Regulation of Tissue-Specific Carboxylesterase Expression by Pregnane X Receptor and Constitutive Androstane Receptor

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ABSTRACT:

The liver- and intestine-enriched carboxylesterase 2 (CES2) enzyme catalyzes the hydrolysis of several clinically important anticancer agents administered as prodrugs. For example, irinotecan, a carbamate prodrug used in the treatment of colorectal cancer, is biotransformed in vivo by CES2 in intestine and liver, thereby producing a potent topoisomerase I inhibitor. Pregnane X receptor (PXR) and constitutive androstane receptor (CAR), two members of the nuclear receptor superfamily of ligand-activated transcription factors, mediate gene activation in response to xenobiotic stress. Together, these receptors comprise a protective response in mammals that coordinately regulate hepatic transport, metabolism, and elimination of numerous xenobiotic compounds. In the present study, microarray analysis was used to identify PXR target genes in duodenum in mice. Here, we show that a gene encoding a member of the CES2 subtype of liver- and intestine-enriched CES enzymes, called *Ces6*, is induced after treatment with pregnenolone 16α-carbonitrile in a PXR-dependent manner in duodenum and liver in mice. Treatment of mice with the CAR activator 1,4-bis[2-(3,5-dichloropyridyloxy)] benzene also induced expression of *Ces6* in duodenum and liver in a CAR-dependent manner, whereas treatment with phenobarbital produced induction of *Ces6* exclusively in liver. These data identify a key role for PXR and CAR in regulating the drug-inducible expression and activity of an important CES enzyme in vivo. Future studies should focus on determining whether these signaling pathways governing drug-inducible CES expression in intestine and liver are conserved in humans.

Carboxylesterase (CES) enzymes comprise a multigene family and are dominantly involved in hydrolysis activity in liver and small intestine of mammals (Satoh and Hosokawa, 1998). These enzymes participate in the biotransformation of a wide range of ester- and amide-containing drugs and prodrugs, including angiotensin-converting enzyme inhibitors, antitumor drugs, and narcotics such as cocaine and heroin (Pindel et al., 1997; Takai et al., 1997; Humerickhouse et al., 2000). Members of the CES family of enzymes also hydrolyze numerous endogenous compounds, including short- and long-chain acyl-glycerols, long-chain acyl-carnitine, and long-chain acyl-CoA esters (Satoh and Hosokawa, 1998).

The mouse *Ces6* gene was first identified in 2004 and encodes a protein of 558 amino acid residues in length (Stok et al., 2004). In this same study, the Ces6 gene product was found to hydrolyze selected pyrethroid compounds, a major class of insecticides used worldwide and extensively in the United States. The closest relative of the mouse Ces6 protein in humans is the CES2 enzyme, because these two orthologous proteins are approximately 61% identical and 72% similar when compared at the amino acid level.

Like the human CES2 enzyme, mouse Ces6 is expressed in a liver-

and intestine-enriched manner. The CES2 enzyme has come under recent scrutiny because it catalyzes the hydrolysis of several clinically used anticancer agents that are administered as prodrugs. In particular, CES2 is a high-affinity, high-velocity enzyme with respect to the prodrug 7-ethyl-10-[4-(1-piperidino)-1-piperidino] carbonyloxy-camptothecin, also called irinotecan, and probably plays a substantial role in irinotecan bioactivation in human liver and intestine at relevant pharmacological concentrations (Humerickhouse et al., 2000). Although much is known regarding the role of CES enzymes in biotransformation of prodrugs, little is known regarding the tissue-specific transcriptional regulation of these important drug-metabolizing enzymes in vivo in tissues such as liver and intestine.

Nuclear receptors comprise a superfamily of ligand-activated transcriptional factors that are involved in diverse physiological, developmental, and metabolic processes. The pregnane X receptor (PXR, NR1I2) and constitutive androstane receptor (CAR, NR1I3) are two closely related members of "xenobiotic-sensing" nuclear receptors among this family. It is now well established that PXR is a key regulator of xenobiotic-inducible *CYP3A* gene expression in liver (reviewed in Goodwin et al., 2002). PXR also regulates the drug-inducible expression and activity of genes encoding key members of the CYP2B and CYP2C subfamily of enzymes in liver, as well as the drug-inducible expression and activity of several glutathione *S*-transferase, sulfotransferase, and UDP-glucuronosyltransferase enzymes in liver (Maglich et al., 2002; Sonoda et al., 2002; Wei et al., 2002; Tully

et al., 2006). PXR target genes also encode key hepatic drug transporter proteins such as organic anion transporting polypeptide 2, multidrug resistance 1/P-glycoprotein, and multidrug resistance proteins 2 and 3 (Geick et al., 2001; Kast et al., 2002; Staudinger et al., 2003). Recent reports indicate a key role for human PXR in regulating the expression of the *CES2* gene in human hepatocytes and the human hepatoma cell line Huh-7 in culture (Yang and Yan, 2007). Other reports suggest a positive role for rat PXR in regulating liver-enriched expression of CES enzymes in rodents (Goetz et al., 2006; Tully et al., 2006; Shi et al., 2008). Earlier research indicates that overexpression of constitutively active human PXR has a positive effect on the expression of mouse genes encoding Ces2 and Ces3 enzymes in mouse liver (Rosenfeld et al., 2003).

The nuclear receptor CAR is also recognized as a xenobiotic-sensing nuclear receptor that is mainly expressed in hepatic tissue. It was originally shown to regulate the phenobarbital (PB)-inducible expression of several genes encoding important members of the CYP2B subfamily of enzymes (Honkakoski et al., 1998). CAR has since been shown to regulate the expression and activity of a number of phase I and phase II metabolic enzymes, as well as the expression and activity of numerous important membrane transporter proteins involved in the elimination of endogenous and exogenous substances, including bilirubin, steroid hormones, and xenobiotics (Ueda et al., 2002). Definitive reports linking CAR activation to drug-inducible CES gene expression in any species or tissue are currently lacking. However, historical reports indicate that treatment of rodents with PB or PB-like inducers significantly increases carboxylesterase expression and activity in liver tissue (Hosokawa et al., 1987). Distinct, yet overlapping functions of PXR and CAR in liver have been described previously (Maglich et al., 2002; Wei et al., 2002), and it is well established that these two receptors form the molecular basis of an important class of drug-drug interactions through their actions in liver. Although much is known regarding the identity and function of PXR target genes in liver, less is known about their function in small intestine.

In the present study, we report the identification of several pregnenolone 16α-carbonitrile (PCN)-inducible genes in duodenum of wild-type mice using microarray analysis. Among the PCN-inducible genes identified here, expression of the gene encoding the Ces6 protein was further characterized with respect to its basal and drug-inducible expression in liver and intestine. By exploiting the *Pxr* knockout (PXR-KO) and *Car* knockout (CAR-KO) mouse models, we reveal that in both duodenum and liver tissues, the drug-inducible expression of *Ces6* is regulated by both PXR and CAR. Our data conclusively show that *Ces6* is a shared PXR and CAR target gene in mice. It is interesting to note that, in small intestine, despite significant expression of the CAR nuclear receptor, expression of the *Ces6* gene is exclusively regulated by intraperitoneal treatment with 1,4-bis[2-(3,5-dichloropyridyloxy)] benzene (TCPOBOP) and not by intraperitoneal treatment of mice with PB. These data suggest that there may be significant differences in the bioavailability of PB and TCPOBOP in intestine after treatment with these two CAR activators that can produce variable results. Alternatively, differences in the mode of CAR activation, either phosphorylation-dependent in the case of PB or direct ligand-mediated activation in the case of TCPOBOP, can probably produce tissue-specific differences when using these two compounds to activate the CAR nuclear receptor in intestine and liver tissue in vivo. In any case, these data reveal that liver- and intestine-enriched CES expression and activity are probably modulated in humans on combination therapy in a clinically significant manner. This is of particular importance because numerous drug development programs seek to take advantage of intestine- and liver-enriched CES

enzymes as convenient targets for delivery of increasing numbers of prodrugs.

Materials and Methods

Animal Care. All the rodents were maintained on standard laboratory chow and allowed food and water ad libitum. All the mice were treated once a day intraperitoneally with either vehicle (corn oil, saline), PCN (100 mg/kg in corn oil), TCPOBOP (3 mg/kg in corn oil), or PB (100 mg/kg in saline) for 4 days. The studies reported here have been carried out in accordance with the Declaration of Helsinki and/or with the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, 1996) as adopted and promulgated by the National Institutes of Health.

Drugs and Chemicals. Unless otherwise stated, all the chemical compounds were purchased from Sigma-Aldrich (St. Louis, MO). Antibody against CES6 was purchased from Abcam Inc. (Cambridge, MA). The anti-Cyp3a23 antibody that cross-reacts with mouse Cyp3a11 protein was used to probe immunoblots (Millipore Bioscience Research Reagents, Temecula, CA). The anti-Cyp2b10 antibody was obtained from Millipore Bioscience Research Reagents.

Microarray Analysis. Total RNA was isolated from duodenums of wild-type mice using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The quality and quantity of the total RNA samples were examined with both UV spectrophotometry using a NanoDrop (ND-1000; Thermo Fisher Scientific Inc., Waltham, MA) and Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA). For the subsequent microarray study, Affymetrix (Santa Clara, CA) Mouse Genome GeneChip 430 2.0 oligonucleotide arrays were used that cover more than 39,000 transcripts from the mouse genome. To carry out the GeneChip analysis, established standard protocols at the University of Kansas Genomics Facility were performed on cRNA target preparation, array hybridization, washing, staining, and image scanning. After they were generated from the Affymetrix GeneChip Operating Software (version 1.2), the microarray data were first subjected to quality assessment. All of the GeneChip data passed the quality control (QC) step because data of all the chips met established Affymetrix GeneChip QC criteria, including low background, low noise, positive detection of QC probe sets such as bioB, percentage of called present in the normal range (40–60%), similar scaling factors across chips, and 3′:5′ ratio. Because of its known response to PCN, *Cyp3a11* was also used as a positive control in this study.

Microarray Data Analysis. To facilitate direct comparison of gene expression data between the PCN- and vehicle-treated samples, the GeneChip data were first subjected to preprocessing, including background correction, probe summarization, and normalization using the Affymetrix MAS5 algorithm. All the chips were scaled to a target signal of 500. Before identification of differentially expressed genes, genes that were called "Absent" by the MAS5 algorithm were filtered out. A volcano plot-based approach was subsequently used to identify PCN-inducible gene expression. The expression value of a PCN-inducible gene had to pass two criteria: 1) the -fold change between the compound treatment and the negative control had to be ≥ 1.5, and 2) the p value from parametric test using all the available error estimates had to be ≤ 0.05.

Real-Time Quantitative Polymerase Chain Reaction. After DNase I treatment, 1 μg of RNA was reverse-transcribed using random primers following the manufacturer's instruction (Promega, Madison, WI). Equal amounts of cDNA were used in real-time quantitative polymerase chain reaction (rt-QPCR) reactions. Reactions included 200 nM fluorogenic probe and 300 nM primers specific for each gene. The fluorogenic probe and primer sets were designed using the Primer3 program (http://frodo.wi.mit.edu/). The fluorogenic probes were synthesized by BioSearch Technologies (Novato, CA). The sequences (5′ to 3′) for the primers and probes are as follows: *Cyp3a11*, forward primer (CAAGGAGATGTTCCCTGTCA), fluorogenic probe (FAM-AGAAGGCAAAGAAAGGCAAGCCTG-BHQ1), reverse primer (CCACGTTCACTCCAAATGAT); and *Cyp2b10*, forward primer (GACTTTGGGATGGGAAAGAG), fluorogenic probe (FAM-TAGTG-GAGGAACTGCGGAAATCCC-BHQ1), reverse primer (CCAAACA-CAATGGAGCAGAT). For the *Ces6* and 18S genes, 1× SYBR Green (Lonza Rockland, Inc., Rockland, ME) was included in the reaction instead of the fluorogenic probe. The sequences (5′ to 3′) for the *Ces6* and 18S are as follows: *Ces6*, forward primer (GTGTGAGAGATGGGACCTCA),

reverse primer (TCATTCATGGAAGCTGATCC); and 18S, forward primer (AGTCCCTGCCCTTTGTACACA), reverse primer (CGATCCGAGGGCCT-CACTA). Cycling conditions were 95°C for 2 min followed by 45 cycles of 95°C for 15 s, 60°C for 15 s, and 68°C for 15 s using the Cepheid Smart Cycler system (Sunnyvale, CA). The -fold induction was calculated as described previously (Staudinger et al., 2003).

Northern Blot Analysis. Total RNA was isolated as described in rt-QPCR, and 20 μg of total RNA per lane was resolved on 3.7% formaldehyde/1% agarose gel in MOPS buffer for Northern blot analysis. Blots were hybridized with ^{32}P-labeled cDNA corresponding to the sequences for mouse *Ces6*, *Cyp3a11*, and *Cyp2b10*, and 18S ribosomal RNA as described previously (Ding and Staudinger, 2005).

Western Blot Analysis. Approximately 250 mg of liver tissue was homogenized using a Dounce Teflon (Thermo Fisher Scientific) homogenizer in 1 ml of homogenization buffer (50 mM Tris-HCl, pH 7.4, at 4°C containing 150 mM KCl and 2 mM EDTA). The homogenate was subjected to centrifugation at 500g for 15 min at 4°C to remove cell debris and nuclei. The supernatant fraction was subjected to centrifugation at 12,000g for 15 min at 4°C. Microsomes were prepared by ultracentrifugation (50,000g for 60 min at 4°C) of the postmitochondrial supernatant fraction. The first microsomal pellet was resuspended in wash buffer (10 mM EDTA containing 150 mM KCl), then reisolated by ultracentrifugation (50,000g for 60 min at 4°C). The washed microsomes were suspended in a small volume of 250 mM sucrose. Liver microsomal protein (20 μg/lane) was resolved on 10% SDS-polyacrylamide gel electrophoresis gels and transferred to polyvinylidene difluoride microporous membranes (Millipore Bioscience Research Reagents) that were probed with anti-Ces6, anti-Cyp3A23, and anti-Cyp2b10 antibodies. Immunodetection was performed using the enhanced chemiluminescence kit (GE Healthcare, Little Chalfont, Buckinghamshire, UK) according to the protocol provided by the manufacturer. Quantitative densitometric analyses of Western blot images were achieved using the digital Eastman Kodak (Rochester, NY) EDAS 290 image acquisition system together with the 1D image analysis software package.

Statistical Analysis. Statistical differences between treatment groups were determined using one-way analysis of variance, followed by the Duncan's multiple range post hoc test.

Results

Basal Expression Profiles of Mouse *Cyp3a11*, *Pxr*, and *Car* in Liver, Duodenum, Jejunum, and Ileum. Liver and small intestine are two major organs that play an important and primary role in regulating the metabolism, transport, excretion, and efflux of xenobiotic compounds. The relative expression levels of mouse genes encoding *Cyp3a11*, *Pxr*, and *Car* in three individual wild-type mice were determined using total RNA isolated from liver, duodenum, jejunum, and ileum (Fig. 1). The expression of the *Cyp3a11* gene was highest in mouse liver, followed by a significant reduction of approximately 60% in duodenum. The *Cyp3a11* transcript in jejunum and ileum was virtually undetectable. The expression level of *Pxr* was also determined. The *Pxr* mRNA was expressed at the highest levels in liver, with lower but easily detectable levels observed in duodenum, jejunum, and ileum. The *Pxr* mRNA was approximately 60% of that observed in liver when examined in duodenum, jejunum, and ileum, respectively. The expression level of *Car* was then determined. The *Car* gene was expressed at the highest level in liver, with slightly lower but easily detectable expression levels observed in duodenum, jejunum, and ileum.

Detection of PCN-Inducible *Cyp3a11* Gene Expression in Duodenum. Before performing microarray analysis, we sought to determine whether the known PXR target gene *Cyp3a11* was induced in small intestine after administration of PCN, a well known rodent PXR activator. Wild-type mice ($n = 3$) were treated for 4 days using an intraperitoneal injection of either vehicle (corn oil) or PCN (100 mg/kg). On the morning of day 5, tissues were harvested, and total RNA was isolated from the entire small intestine and resolved on an
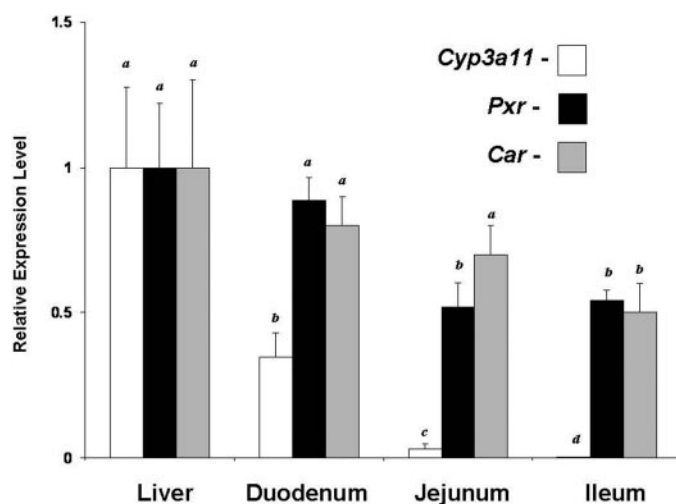

FIG. 1. The basal expression of *Cyp3a11*, *Pxr*, and *Car* gene expression levels in liver, duodenum, jejunum, and ileum. Total RNA was isolated from liver, duodenum, jejunum, and ileum of 6-week-old wild-type mice. rt-QPCR analysis was performed to measure the relative abundance of the transcripts using reverse-transcribed cDNA from all the tissues examined. All of the data are normalized to 18S levels and are expressed relative to that observed in liver and represent average values ± S.E.M. ($n = 3$). Letters different from each other indicate a statistical difference between treatment groups ($p < 0.05$).

agarose gel for subsequent Northern blotting using a cDNA probe to detect *Cyp3a11* expression levels. This analysis revealed that *Cyp3a11* gene expression was induced in small intestine by treatment with PCN (data not shown). These data indicate that PXR-mediated target gene activation is completely intact in the small intestine of wild-type mice. Induction of *Cyp3a11* gene expression was absent from duodenal tissue isolated from PXR-KO mice (data not shown). Taken together with the data obtained in Fig. 1, we reasoned that treatment of wild-type mice with PCN should produce robust induction of PXR target genes in the duodenum and that microarray analysis should detect PXR-mediated gene activation.

Detection of PCN-Responsive Genes in Mouse Duodenum by Microarray. To identify novel PXR target genes in mouse duodenum, we performed microarray analyses using GeneChip Mouse Genome 430 2.0 oligonucleotide arrays from Affymetrix, which interrogate more than 39,000 transcripts encompassing the entire mouse genome. As expected, the expression of the prototypical PXR target gene *Cyp3a11* was increased approximately 1.8-fold after treatment with the known PXR activator PCN in duodenum. Several additional *CYP* genes were increased by treatment with PCN, including *Cyp2C55*, *Cyp2C29*, and *Cyp3a25*, by approximately 3.9-, 3.3-, and 2.0-fold, respectively. Seven genes encoding distinct members of the glutathione *S*-transferase family of enzymes were up-regulated after treatment with PCN. Three different glutathione *S*-transferase μ-isoenzymes were increased after treatment with PCN, including the Gstm1, Gstm3, and Gstm6 isoforms by 5.3-, 2.6-, and 1.9-fold, respectively. The glutathione *S*-transferase Gsta2 and Gsta4 isoforms were increased after treatment with PCN by 2.2- and 1.9-fold, respectively. The microsomal glutathione *S*-transferase Mgst1 and Mgst2 enzymes were increased 1.6- and 2.0-fold, respectively. The gene encoding microsomal epoxide hydrolase was increased approximately 7.0-fold after treatment with PCN. Table 1 shows a complete listing of selected genes identified after this analysis. The gene that was induced to the highest absolute level as judged by the overall strength of the signal detected from the PCN-treated sample after *Cyp3a11* and *Gsta2* was *Ces6*. Therefore, we chose to further characterize the potential

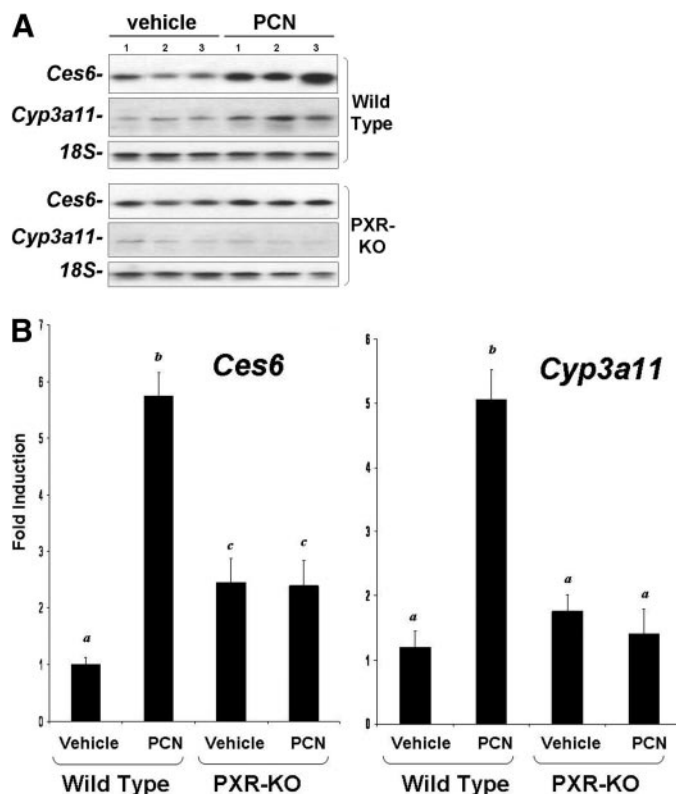| Gene	-Fold Induction	Description
Ephx1	7.0	Epoxide hydrolase 1, microsomal
Gstm1	5.3	Glutathione *S*-transferase, μ 1
Gstm3	2.6	Glutathione *S*-transferase, μ 3
Gstm6	1.9	Glutathione *S*-transferase, μ 6
Cyp2c55	3.9	Cytochrome P450, family 2, subfamily c, polypeptide 55
Cyp2c29	3.3	Cytochrome P450, family 2, subfamily c, polypeptide 29
Cyp3a25	2.0	Cytochrome P450, family 3, subfamily a, polypeptide 25
Cyp3a11	1.8	Cytochrome P450, family 3, subfamily a, polypeptide 11
Ces6	3.2	Carboxylesterase 6
Akr1b7	3.0	Aldo-keto reductase family 1, member B7
Hsd17b11	2.4	Hydroxysteroid 17-β dehydrogenase 9
Gsta4	2.2	Glutathione *S*-transferase, α 4
Gsta2	1.9	Glutathione *S*-transferase, α 2 (Yc2)
Mgst2	2.0	Microsomal glutathione *S*-transferase 2
Mgst1	1.6	Microsomal glutathione *S*-transferase 1
Abca1	2.0	ATP-binding cassette, subfamily A (ABC1), member 1
Aldh1a7	1.9	Aldehyde dehydrogenase family 1, subfamily A1
Pdk4	1.8	Pyruvate dehydrogenase kinase 4
Sgk	1.7	Serum/glucocorticoid regulated kinase
Hpgd	1.7	Hydroxyprostaglandin dehydrogenase 15 (NAD)
Dbi	1.6	Diazepam binding inhibitor

FIG. 2. Induction of *Ces6* and *Cyp3a11* gene expression by PCN treatment in mouse duodenum is PXR-dependent. Total RNA was isolated from the duodenum of wild-type and PXR-KO mice ($n = 3$) treated intraperitoneally with vehicle (corn oil) or PCN (100 mg/kg) for 4 days. A, Northern blot analysis was performed using 20 μg of total RNA/lane, and the blots were probed sequentially with ^{32}P-labeled cDNA fragments encoding *Ces6*, *Cyp3a11*, and 18S ribosomal RNA. Each lane represents an individual animal. B, rt-QPCR analyses were performed to determine the expression levels of *Ces6* and *Cyp3a11*. All the data are normalized to 18S levels and represent the average values $\pm$ S.E.M. ($n = 5$) and are expressed as -fold induction over vehicle control. Letters different from each other indicate a statistical difference between treatment groups ($p < 0.05$).

role of PXR and CAR in modulating the drug-inducible expression of this gene in both liver and intestine.

Regulation of Ces6 mRNA by PCN in Mouse Duodenum Is PXR-Dependent. To determine whether *Ces6* represents a bona fide PXR target gene in duodenum, we performed both Northern blot and rt-QPCR analysis using wild-type and PXR-KO mice. Northern blot analysis using total RNA isolated from the duodenum of three individual animals was performed to determine the relative expression levels of both *Cyp3a11* and *Ces6* (Fig. 2A). Treatment of wild-type mice with PCN produced marked increases in *Ces6* and *Cyp3a11* mRNA levels; however, PCN-inducible increases in the expression of both the *Ces6* and *Cyp3a11* mRNAs were totally absent when the analysis was performed using total RNA isolated from the duodenum of individual PXR-KO mice. The results obtained using Northern blot analysis were quantified using rt-QPCR (Fig. 2B). The *Ces6* (left) and *Cyp3a11* (right) mRNAs exhibited a significant increase in their expression level, respectively, with each gene exhibiting an approximate increase of 5-fold in wild-type mice. In contrast, no significant increases in expression were detected when total RNA was analyzed from the duodenum of PXR-KO mice.

Regulation of Drug-Inducible Ces6 Is PXR- and CAR-Dependent. A number of studies have shown that PCN treatment induces *Cyp3a11* gene expression in both mouse liver and intestine (Masuyama et al., 2001; Staudinger et al., 2001b; Matheny et al., 2004). Other research has shown that PXR and CAR share distinct but overlapping target genes in liver (Moore et al., 2000; Xie et al., 2000; Goodwin et al., 2001; Wei et al., 2002). We next investigated whether induction of *Ces6* gene expression in liver and intestine by treatment with PCN, TCPOBOP, or PB is dependent on PXR or CAR. Therefore, we treated wild-type, PXR-KO, and CAR-KO mice with vehicle, PCN, TCPOBOP, or PB and isolated total RNA from liver and duodenum and microsomes from liver for subsequent analysis of gene expression and protein levels, respectively. We first analyzed *Ces6* and *Cyp3a11* gene expression levels using Northern blot analysis (Fig. 3A). As expected, treatment of wild-type mice with PCN produced increased levels of *Cyp3a11* gene expression in liver in a PXR-

dependent manner. Treatment with PCN also produced increased *Ces6* mRNA levels in liver in a PXR-dependent manner. Subsequent quantitative analysis using rt-QPCR analysis revealed that statistically significant induction of both *Ces6* (left) and *Cyp3a11* (right) gene expression by PCN treatment in mouse liver is PXR-dependent (Fig. 3B).

We next examined the relative expression levels of microsomal Ces6 and Cyp3a11 protein in mouse liver. Hepatic microsomes were isolated and resolved on an SDS-polyacrylamide gel electrophoresis gel for subsequent Western blot analysis. Expression of both the Ces6 and Cyp3a11 proteins was induced by PCN treatment in a PXR-dependent manner in hepatic microsomes (Fig. 4A). Quantitative analyses of these data indicate that both Ces6 (left) and Cyp3a11 (right) protein levels are significantly induced in liver in a PXR-dependent manner (Fig. 4B). Deletion of the PXR protein in vivo significantly increased the basal expression of both Ces6 and Cyp3a11 protein levels.

We next sought to determine whether expression of the *Ces6* gene in liver is regulated by the nuclear receptor CAR. Mice were treated for 4 days intraperitoneally with vehicle, TCPOBOP, or PB. Liver and duodenum were removed, and total RNA and protein were isolated on the morning of day 5. Gene expression levels were qualitatively and quantitatively determined using Northern blot and rt-QPCR analyses, respectively. In liver, the expression of genes encoding *Cyp2b10* and *Ces6* was increased after treatment with TCPOBOP in a CAR-
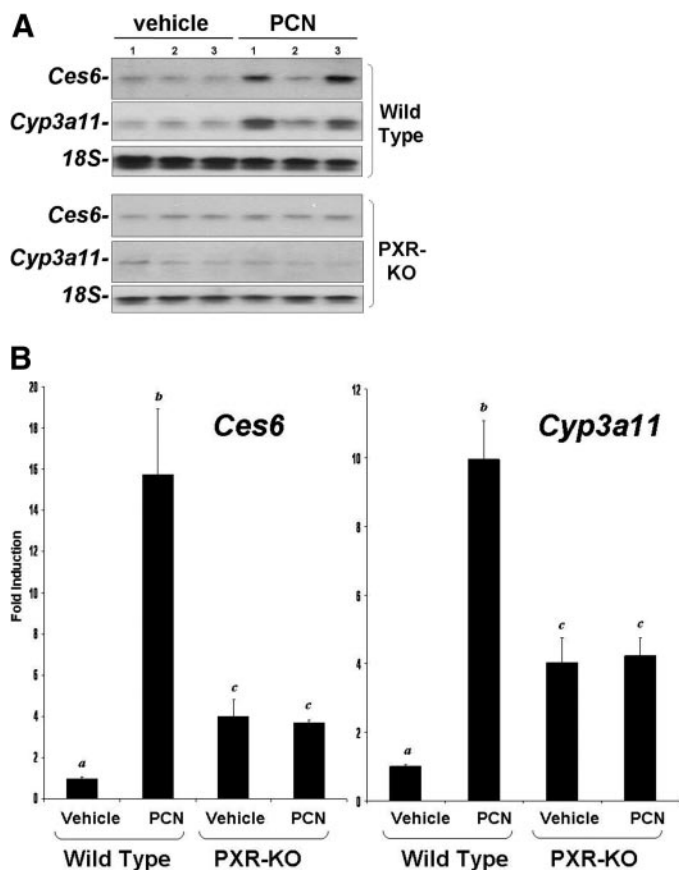
FIG. 3. Induction of *Ces6* and *Cyp3a11* gene expression by PCN treatment in mouse liver is PXR-dependent. Total RNA was isolated from the liver of wild-type and PXR-KO mice ($n = 3$) treated intraperitoneally with corn oil (vehicle) or PCN (100 mg/kg) for 4 days. A, Northern blot analysis was performed using 20 μg of total RNA/lane, and the blots were probed sequentially with ^{32}P-labeled cDNA fragments encoding *Ces6*, *Cyp3a11*, and 18S ribosomal RNA. Each lane represents an individual animal. B, rt-QPCR analyses were performed to determine the expression of *Cyp3a11* and *Ces6*. All of the data are normalized to 18S levels and represent the average values ± S.E.M. ($n = 5$) and are expressed as -fold induction over vehicle control. Letters different from each other indicate a statistical difference between treatment groups ($p < 0.05$).

FIG. 4. Expression of Ces6 and Cyp3a11 protein is induced by PCN in a PXR-dependent manner in mouse liver. Hepatic microsomes were from the liver of wild-type and PXR-KO mice ($n = 3$) treated intraperitoneally with corn oil (vehicle) or PCN (100 mg/kg) for 4 days. A, Western blot analysis was performed to determine the expression levels of Ces6, Cyp3a11, and β-actin protein, respectively. Each lane represents an individual animal. B, the results from A were quantified using scanning densitometry. All the data are normalized to β-actin levels and represent the average values ± S.E.M. ($n = 3$) and are expressed as -fold induction over vehicle control. Letters different from each other indicate a statistical difference between treatment groups ($p < 0.05$).

dependent manner in liver (Fig. 5A). Quantitative analysis using rt-QPCR confirmed that both *Ces6* and *Cyp2b10* gene expression levels were significantly induced by treatment with TCPOBOP in liver and duodenum in a CAR-dependent manner (Fig. 5B).

We next examined Ces6 and Cyp2B10 protein levels in mouse hepatic microsomes qualitatively and quantitatively using Western blot analysis and photodensitometry, respectively. These analyses revealed that treatment of mice with TCPOBOP produced robust increases in hepatic microsomal Ces6 and Cyp2b10 proteins in a CAR-dependent manner in liver microsomal fractions (Fig. 6A). Densitometric analysis showed that the increased levels of Ces6 (left) and Cyp2b10 (right) protein observed in wild-type mice treated with TCPOBOP were statistically significant (Fig. 6B). Similar results in liver were obtained when PB treatment was used to activate the CAR nuclear receptor protein in vivo. Qualitative analysis using Northern blotting revealed that PB treatment produced robust increases in the expression levels of the genes encoding *Ces6* and *Cyp2b10* in a CAR-dependent manner in liver (Fig. 7A). Quantitative analysis using rt-QPCR revealed that treatment with PB produced significant increases in the *Ces6* and *Cyp2b10* genes in liver (Fig. 7B). Examination of Ces6 and Cyp2B10 protein levels in mouse hepatic microsomes was accomplished qualitatively and quantitatively using
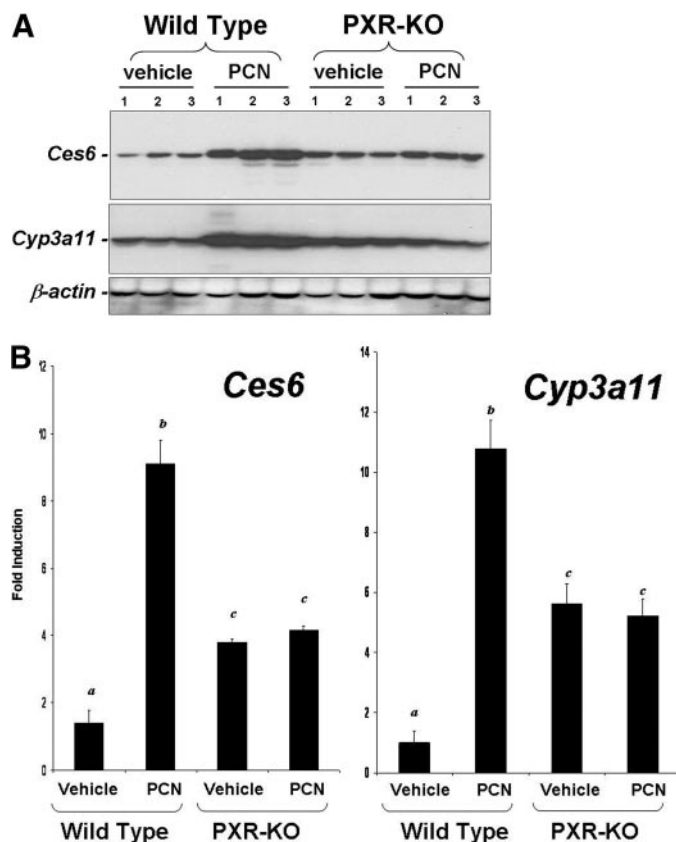
Western blot analysis and photodensitometry, respectively. These analyses revealed that treatment of mice with PB produced robust increases in hepatic microsomal Ces6 and Cyp2b10 proteins in a CAR-dependent manner in liver microsomal fractions (Fig. 8A). Densitometric analysis showed that the increased levels of Ces6 (left) and Cyp2b10 (right) protein observed in wild-type mice treated with TCPOBOP were statistically significant (Fig. 8B). It is interesting to note that no increases in the basal levels of either Ces6 or Cyp2b10 proteins were observed in CAR-KO mice. This is in stark contrast with that observed in the PXR-KO mice. This observation probably indicates that the CAR protein does not play an active repressive role in a nonstimulated state in liver. Treatment of all the genotypes of mice examined here with PB did not produce any significant changes in expression levels of genes encoding *Ces6*, *Cyp3a11*, or *Cyp2b10* in duodenum (data not shown). These data indicate that the drug-inducible expression and activity of the Ces6 and Cyp2b10 gene products are regulated by both PXR and CAR nuclear receptor proteins. Taken together, these data lead to a model in which drug-inducible activation of intestinal CES activity in intestine would be expected to increase the conversion of prodrugs to the active drug, thereby increasing the transport to portal vein and liver (Fig. 9). In the liver, high levels of cytochrome P450 and CES activity would be expected to further increase metabolism of coadministered drugs,
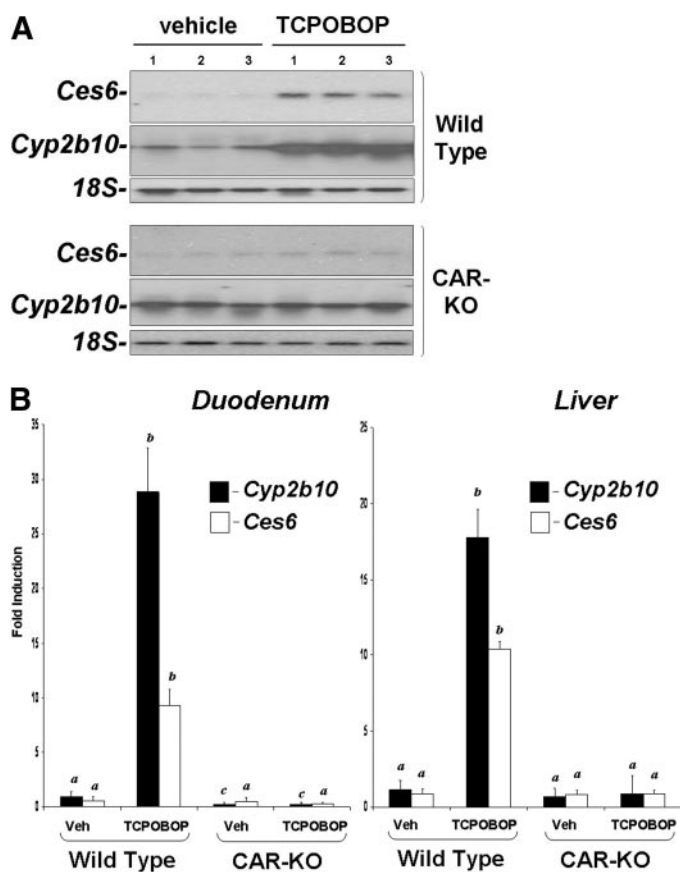
FIG. 5. Induction of *Ces6* and *Cyp2b10* gene expression by TCPOBOP treatment in mouse liver is CAR-dependent. Total RNA was isolated from the liver of wild-type and CAR-KO mice ($n = 3$) treated intraperitoneally with corn oil (vehicle) or TCPOBOP (3 mg/kg) for 4 days. A, Northern blot analysis was performed using 20 μg of total RNA/lane, and the blots were probed sequentially with ^{32}P-labeled cDNA fragments encoding *Ces6*, *Cyp2b10*, and 18S ribosomal RNA. Each lane represents an individual animal. B, rt-QPCR analyses were performed to determine the expression levels of *Ces6* and *Cyp2b10* in duodenum and liver tissues. All the data are normalized to 18S levels and represent the average values $\pm$ S.E.M. ($n = 5$) and are expressed as -fold induction over vehicle control. Letters different from each other indicate a statistical difference between treatment groups ($p < 0.05$).

FIG. 6. Expression of Ces6 and Cyp2b10 protein is induced by TCPOBOP in a CAR-dependent manner in mouse liver. Hepatic microsomes were from the liver of wild-type and CAR-KO mice ($n = 3$) treated intraperitoneally with corn oil (vehicle) or TCPOBOP (3 mg/kg) for 4 days. A, Western blot analysis was performed to determine the expression levels of Ces6, Cyp2b10, and β-actin protein, respectively. Each lane represents an individual animal. B, the results from A were quantified using scanning densitometry. All data are normalized to β-actin levels and represent the average values $\pm$ S.E.M. ($n = 3$) and are expressed as -fold induction over vehicle control. Letters different from each other indicate a statistical difference between treatment groups ($p < 0.05$).

leading to increased prospects for drug-drug interaction in patients on combination therapy.

Discussion

The first-pass effect is a phenomenon of drug metabolism whereby the concentration of a drug is greatly reduced before it reaches the systemic circulation. This effect is largely mediated by drug-metabolizing enzymes and drug transporter proteins in the small intestine and liver of mammals. When administered in the active form, these tissues metabolize many drugs to such an extent that only a small amount of the active drug emerges from the liver to the rest of the circulatory system. Increasingly, prodrugs are designed using a rational approach that takes advantage of the enzymes and transporter proteins in these tissues in such a manner that promotes their biotransformation and absorption after oral administration. In particular, introduction of an ester group generally improves bioavailability because of increased transport. Ester-containing prodrugs, including several angiotensin-converting enzyme inhibitors, antitumor drugs, and narcotics, are acted on by CES enzymes in this manner. In this regard, CES enzymes are considered to be one of the major determinants of the metabolism and disposition of ester-containing drugs through their actions in liver and intestine.
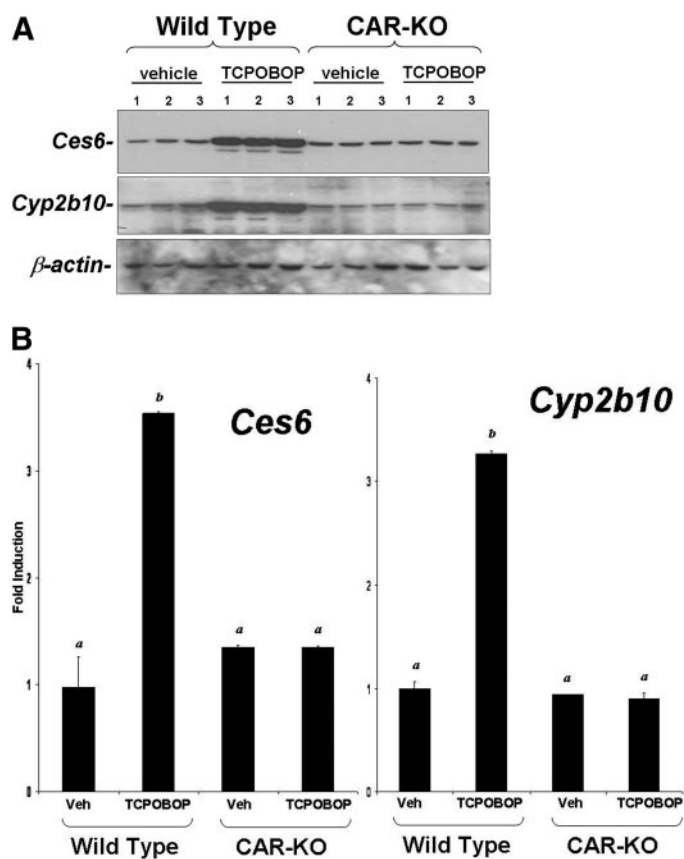
Most of what is known regarding the liver- and intestine-enriched CES enzymes (CES1 and CES2 subfamilies) consists of characterization of their respective substrate specificities. In particular, the CES1 subfamily of CES enzymes mainly hydrolyzes substrates with small alcohol and large acyl groups. In contrast, the CES2 subfamily of CES enzymes mainly hydrolyzes substrates with large alcohol and small acyl groups. Whereas much is known regarding their substrate selectivity, little is known regarding the regulation of expression of these important drug-metabolizing enzymes in liver and intestine, although it is a topic of intense study in several laboratories.

Because expression of the prototypical PXR target gene, *Cyp3a11*, was still detectable in duodenum when compared with that observed in jejunum or ileum (Fig. 1), we chose to further analyze the expression of PCN-inducible genes in this particular tissue. We show here that treatment of mice with PCN, a known PXR activator, induces the expression of multiple genes in duodenum involved in the regulation of drug metabolism and disposition. It is important to note here that we did not observe regulation of CAR target gene expression after treatment of mice with PB, a known indirect and phosphorylation-dependent activator of CAR, in the intestine, despite significant expression of the *CAR* gene itself in intestine. In contrast, treatment of mice with the direct activating ligand of CAR, TCPOBOP, produced significant increases in the expression of CAR target genes examined
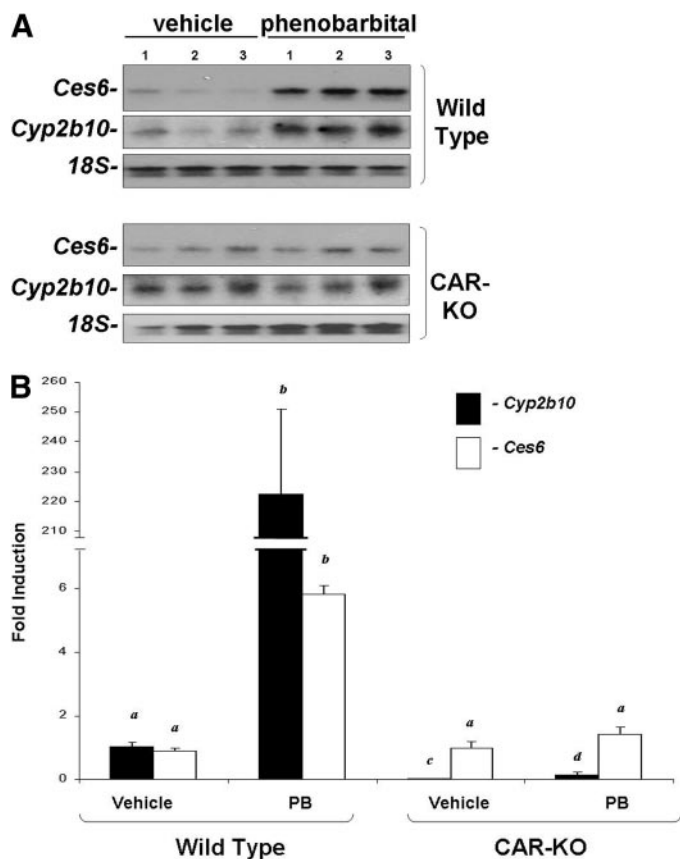
FIG. 7. Induction of *Ces6* and *Cyp2b10* gene expression by PB treatment in mouse liver is CAR-dependent. Total RNA was isolated from the liver of wild-type and CAR-KO mice ($n = 3$) treated intraperitoneally with saline (vehicle) or PB (100 mg/kg) for 4 days. A, Northern blot analysis was performed using 20 μg of total RNA/lane, and the blots were probed sequentially with ^{32}P-labeled cDNA fragments encoding *Ces6*, *Cyp2b10*, and 18S ribosomal RNA. Each lane represents an individual animal. B, rt-QPCR analyses were performed to determine the expression of *Cyp2b10* and *Ces6*. All of the data are normalized to 18S levels and represent the average values $\pm$ S.E.M. ($n = 5$) and are expressed as -fold induction over vehicle control. Letters different from each other indicate a statistical difference between treatment groups ($p < 0.05$).

FIG. 8. Expression of Ces6 and Cyp2b10 protein is induced by PB in a CAR-dependent manner in mouse liver. Microsomes were isolated from the livers of wild-type and CAR-KO mice treated with saline (vehicle) or PB for 4 days ($n = 3$). A, Western blot analysis was performed to determine the expression levels of Ces6, Cyp2b10, and β-actin protein, respectively. Each lane represents an individual animal. B, the results from A were quantified using scanning densitometry. All of the data are normalized to β-actin levels and represent the average values $\pm$ S.E.M. ($n = 3$) and are expressed as -fold induction over vehicle control. Letters different from each other indicate a statistical difference between treatment groups ($p < 0.05$).

here. Therefore, it is now tempting to speculate that the two different modes of activation by these two CAR-activating compounds are responsible for the distinct CAR-mediated gene activation profiles in liver when compared with that observed in intestine. Alternatively, differences in the bioavailability of these two compounds in vivo after intraperitoneal administration could, in principle, be responsible for the apparent disparate results observed in this study. It is interesting to note that deletion of *Pxr* produced significant increases in the level of *Ces6* gene expression in both duodenum and liver. Although the trend was clearly toward elevated expression, the level of *Cyp3a11* gene expression did not reach statistical significance in duodenum in PXR-KO mice. Nonetheless, these data indicate a likely repressive role for nonliganded PXR protein in duodenum, similar to what was observed for Cyp3a11 in this study (Fig. 4B) and to what has been previously reported by our group in liver tissue (Staudinger et al., 2001a).

It is well established that relatively small increases in gene expression noted using microarray technology can sometimes translate into very big changes in protein levels. This is especially true with respect to genes that encode proteins that participate in drug metabolism pathways. In particular, certain cytochrome P450 genes, as well as those encoding other drug-metabolizing enzymes such as the glutathione *S*-transferase enzymes, are known to be highly regulated at the
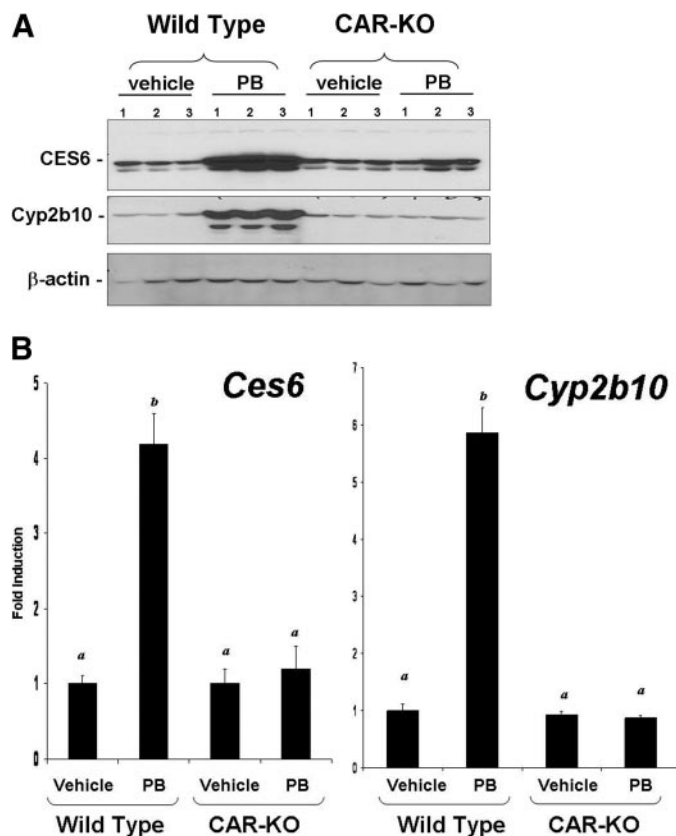
level of transcription. Among the PCN-inducible genes in duodenum reported here, several encode glutathione *S*-transferase enzymes (*Gstm1*, *Gstm3*, *Gstm6*, *Gsta2*, *Gsta4*, *Mgst1*, and *Mgst2*) and drug-metabolizing enzymes Cyp2C (*Cyp2c55* and *Cyp2c29*) and Cyp3A (*Cyp3a25* and *Cyp3a11*) family members, many of which have been previously identified as PXR target genes in liver (Maglich et al., 2002; Rosenfeld et al., 2003). It is interesting to note that expression of the gene encoding epoxide hydrolase was induced in duodenum after treatment with PCN. Epoxide hydrolase is well known to be induced by compounds that produce electrophilic and oxidative stress via the Nrf2-Maf transcription factor complex (Goldring et al., 2004); however, relatively little is known regarding the regulation of this gene by PXR in duodenum. Our observation of significant up-regulation of epoxide hydrolase by PXR agonist treatment in small intestine is in agreement with a recent report that used PXR activators in rat model systems (Hartley et al., 2004). More research will need to be conducted to verify this finding and determine its potential biological relevance to drug metabolism and disposition in mammals in both liver and intestine.

The Abca1 gene product functions as a cholesterol efflux pump in the cellular lipid removal pathway. Mutations in Abca1 have been associated with Tangier's disease and familial high-density lipoprotein deficiency in humans (Tam et al., 2006). It is interesting that our analysis identified Abca1 as a PCN-inducible gene in duodenum
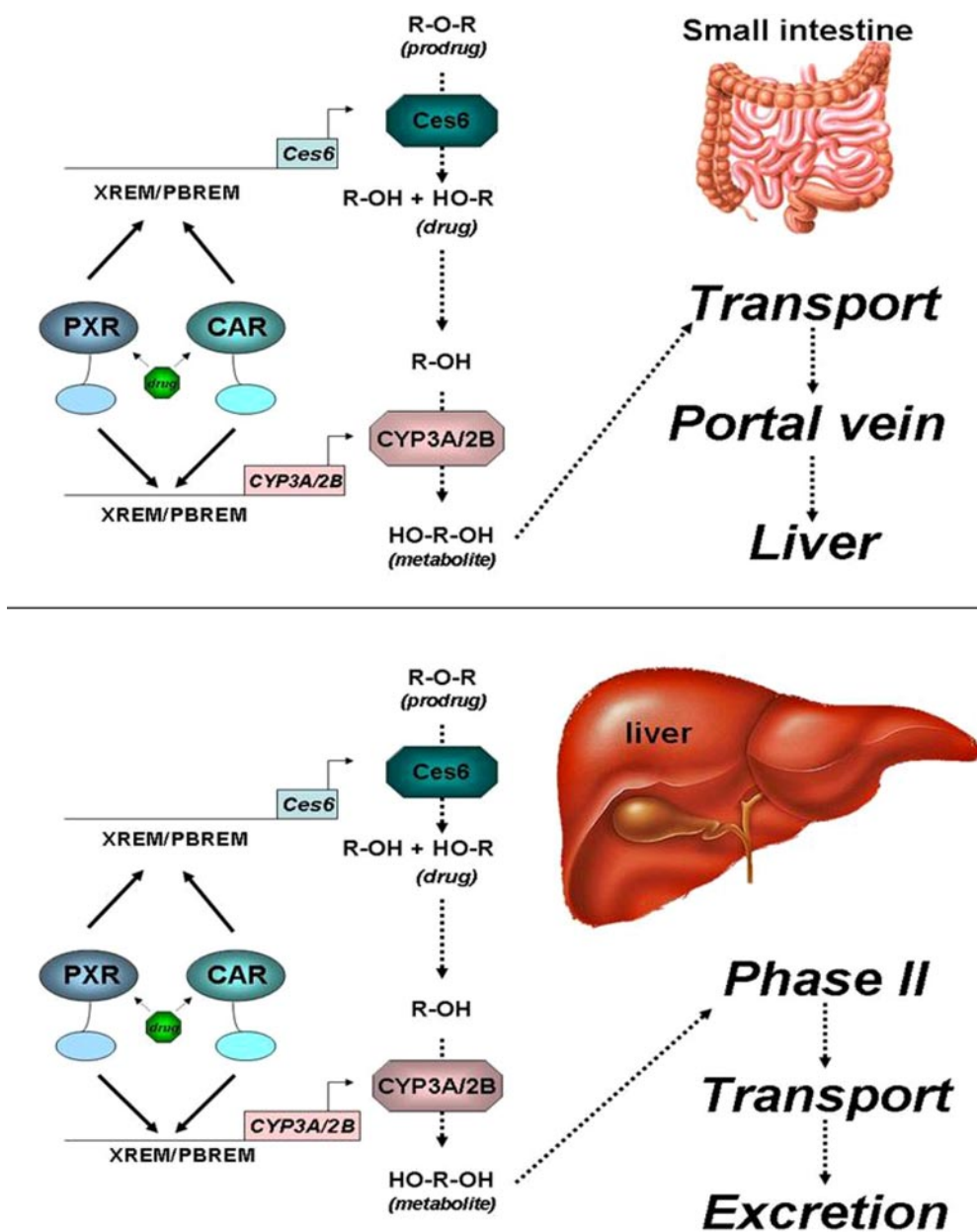
Fig. 9. Model of coordinated PXR- and CAR-mediated gene activation in liver and intestine. Activation of PXR and CAR in intestine produces elevated levels of Ces6 and cytochrome P450 drug-metabolizing activity (top). This would be expected to accelerate conversion of prodrug to active drug and increase uptake into the portal circulation. The liver would then mediate further uptake metabolism and excretion into bile and elimination in feces, or back into blood for eventual elimination through the kidney and in urine (bottom).

because it has previously been identified as down-regulated by PXR agonist treatment in transformed human liver cell lines, as well as in rodent hepatocytes (Sporstøl et al., 2005; de Haan et al., 2009). Still, other studies indicate that Abca1 is up-regulated in small intestine in mice in a PXR-dependent manner (Cheng and Klaassen, 2006). Another study indicates that treatment of intestinal cell lines Caco2 and Ls174T with the PXR agonist rifampicin induces Abca1 expression but does not affect expression of this gene in liver cell lines (Li et al., 2007). It is clear that more research is necessary to determine the molecular basis by which this important cholesterol efflux transporter is regulated differentially in hepatic versus intestinal tissue; however, our data are consistent with others that indicate that this gene is up-regulated in intestine after treatment with PXR agonists.

Our analysis also identified the acyl-CoA-binding protein, or diazepam binding inhibitor, as a PCN-responsive gene. The acyl-CoA-binding protein/diazepam binding inhibitor gene encodes a 10-kDa intracellular protein that specifically binds acyl-CoA esters with high affinity. This small protein is expressed in most cell types at low levels; however, its expression is inducible by metabolic and xenobiotic signals through sterol regulatory element binding protein and peroxisome proliferator-activated receptor (PPAR)-α signaling in hepatocytes, respectively (Sandberg et al., 2005). Our data indicate that PXR also probably regulates the drug-inducible expression of this important gene in small intestine.

Other genes of note up-regulated in mouse intestine after treatment with PCN include 17-β-hydroxysteroid dehydrogenase type 11 (Hsd17b11), a member of the short-chain dehydrogenase/reductase family. The Hsd17b11 gene product is involved in the activation and inactivation of gender steroid hormones in liver and intestine. It is interesting to note that treatment of mice with the potent peroxisome proliferator Wy14,643 induced expression of this gene product in both liver and intestine (Yokoi et al., 2007), presumably through activation of PPARα. However, because several PPARα agonists are also PXR agonists, it is possible that induction of Hsd17b11 gene expression by treatment with Wy14,643 occurs, in part, through activation of PXR by this compound.

Another PCN-inducible gene detected in duodenum by our analysis is pyruvate dehydrogenase kinase, isozyme 4. This gene encodes a member of the PDK protein kinase family that inhibits the pyruvate dehydrogenase complex by phosphorylating one of its subunits. Activation of this gene by glucocorticoid receptor, PPARδ, and farnesoid X receptor agonists contributes to the regulation of glucose metabolism in several tissues (Savkur et al., 2005; Shearer et al., 2008).

The nuclear receptors PXR and CAR were originally identified and characterized as "xenobiotic sensors"; however, more recent research indicates a wider role for these two receptors in regulation of the response to metabolic and nutritional stress (reviewed in Moreau et al., 2008). In any case, taken together our data indicate that activation of PXR target gene expression in intestine regulates the expression of genes involved in modulating drug metabolism, the response to oxidative stress, as well as the disposition of steroids, glucose, and cholesterol homeostasis. Future research should seek to unravel the molecular basis for the differential interaction between nuclear receptor signaling and gene activation pathways in a tissue-selective manner. Additional whole animal studies should be performed to test our model that would include CES activity assays, as well as monitoring prodrug and drug plasma levels after administration of PXR and CAR activators in vivo. Additional studies should be performed using primary hepatocytes and immortalized cell lines to determine whether the signaling pathways investigated here are evolutionarily conserved in humans. Together, these issues represent interesting research opportunities for the future.

References

Cheng X and Klaassen CD (2006) Regulation of mRNA expression of xenobiotic transporters by the pregnane x receptor in mouse liver, kidney, and intestine. *Drug Metab Dispos* **34:**1863–1867.

de Haan W, de Vries-van der Weij J, Mol IM, Hoekstra M, Romijn JA, Jukema JW, Havekes LM, Princen HM, and Rensen PC (2009) PXR agonism decreases plasma hdl levels in apoE3-Leiden.CETP mice. *Biochim Biophys Acta* **1791:**191–197.

Ding X and Staudinger JL (2005) Induction of drug metabolism by forskolin: the role of the pregnane X receptor and the protein kinase a signal transduction pathway. *J Pharmacol Exp Ther* **312:**849–856.

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.

Goetz AK, Bao W, Ren H, Schmid JE, Tully DB, Wood C, Rockett JC, Narotsky MG, Sun G, Lambert GR, et al. (2006) Gene expression profiling in the liver of CD-1 mice to characterize the hepatotoxicity of triazole fungicides. *Toxicol Appl Pharmacol* **215:**274–284.

Goldring CE, Kitteringham NR, Elsby R, Randle LE, Clement YN, Williams DP, McMahon M, Hayes JD, Itoh K, Yamamoto M, et al. (2004) Activation of hepatic Nrf2 in vivo by acetaminophen in CD-1 mice. *Hepatology* **39:**1267–1276.

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.

Goodwin B, Redinbo MR, and Kliewer SA (2002) Regulation of cyp3a gene transcription by the pregnane x receptor. *Annu Rev Pharmacol Toxicol* **42:**1–23.

Hartley DP, Dai X, He YD, Carlini EJ, Wang B, Huskey SE, Ulrich RG, Rushmore TH, Evers R, and Evans DC (2004) Activators of the rat pregnane X receptor differentially modulate hepatic and intestinal gene expression. *Mol Pharmacol* **65:**1159–1171.

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.

Hosokawa M, Maki T, and Satoh T (1987) Multiplicity and regulation of hepatic microsomal carboxylesterases in rats. *Mol Pharmacol* **31:**579–584.

Humerickhouse R, Lohrbach K, Li L, Bosron WF, and Dolan ME (2000) Characterization of CPT-11 hydrolysis by human liver carboxylesterase isoforms hCE-1 and hCE-2. *Cancer Res* **60:**1189–1192.

Institute of Laboratory Animal Resources (1996) *Guide for the Care and Use of Laboratory Animals* 7th ed. Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, Washington DC.

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.

Li T, Chen W, and Chiang JY (2007) PXR induces CYP27A1 and regulates cholesterol metabolism in the intestine. *J Lipid Res* **48:**373–384.

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.

Masuyama H, Hiramatsu Y, Mizutani Y, Inoshita H, and Kudo T (2001) The expression of pregnane X receptor and its target gene, cytochrome P450 3A1, in perinatal mouse. *Mol Cell Endocrinol* **172:**47–56.

Matheny CJ, Ali RY, Yang X, and Pollack GM (2004) Effect of prototypical inducing agents on P-glycoprotein and CYP3A expression in mouse tissues. *Drug Metab Dispos* **32:**1008–1014.

Moore LB, Parks DJ, Jones SA, Bledsoe RK, Consler TG, Stimmel JB, Goodwin B, Liddle C, Blanchard SG, Willson TM, et al. (2000) Orphan nuclear receptors constitutive androstane receptor and pregnane X receptor share xenobiotic and steroid ligands. *J Biol Chem* **275:**15122–15127.

Moreau A, Vilarem MJ, Maurel P, and Pascussi JM (2008) Xenoreceptors CAR and PXR activation and consequences on lipid metabolism, glucose homeostasis, and inflammatory response. *Mol Pharm* **5:**35–41.

Pindel EV, Kedishvili NY, Abraham TL, Brzezinski MR, Zhang J, Dean RA, and Bosron WF (1997) Purification and cloning of a broad substrate specificity human liver carboxylesterase that catalyzes the hydrolysis of cocaine and heroin. *J Biol Chem* **272:**14769–14775.

Rosenfeld JM, Vargas R Jr, Xie W, and Evans RM (2003) Genetic profiling defines the xenobiotic gene network controlled by the nuclear receptor pregnane X receptor. *Mol Endocrinol* **17:**1268–1282.

Sandberg MB, Bloksgaard M, Duran-Sandoval D, Duval C, Staels B, and Mandrup S (2005) The gene encoding acyl-CoA-binding protein is subject to metabolic regulation by both sterol regulatory element-binding protein and peroxisome proliferator-activated receptor alpha in hepatocytes. *J Biol Chem* **280:**5258–5266.

Satoh T and Hosokawa M (1998) The mammalian carboxylesterases: from molecules to functions. *Annu Rev Pharmacol Toxicol* **38:**257–288.

Savkur RS, Bramlett KS, Michael LF, and Burris TP (2005) Regulation of pyruvate dehydrogenase kinase expression by the farnesoid X receptor. *Biochem Biophys Res Commun* **329:**391–396.

Shearer BG, Patel HS, Billin AN, Way JM, Winegar DA, Lambert MH, Xu RX, Leesnitzer LM, Merrihew RV, Huet S, et al. (2008) Discovery of a novel class of PPARdelta partial agonists. *Bioorg Med Chem Lett* **18:**5018–5022.

Shi D, Yang J, Yang D, and Yan B (2008) Dexamethasone suppresses the expression of multiple rat carboxylesterases through transcriptional repression: evidence for an involvement of the glucocorticoid receptor. *Toxicology* **254:**97–105.

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 U S A* **99:**13801–13806.

Sporstøl M, Tapia G, Malerød L, Mousavi SA, and Berg T (2005) Pregnane X receptor-agonists down-regulate hepatic ATP-binding cassette transporter A1 and scavenger receptor class B type I. *Biochem Biophys Res Commun* **331:**1533–1541.

Staudinger J, Liu Y, Madan A, Habeebu S, and Klaassen CD (2001a) 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. (2001b) The nuclear receptor PXR is a lithocholic acid sensor that protects against liver toxicity. *Proc Natl Acad Sci U S A* **98:**3369–3374.

Staudinger JL, Madan A, Carol KM, and Parkinson A (2003) Regulation of drug transporter gene expression by nuclear receptors. *Drug Metab Dispos* **31:**523–527.

Stok JE, Huang H, Jones PD, Wheelock CE, Morisseau C, and Hammock BD (2004) Identification, expression, and purification of a pyrethroid-hydrolyzing carboxylesterase from mouse liver microsomes. *J Biol Chem* **279:**29863–29869.

Takai S, Matsuda A, Usami Y, Adachi T, Sugiyama T, Katagiri Y, Tatematsu M, and Hirano K (1997) Hydrolytic profile for ester- or amide-linkage by carboxylesterases pI 5.3 and 4.5 from human liver. *Biol Pharm Bull* **20:**869–873.

Tam SP, Mok L, Chimini G, Vasa M, and Deeley RG (2006) ABCA1 mediates high-affinity uptake of 25-hydroxycholesterol by membrane vesicles and rapid efflux of oxysterol by intact cells. *Am J Physiol Cell Physiol* **291:**C490–C502.

Tully DB, Bao W, Goetz AK, Blystone CR, Ren H, Schmid JE, Strader LF, Wood CR, Best DS, Narotsky MG, et al. (2006) Gene expression profiling in liver and testis of rats to characterize the toxicity of triazole fungicides. *Toxicol Appl Pharmacol* **215:**260–273.

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.

Wei P, Zhang J, Dowhan DH, Han Y, and Moore DD (2002) Specific and overlapping functions of the nuclear hormone receptors CAR and PXR in xenobiotic response. *Pharmacogenomics J* **2:**117–126.

Xie W, Barwick JL, Simon CM, Pierce AM, Safe S, Blumberg B, Guzelian PS, and Evans RM (2000) Reciprocal activation of xenobiotic response genes by nuclear receptors SXR/PXR and CAR. *Genes Dev* **14:**3014–3023.

Yang J and Yan B (2007) Photochemotherapeutic agent 8-methoxypsoralen induces cytochrome P450 3A4 and carboxylesterase HCE2: evidence on an involvement of the pregnane X receptor. *Toxicol Sci* **95:**13–22.

Yokoi Y, Horiguchi Y, Araki M, and Motojima K (2007) Regulated expression by PPARalpha and unique localization of 17beta-hydroxysteroid dehydrogenase type 11 protein in mouse intestine and liver. *FEBS J* **274:**4837–4847.

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