369–3374.
Stieger B and Meier PJ (1998) Bile acid and xenobiotic transporters in liver. *Curr Opin Cell Biol* **10:**462–467.
Sueyoshi T, Kawamoto T, Zelko I, Honkakoski P, and Negishi M (1999) The repressed nuclear receptor CAR responds to phenobarbital in activating the human CYP2B6 gene. *J Biol Chem* **274,** 6043–6046.
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.
Synold TW, Dussault I, and Forman BM (2001) The orphan nuclear receptor SXR coordinately regulates drug metabolism and efflux. *Nat Med* **7:**584–590.
Tzameli I, Pissios P, Schuetz EG, and Moore DD (2000) The xenobiotic compound 1,4-bis[2-(3, 5-dichloropyridyloxy)]benzene is an agonist ligand for the nuclear receptor CAR. *Mol Cell Biol* **20:**2951–2958.
Ueda A, Hamadeh HK, Webb HK, Yamamoto Y, Sueyoshi T, Afshari CA, Lehmann JM, and Negishi M (2002) Diverse roles of the nuclear orphan receptor CAR in regulating hepatic genes in response to phenobarbital. *Mol Pharmacol* **61:**1–6.
Vasiliou V, Pappa A, and Petersen DR (2000) Role of aldehyde dehydrogenases in endogenous and xenobiotic metabolism. *Chem Biol Interact* **129:**1–19.
Waxman DJ (1999) P450 gene induction by structurally diverse xenochemicals: central role of nuclear receptors CAR, PXR and PPAR. *Arch Biochem Biophys* **369:**11–23.
Wei P, Zhang J, Dowhan DH, and Moore DD (2002) Specific and overlapping functions of the nuclear hormone receptors CAR and PXR in xenobiotic response. *Pharmacogenomics J* **2:**117–126.
Wei P, Zhang J, Egan-Haffey M, Liang S, and Moore DD (2000) The nuclear receptor CAR mediates specific xenobiotic induction of drug metabolism. *Nature (Lond)* **407:**920–923.
Xie W, Barwick JL, Downes M, Blumberg B, Simon CM, Nelson MC, Neuschwander-Tetri BA, Brunt EM, Guzelian PS, and Evans RM (2000a) Humanized xenobiotic response in mice expressing nuclear receptor SXR. *Nature (Lond)* **406:**435–439.
Xie W, Barwick JL, Simon CM, Pierce AM, Safe S, Blumberg B, Guzelian PS, and Evans RM (2000b) Reciprocal activation of xenobiotic response genes by nuclear receptors SXR/PXR and CAR. *Genes Dev* **14:**3014–3023.
Xie W, Radominska-Pandya A, Shi Y, Simon CM, Nelson MC, Ong ES, Waxman DJ, and Evans RM (2001) An essential role for nuclear receptors SXR/PXR in detoxification of cholestatic bile acids. *Proc Natl Acad Sci USA* **98:**3375–3380.

Address correspondence to: Dr. Steven A. Kliewer, Department of Molecular Biology, University of Texas Southwestern Medical Center, Building NC7.214, 5323 Harry Hines Blvd., Dallas, TX 75390-8594. E-mail: steven.kliewer@utsouthwestern.edu