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REGULATION OF MOUSE ORGANIC ANION TRANSPORTING POLYPEPTIDES (Oatps) IN LIVER BY PROTOTYPICAL MICROSOMAL ENZYME INDUCERS THAT ACTIVATE DISTINCT TRANSCRIPTION FACTOR PATHWAYS

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Running Title Page:

Running title: Chemical regulation of mouse Oatps

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Abstract

Drug metabolizing enzymes and transporters are key factors that affect disposition of xenobiotics. Phase-I enzyme induction by classes of microsomal enzyme inducers occurs via activation of transcription factors such as AhR, CAR, PXR, PPARα, and Nrf2. However, regulation of Oatp uptake transporters by these factors is poorly understood. Hepatic Oatp uptake of some chemicals must occur prior to biotransformation, thus we hypothesize that expression of Oatps and biotransformation enzymes is coordinately regulated in liver. In the present study, the effects of known chemical activators of AhR, CAR, PXR, PPARα, and Nrf2 on the hepatic mRNA expression of mouse Oatps and drug metabolizing enzymes were quantified by the branched DNA assay. All chemicals increased the expression of their well-characterized target drug metabolizing enzymes: AhR ligands increased Cyp1A1; CAR activators increased Cyp2B10; PXR ligands increased Cyp3A11; PPARα ligands increased Cyp4A14; and Nrf2 activators induced NAD(P)H:quinone oxidoreductase (Nqo)1. AhR ligands (TCDD, polychlorinated biphenyl 126, and β-naphthoflavone) increased Oatp2b1 and 3a1 mRNA expression in liver. CAR activators (phenobarbital, TCPOBOP, and diallyl sulfide) decreased Oatp1a1 mRNA expression. Two PXR ligands (PCN and spironolactone) increased Oatp1a4 mRNA expression in liver, whereas PXR ligands (PCN, spironolactone, and dexamethasone) and PPARα ligands (clofibrate, ciprofibrate, and diethylhexylphthalate) decreased Oatp1a1, 1b2, 2a1, and 2b1 mRNA expression in liver. Nrf2 activators (oltipraz, ethoxyquin, and butylated hydroxyanisole) down-regulated Oatp1a1, but up-regulated Oatp2b1 mRNA expression. Therefore, only a few transcription factor activators increased Oatp expression, and surprisingly, many decreased Oatp expression.

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Introduction

Induction of phase-I enzymes by microsomal enzyme inducers (MEIs) can be attributed to activation of signal-transduction pathways. Many MEIs exert their effects through activation of various orphan nuclear receptors, subsequently enhancing target gene transcription (Sonoda et al., 2003; Denison et al., 2003). For example, TCDD binds to the aryl hydrocarbon receptor (AhR), which subsequently releases the AhR from the cytosolic tethering protein HSP90, allowing for AhR translocation to the nucleus, heterodimerization with ARNT, and binding to xenobiotic response elements in the Cyp1A1 promoter. Similarly, constitutive androstane receptor (CAR) ligands induce Cyp2B10, pregnane-X receptor (PXR) ligands induce Cyp3A11, and peroxisome proliferator-activated receptor α (PPARα) ligands induce Cyp4A14. The NF-E2 related factor 2 (Nrf2) pathway is more complex, where both antioxidants and oxidants can either alter the tethering protein Keap1 by sulfhydryl modifications, or alter the cellular oxidative state in such a way to allow Nrf2 to be released, allowing for subsequent translocation of Nrf2 to the nucleus, heterodimerization with small Maf proteins, and binding to EpRE/ARE in the promoter regions of genes, such as NAD(P)H quinone oxidoreductase 1 (Nqo1) (Jaiswal et al., 2000).

Examples of receptor-mediated regulation of Oatp transporters have also been reported in the literature, such as the induction of Oatp1a4, via PXR activation and subsequent binding to the 5´-flanking region of the rat *Oatp1a4* gene (Guo et al., 2002b). Similar observations in wildtype mice, but not in PXR-null mice have also been reported (Staudinger et al., 2001; Sonoda et al., 2002). Other observations, such as the decrease in Oatp1a4 expression in rats after treatment with the AhR ligand polychlorinated biphenyl (PCB)126, suggest a receptor-mediated mechanism (Guo et al., 2002a).

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Hepatic uptake must occur for some chemicals prior to undergoing biotransformation. Oatp uptake transporters function as an important part of the "first-pass" extraction of chemicals from systemic circulation into liver. Therefore, the potential for co-regulation of drug metabolizing enzymes and transporters exists because uptake and biotransformation of drugs could theoretically occur by a coordinated receptor activation mechanism. One such example exists with the PXR ligand pregnenolone-16α-carbonitrile (PCN), which markedly enhances both plasma disappearance and biliary excretion of cardiac glycosides in rats (Klaassen, 1974). Recently, the uptake transporter Oatp1a4, which transports cardiac glycosides with high affinity (Noé et al., 1997), has been shown to be induced by PCN in rats (Rausch-Derra et al., 2001; Guo et al., 2002). Cardiac glycosides are taken up into the liver by Oatp1a4 in rodents, biotransformed by Cyp3A and UDP-glucuronosyltransferases (UGTs), and excreted into bile via the canalicular transporter Mdr1. Activation of PXR by PCN leads to coordinate induction of Oatp1a4, Cyp3A, and UGTs, as well as biliary excretion of cardiac glycosides (Klaassen, 1974; Rausch-Derra et al., 2001; Guo et al., 2002; Chen et al., 2003). Another example of coordinate regulation of transporters and drug metabolizing enzymes is with phenobarbital (PB), a constitutive androstane receptor (CAR) activator. PB increases the plasma clearance of the Oatp substrate bilirubin (Klaassen, 1970), thus implicating an uptake transporter, and furthermore induces bilirubin UDP-glucuronosyltransferase UGT1A1 in liver (Ritter et al., 1999).

There is limited information linking Oatp transporter regulation with phase-I enzymes via transcription factor activation. Therefore, we hypothesize that the expression of drug-metabolizing enzymes and Oatps might be coordinately regulated in liver. In the present study, we investigate the effect of MEIs with known mechanisms of induction on mouse Oatp

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expression, in order to determine whether there is a correlation in the regulation of drug metabolizing enzymes with Oatps in mouse liver.

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Materials and Methods

Chemicals. 2, 3, 7, 8-Tetrachlorodibenzo-*p*-dioxin (TCDD) was a gift from Dr. Karl Rozman (University of Kansas Medical Center, KS). Oltipraz was a gift from Dr. Steven Safe (Texas A&M University, TX). Polychlorinated biphenyl 126 (PCB 126) was obtained from AccuStandard (New Haven, CT). All other chemicals, unless otherwise indicated, were purchased from Sigma-Aldrich Co. (St. Louis, MO).

Animals and Treatments. Eight-week-old male C57BL/6 mice were purchased from Jackson laboratories (Bar Harbor, Maine). Groups of five mice were administered one of the following chemicals once daily for 4 days: AhR ligands: TCDD (40 μg/kg, ip in corn oil), β-naphthoflavone (BNF, 200 mg/kg, ip in corn oil), and PCB126 (300 μg/kg, po in corn oil); CAR activators: phenobarbital (PB, 100 mg/kg, ip in saline), TCPOBOP (3 mg/kg, ip in corn oil), and diallyl sulfide (DAS, 200 mg/kg, ip in corn oil); PXR ligands: pregnenolone-16α-carbonitrile (PCN, 200 mg/kg, ip in corn oil), spironolactone (SPR, 200 mg/kg, ip in corn oil), and dexamethasone (DEX, 75 mg/kg, ip in corn oil); PPARα ligands: clofibric acid (CLFB, 500 mg/kg, ip in saline), ciprofibrate (CPFB, 40 mg/kg, ip in saline), and diethylhexylphthalate (DEHP, 1000 mg/kg, po in corn oil); Nrf2 activators: butylated hydroxyanisole (BHA, 350 mg/kg, ip in corn oil), ethoxyquin (ETHOXYQ, 250 mg/kg, po in corn oil), and oltipraz (OPZ, 150 mg/kg, po in corn oil). Four different vehicle control groups (corn oil by ip, corn oil by po, saline by ip, and saline by po) were used. No statistical difference between these groups was observed, thus these groups were averaged together as a single vehicle control group. All injections were administered in a volume of 10 ml/kg. Livers were removed on day 5, snap-frozen in liquid nitrogen, and stored at -80°C.

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RNA Isolation. Total RNA was isolated using RNAzol Bee® reagent (TelTest Inc., Friendswood, TX) per the manufacturer's protocol. The concentration of total RNA in each sample was quantified spectrophotometrically at 260 nm. The integrity of each RNA sample was evaluated by formaldehyde-agarose gel electrophoresis before analysis.

Branched DNA (bDNA) Signal Amplification Assay. The mRNA for mouse Oatps (Oatp1a1, 1a4, 1a5, 1a6, 1b2, 1c1, 2a1, 2b1, 3a1, and 4a1) and 5 drug metabolizing enzymes (Cyp1A1, Cyp2B10, Cyp3A11, Cyp4A14, and Nqo1) was quantified using the bDNA assay (Quantigene® bDNA signal amplification kit; Bayer Diagnostics, East Walpole, MA), with modifications according to Hartley and Klaassen (2000). The gene sequences of mouse Oatps and biotransformation enzymes were accessed from GenBank. Multiple oligonucleotide probe sets (containing capture probes, label probes, and blocker probes) specific to a single mRNA transcript were designed using ProbeDesigner® software, version 1.0 (Bayer Corp., Emeryville, CA). Every probe developed in ProbeDesigner® was submitted to the National Center for Biotechnology Information for nucleotide comparison by the basic local alignment search tool (BLASTn; NCBI, Bethesda, MD) to ensure minimal cross-reactivity with other known mouse sequences and expressed sequence tags. Oligonucleotides with a high degree of similarity (>80%) to other mouse gene transcripts were eliminated from the design. Probes were designed with a melting temperature of approximately 63°C, enabling hybridization conditions to be held constant (i.e., 53°C) during each hybridization step and for each probe set. The probesets for mouse Oatps are published presently (Cheng et al., 2005). Probesets for mouse cytochrome P450s (Cyps) and Nqo1 are shown in Table 2. All probes were synthesized (i.e., 50-nmol synthesis scale) by Operon Technologies (Palo Alto, CA), and obtained desalted and lyophilized. Total RNA (1 µg/µl) was added to each well (10 µl/well) of a 96-well plate

containing 50 µl of capture hybridization buffer and 50 µl of each diluted probe set. For each gene, total RNA was allowed to hybridize to the probe set overnight at 53°C. Subsequent hybridization steps were carried out as per the manufacturer's protocol, and luminescence was quantified with a Quantiplex® 320 bDNA luminometer interfaced with Quantiplex® data management software, version 5.02, for analysis of luminescence from 96-well plates. The luminescence for each well is reported as relative light units (RLUs) per 10 µg total RNA.

Statistics. The data are expressed as mean ± S.E. Statistical significance was determined by one-way analysis of variance, followed by Duncan's post hoc test. Statistical significance was set at $P < 0.05$.

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Results

Effects of the Prototypical Microsomal Enzyme Inducers on the mRNA Levels of Mouse Biotransformation Enzymes.

Male C57BL/6 mice were dosed with five classes of prototypical drug-metabolizing enzyme inducers. Each class contains three chemicals that are known to transcriptionally activate a specific signaling pathway. The doses were chosen according to what is commonly cited in the literature to activate specific transcription factors that target specific biotransformation enzymes (Cyp1A1, Cyp2B10, Cyp3A11, Cyp4A14, and Nqo1). To confirm that the doses selected were effective, the mRNA levels of these marker genes were quantified (Fig. 1). The mRNA levels of Cyp1A1 were increased by treatment with the AhR ligands TCDD (1230-fold), BNF (1230-fold), and PCB 126 (1170-fold). The mRNA levels of Cyp2B10 were increased by the CAR activators (PB, 15-fold; TCPOBOP, 150-fold; DAS, 22-fold). The mRNA levels of Cyp3A11 were increased by the PXR ligands (PCN, 5-fold; SPR, 5-fold; and DEX, 5-fold). The mRNA levels of Cyp4A14 were increased by the PPARα ligands (CLFB, 22-fold; CPFB, 93-fold; and DEHP, 87-fold). The mRNA levels of Nqo1 were increased by Nrf2 activators (BHA, 3-fold; ETHOXYQ, 5-fold; and OPZ, 2-fold).

In addition to the well-characterized induction profile of drug-metabolizing enzymes by the five classes of MEIs, as shown in Fig. 1, there exists some non-specificity of the transcription factor activators, as some of the chemicals administered induced the expression of genes in addition to their known target gene. As shown in Fig. 1, AhR ligands markedly induced Cyp1A1 as predicted, but these compounds as a class also induced Cyp3A11 and Nqo1 mRNA expression. CAR activators up-regulated not only Cyp2B10, but also Cyp3A11 and Nqo1 expression. PXR ligands were relatively specific in that they only induced Cyp3A11, with the exception of DEX which also induced Cyp2B10. PPARα ligands were

also relatively specific in that they primarily induced Cyp4A14, except DEHP, which also induced Cyp3A11 expression. Nrf2 activators mediated not only Nqo1 induction, but also Cyp3A11 induction, and ETHOXYQ increased Cyp2B10. Most microsomal enzyme inducers increased Cyp3A11.

Effects of the Prototypical Microsomal Enzyme Inducers on the mRNA Levels of Mouse Oatps.

The regulation of mouse Oatps by microsomal enzyme inducers is shown in Figs 2, 3, and 4. The effects of five classes of transcriptional activators on mouse Oatp1a1 mRNA expression are shown in Fig. 2. CAR activators, PXR ligands, and PPARα ligands, and the majority of AhR activators and Nrf2 activators decreased Oatp1a1 expression. Thus Oatp1a1 mRNA expression was decreased by all five classes of transcriptional activators tested.

Oatp1a4 is a sinusoidal uptake transporter that is known for transporting digoxin with high affinity (Noé et al, 1997). Mouse Oatp1a4 is up-regulated by PCN treatment (Staudinger et al, 2001), which was also observed in the present study. Another PXR ligand, spironolactone, induced Oatp1a4 mRNA expression approximately two fold. The Nrf2 activator BHA also induced Oatp1a4 mRNA about two fold. Otherwise, Oatp1a4 mRNA was not significantly altered by treatment with any of the AhR, CAR, or PPARα activators (Fig. 2).

Regulation of Oatp1a5 in liver by classes of microsomal enzyme inducers is shown in Fig. 2. The expression of Oatp1a5 is minimal in liver, which has been reported previously (Choudhuri et al., 2003). The majority of CAR and PXR ligands decreased Oatp1a5 mRNA levels.

Like Oatp1a5, Oatp1a6 is minimally expressed in liver. Regulation of Oatp1a6 by the microsomal enzyme inducers is shown in Fig. 2. There was no significant alteration of

Oatp1a6 expression following the treatment with any class of compound except for PPARα activators, which slightly decreased Oatp1a6.

Regulation of mouse Oatp1b2, a liver-predominant Oatp, is shown in Fig. 3. Both PXR and PPARα ligands decreased Oatp1b2 mRNA expression. The AhR, CAR, and Nrf2 activators did not alter Oatp1b2 mRNA expression as class. Individually, the AhR ligand PCB126, and the Nrf2 activator OPZ increased Oatp1b2 mRNA expression, whereas the AhR ligand TCDD and the CAR activator DAS decreased Oatp1b2 expression.

Regulation of Oatp1c1, which is minimally expressed in liver, by the 5 classes of transcription factor activators, is shown in Fig. 3. None of the five classes of chemicals altered Oatp1c1 expression.

Chemical regulation of Oatp2a1, a prostaglandin transporter, is shown in Fig. 3. Both PXR and PPARα ligands caused a class-wide decrease in Oatp2a1 mRNA expression. Furthermore, an AhR ligand, BNF, and an Nrf2 activator, BHA, increased Oatp2a1 mRNA expression, but other AhR and Nrf2 activators did not alter Oatp2a1 mRNA expression.

Oatp2b1 is also a liver-predominant Oatp. As shown in Fig. 4, expression of Oatp2b1 was decreased by PXR and PPARα ligands, and increased by AhR and Nrf2 activators. Two CAR activators, PB and DAS, decreased Oatp2b1 mRNA expression. However, TCPOBOP, a third CAR activator, increased Oatp2b1 mRNA expression.

Oatp3a1 and 4a1 are minimally expressed in liver. Regulation of these two transporters by the five classes of transcription factor activators is shown in Fig. 4. All AhR ligands increased Oatp3a1 mRNA expression, whereas PXR ligands decreased Oatp3a1 mRNA expression. As also shown in Fig. 4, none of the five classes of ligands (AhR ligands, CAR activators, PXR ligands, PPARα ligands, and Nrf2 activators) caused a class-specific

alteration of Oatp4a1 expression. Because of the minimal expression of Oatp3a1 and 4a1 in liver, any change in the function of these transporters associated with alterations in mRNA expression may be biologically irrelevant.

Discussion

Virtually all chemicals were once believed to enter the liver through passive diffusion; however, an emerging role for the Oatp family in hepatic uptake suggests a more important role for transporters than once believed. In vivo, uptake transporters such as OATPs/Oatps are necessary for some chemicals to cross the membrane in order to access phase-I and -II enzymes. However, in vitro assays such as microsomal preparations allow for direct access of a substrate to these microsomal enzymes. Therefore, regulation of uptake transport by microsomal enzyme inducers may lead to increased availability of drugs and chemicals for metabolism. Therefore, integration of the regulation of the four processes (uptake, phase-I metabolism, phase-II metabolism, and efflux) may increase the efficacy of detoxication. This study examines the potential of transcription factors that activate phase-I drug metabolism to also affect the regulation of Oatp hepatic uptake transporters.

Phase-I drug metabolizing enzymes often have low expression in naïve liver, but can be induced tens to hundreds of fold after exposure to chemicals that activate specific transcription factors. Except for Cyp3A11, the basal expression of phase-I enzymes examined in this study (Cyp1A1, Cyp2B10, Cyp4A14, and Nqo1) is very low in liver. However, AhR ligands (TCDD, BNF, and PCB126) induced Cyp1A1 over 1,000 fold, CAR activators produced 20-times more Cyp2B10 mRNA, and PPARα ligands increased Cyp4A14 mRNA 120 fold (Fig 1). The most robust up-regulation of these cytochrome P-450s, such as the induction of Cyp1a1 after TCDD exposure, has been extensively examined (Jaiswal et al, 1985**;** Jones et al. 1986). Furthermore, transcriptional up-regulation of Cyp1A1 seems to be mono-specific, as Cyp1A1 is almost exclusively regulated by AhR. However, more recent studies have demonstrated that some phase-I enzymes, such as Cyp3A11 and Nqo1, may be

regulated by several transcription factor activators, as observed in the present work. Increases in Cyp3A4 expression after exposure to inducers are thought to occur primarily through PXR activation, and subsequent binding to ER-6 response elements in the 5' flanking regions of Cyp3A4 (Xie et al., 2000; Lehmann et al., 1998). In humans, exposure to rifampicin or RU486 can evoke a similar increase in Cyp3A4 via PXR activation, whereas in rodents, Cyp3A11 is up-regulated by chemicals such as PCN and DEX (Lehmann et al, 1998; Staudinger et al, 2001). However, the expression of Cyp3A4 can also be markedly increased after treatment with PB or DEX (Fig 1) (Burk et al., 2004; Zhang et al., 2004). Known up-regulation of Cyp3A after activation of CAR or the glucocorticoid receptor (Xie et al, 2000; Pascussi et al, 2001) demonstrates that multiple receptors can regulate Cyp3A11 by different classes of chemicals, such as CAR activators (PB, TCPOBOP) and Nrf2 activators (OPZ, and ETHOXYQ). However, the induction of Cyp3A11 by AhR ligands and Nrf2 activators suggest a novel mechanism of Cyp3A11 regulation (Fig 1). Overlap of Nrf2/AhR activators has been observed previously for other phase-I and II enzymes, including UGT1A6 by oltipraz, and induction of Nqo1 by TCDD (Auyeung et al, 2003; Ma et al, 2004). It has been suggested that oxidative stress can induce CYP3A4 expression in the human erythroleukemia cell line K562 (Negai et al, 2004). The protective role of Nrf2 in oxidative stress has been well established, and these results, when taken together, may suggest a role for Nrf2 in regulation of Cyp3A11.

Another novel observation from this study is that TCPOBOP, an efficacious CAR activator, and DAS, a garlic component, up-regulate Nqo1 mRNA expression (Fig 1). The mechanism of TCPOBOP and DAS induction of Nqo1 is not known, but it could be due to generation of reactive oxidants by the increased amount of Cyp2B. A precedent for this

possibility is the induction of Nqo1 by TCDD. Nrf2 is an important regulator of Nqo1 expression, but Nqo1 can also be up-regulated in response to AhR ligands such as TCDD (Jaiswal, 1991; Marchand et al., 2004). Recent studies have shown that chemicals with strong AhR ligand binding, such as TCDD, induce Nqo1 via Nrf2 (Ma et al., 2004). Nqo1 induction is thought to be a result of increased Cyp1A1 activity, which leads to an increased production of oxidative metabolites. These reactive species activate Nrf2, which ultimately up-regulate Nqo1. Therefore, CAR activators may also indirectly cause oxidative stress by up-regulation of phase I enzymes, and this generation of oxidative stress may ultimately increase expression of Nrf2 target genes (Fig 1)

Information on chemical regulation of Oatp transporters is limited, but there are some reports on the regulation of Oatps after exposure to various challenges. For example, PCN treatment increases hepatic Oatp1a4 mRNA expression in rats (Rausch-Derra et al., 2001; Guo et al., 2002), and in mice (Staudinger et al., 2001). Lipopolysaccharide dramatically decreases Oatp1a1, 1a4, and 1b2 mRNA expression in rats (Cherrington et al., 2004) and Oatp1b2 in mice (Li et al., 2004). Cholic acid, an Oatp substrate, increases Oatp1a4, but decreases Oatp1b2 protein expression in rats (Rost et al., 2003). Carbon tetrachloride decreases mRNA expression of Oatp1a1 and 1a4 in rats and mice, but not 1b2 (Geier et al., 2002; Aleksunes et al., 2005). Thus, reports on Oatp regulation are few, with the data predominately from rats. The present study provides a systematic evaluation of the effects of MEIs on Oatp expression in mice. The effects of altered expression of Oatp transporters will ultimately aid in predicting drug-drug interactions at the level of hepatic uptake, whereas therapeutic modulation of Oatp expression by chemicals may also be clinically beneficial in hepatic disease states such as cholestasis or fibrosis.

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Oatp transporters, in general, are not up-regulated by microsomal enzyme inducers as are the Cyps, and more often are decreased than increased. The AhR class of ligands up-regulated Oatp2b1 and 3a1 expression to a modest extent (Figs 3 and 4). CAR ligands down-regulated Oatp1a1 and 1a5 mRNA expression, and slightly down-regulated Oatp2b1 and Oatp1b2 (Figs 2, 3, and 4). PXR ligands altered the expression of several Oatps, including down-regulation of Oatp1a1, 1a5, 1b2, 2a1, 2b1, and 3a1, although the decreases in expression were modest (Figs 2, 3, and 4). Conversely, the PXR ligands induced expression of Oatp1a4. PPARα activators also decreased Oatp1a1, 1a6, 1b2, 2a1, and 2b1 mRNA expression (Fig 2, 3, and 4). Finally, the majority of Nrf2 activators decreased Oatp1a1 mRNA expression (Fig 2), whereas, only Oatp2b1 was increased by Nrf2 class of activators (Fig 4).

Increased uptake and biotransformation can either enhance the elimination and detoxication, or enhance the hepatotoxicity of chemicals (Nozawa et al., 2004). For example microcystin and phalloidin, two Oatp substrates, cause hepatotoxicity after accumulating in the liver (Hagenbuch and Meier, 2003). Conversely, pretreatment of rats with PCN or SPR (PXR ligands) decreases the toxicity of digoxin by increasing hepatic uptake and biotransformation, via PXR activation and subsequent up-regulation of Oatp1a4 and Cyp3A11. Similarly, induction of Oatp1A4 and Cyp3A11, and down-regulation of Cyp7A1 have been observed after PXR activation by lithocholic acid. This mechanism is believed to occur as a feedback pathway to protect the liver from bile acid toxicity by decreasing bile acid synthesis, and increasing uptake and metabolism of hydrophobic bile acids by increasing Oatp1a4 and Cyp3A11 levels (Staudinger et al, 2001).

In summary, the present study examines expression of some phase-I enzymes known to be regulated by specific transcription factors, in parallel with Oatp expression. The induction

profile of biotransformation enzymes by these prototypical MEIs was generally similar to that reported in the literature, however, some novel observations were observed in this study, including the induction of: Cyp3A11 by AhR and Nrf2 activators, Cyp2B10 by ETHOXYQ, and Nqo1 by CAR activators. In general, the MEIs used in this study induced their corresponding phase-I enzyme to a far greater extent than any up-regulation observed with the Oatp family members. Furthermore, no significant down-regulation was observed with the phase-I enzymes, whereas several of the Oatps were down-regulated after MEI exposure. The Oatps were generally not induced by chemical activators after treatment. However, down-regulation in hepatic Oatp expression, especially of the highly expressed Oatp1a1 and 1b2, was observed after exposure to a variety of compounds. This could suggest that a hepato-protective mechanism may exist to protect against further hepatic uptake by Oatp transporters after MEI exposure. Down-regulation of Oatps would result in decreased uptake of substrates into liver, thus decreasing the chemical challenge to the liver and serving to protect hepatocytes from chemical toxicity. Thus, the effects of MEI exposure on phase-I enzyme regulation are generally opposite to the effects on Oatp expression, with the notable exception of Cyp3A11 and Oatp1a4, which are up-regulated in parallel after PXR activation.

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Footnotes:

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Legends for Figures:

Fig. 1 mRNA expression of Cyp1A1, Cyp2B10, Cyp3A11, Cyp4A14, NAD(P)H: quinone oxidoreductase (Nqo) 1 in C57BL/6 mouse liver after administration of prototypical phase-I and phase-II drug-metabolizing enzyme inducers: 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), β-naphthoflavone (BNF), polychlorinated biphenyl (PCB) 126, phenobarbital (PB), 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP), diallyl sulfide (DAS), pregnenolone 16α-carbonitrile (PCN), spironolactone (SPR), dexamethasone (DEX), clofibrate (CLFB), ciprofibrate (CPFB), diethylhexylphthalate (DEHP), butylated hydroxyanisole (BHA), ethoxyquin (ETHOXYQ), and oltipraz (OPZ). Total RNA from five chemically treated male livers was analyzed by the bDNA assay. All data were expressed as mean ± S.E. for five animals in each group, except for control groups, which were combined from the four individual control groups after it was determined that they were not statistically different. Asterisk indicates statistically significant difference between treated and control mice ($p<0.05$).

Fig. 2 Expression of Oatp1a1, 1a4, 1a5, and 1a6 mRNA in liver after treatment with prototypical drug-metabolizing enzyme inducers. The abbreviations used are the same as in Fig. 1. Total RNA from liver of chemically treated male C57BL/6 mice (n = 5/treatment) was analyzed by the bDNA assay. All data were expressed as mean ± S.E. for five animals in each group, except for control groups, which were combined from the four individual control groups after it was determined that they were not statistically different. Asterisk indicates statistically significant difference between treated and control mice ($p<0.05$).

Fig. 3 Expression of Oatp1b2 (top), 1c1 (middle) and 2a1 (bottom) mRNA in liver after treatment with prototypical drug-metabolizing enzyme inducers. The abbreviations used are the same as in Fig. 1. Total RNA from liver of chemically treated male C57BL/6 mice (n =

5/treatment) was analyzed by the bDNA assay. All data were expressed as mean ± S.E. for five animals in each group, except for control groups, which were combined from the four individual control groups after it was determined that they were not statistically different. Asterisk indicates statistically significant difference between treated and control mice ($p<0.05$).

Fig. 4 Expression of Oatp2b1 (top), 3a1 (middle), and 4a1 (bottom) mRNA in liver after treatment with prototypical drug-metabolizing enzyme inducers. The abbreviations used are the same as in Fig. 1. Total RNA from liver of chemically treated male C57BL/6 mice (n = 5/treatment) was analyzed by the bDNA assay. All data were expressed as mean ± S.E. for five animals in each group, except for control groups, which were combined from the four individual control groups after it was determined that they were not statistically different. Asterisk indicates statistically significant difference between treated and control mice ($p<0.05$).

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Table 1. Summary of the regulation of mouse biotransformation enzymes and Oatps in liver after treatment with prototypical activators of transcription factor signaling pathways

	AhR ligands	CAR activators	PXR ligands	PPARα ligands	Nrf2 activators
Cyp1A1	+(3/3)	0	0	0	0
Cyp2B10	0	+(3/3)	0	0	0
Cyp3A11	+(2/3)	+(3/3)	+(3/3)	0	+(3/3)
Cyp4A14	0	0	0	+(3/3)	0
Nqo1	+(3/3)	+(2/3)	0	0	+(3/3)
Oatp1a1	-(2/3)	-(3/3)	-(3/3)	-(3/3)	-(2/3)
Oatp1a4	0	0	+(2/3)	0	0
Oatp1a5	0	-(2/3)	-(2/3)	0	0
Oatp1a6	0	0	0	-(2/3)	0
Oatp1b2	0	0	-(3/3)	-(3/3)	0
Oatp1c1	0	0	0	0	0
Oatp2a1	0	0	-(3/3)	-(3/3)	0
Oatp2b1	+(2/3)	0	-(2/3)	-(3/3)	+(2/3)
Oatp3a1	+(3/3)	0	-(3/3)	0	0
Oatp4a1	0	0	0	0	0

Note: "+" indicates up-regulation of mouse Oatp mRNA expression; "-" indicates down-regulation of mouse Oatp mRNA expression; "0" indicates no effects on mouse Oatp mRNA expression; "2/3" indicates 2 ligands have effects of the 3 ligands in a transcription pathway.

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Table 2. List of oligonucleotide probes generated for use in bDNA signal amplification assay.

Target Sequence[a]	Function[b]	Probe Sequence
Cyp1A1 (# NM_010902)		
1129-1152	CE	gttcctcctggatctttctctgtaTTTTTctcttggaaagaaagt
1217-1237	CE	gtctccagaatgaaggcctccTTTTTctcttggaaagaaagt
1386-1404	CE	tttcaggccggaactcgttTTTTTctcttggaaagaaagt
1486-1504	CE	cggccaatggtctctccgaTTTTTctcttggaaagaaagt
1505-1526	CE	caggaagagaaagacctccgatTTTTTctcttggaaagaaagt
1086-1106	LE	catgaggctccacgagatagcTTTTTaggcataggacccgtgtct
1107-1128	LE	ccctagggttggttaccaggtaTTTTTaggcataggacccgtgtct
1153-1177	LE	cgatctctgccaatcactgtgtctaTTTTTaggcataggacccgtgtct
1195-1216	LE	agatagggcagctgaggtctgtTTTTTaggcataggacccgtgtct
1346-1366	LE	cggtcatggttaacctgccacTTTTTaggcataggacccgtgtct
1367-1385	LE	tgggtcaccccacagttccTTTTTaggcataggacccgtgtct
1468-1485	LE	tgcactttcgcttgcccaTTTTTaggcataggacccgtgtct
1527-1545	LE	tttgctgcagcaagatggcTTTTTaggcataggacccgtgtct
1178-1194	BL	cagaaagccggggctgc
1238-1257	BL	cgaaggatgaatgccggaag
1258-1275	BL	gggggatggtgaagggga
1276-1300	BL	agacttgtatctcttgtggtgctgt
1301-1323	BL	ccttggggatatagaagccattc
1324-1345	BL	tggttcacaaagacacagcacc
1405-1422	BL	cgctgggggtgagaaacc
1423-1442	BL	caagcgcttgtccagagtgc
1443-1467	BL	aaccaaagagagtgaccttctcact
Cyp2B10 (# NM_009998)		
159-182	CE	agctgaattaaagacttgaggaggTTTTTctcttggaaagaaagt
205-224	CE	ggtcccaggtgcactgtgaaTTTTTctcttggaaagaaagt
286-303	CE	ccggccagagaaagcctcTTTTTctcttggaaagaaagt
969-993	CE	aatctccttttggactttctctgtaTTTTTctcttggaaagaaagt
1035-1053	CE	gtatggcattttggtgcggTTTTTctcttggaaagaaagt
1614-1633	CE	cttggagccctggagatttgTTTTTctcttggaaagaaagt
183-204	LE	cacatcgccatattttctcgaTTTTTaggcataggacccgtgtct
225-245	LE	cacagcataaccacaggccttTTTTTaggcataggacccgtgtct
246-267	LE	ctcccttatggtgtctgttccaTTTTTaggcataggacccgtgtct
304-324	LE	ctcaacgacagcaactgtcccTTTTTaggcataggacccgtgtct
994-1014	LE	gtgtgagccgatcacctgatcTTTTTaggcataggacccgtgtct
1015-1034	LE	tcatcaagggttggtagccgTTTTTaggcataggacccgtgtct
1054-1075	LE	tctcgtggatgactgcatctgaTTTTTaggcataggacccgtgtct
1568-1587	LE	ccaccagctgtctcagaggcTTTTTaggcataggacccgtgtct
1588-1613	LE	gagacatgcaataggagtactgatttTTTTTaggcataggacccgtgtct
268-285	BL	ggcttggcccaccagagc

Cyp3A11 (# NM_007818)

1094-1111	CE	ggaggtgccttgttgggcTTTTTctcttggaaagaaagt
1162-1185	CE	caatgggatataatctgagggtttTTTTTctcttggaaagaaagt
1266-1288	CE	tcatggtgaagagcataagatggTTTTTctcttggaaagaaagt
1379-1397	CE	cctgggtccattcccaaagTTTTTctcttggaaagaaagt
1586-1609	CE	aagaactccttgagggagactcatTTTTTctcttggaaagaaagt
1049-1071	LE	gcagtttttctggatatcagggTTTTTaggcataggacccgtgtct
1072-1093	LE	agagcctcatcgatctcatcctTTTTTaggcataggacccgtgtct
1112-1136	LE	catctccatcacagtatcatacgtgTTTTTaggcataggacccgtgtct
1137-1161	LE	cattaagcaccatatccaggtattcTTTTTaggcataggacccgtgtct
1216-1242	LE	ggatatacacaccattgagttcaacatTTTTTaggcataggacccgtgtct
1243-1265	LE	aatcatcactgttgacccttggTTTTTaggcataggacccgtgtct
1353-1378	LE	ggcagatatacataaggatcaatgctTTTTTaggcataggacccgtgtct
1398-1417	LE	aacctcatgccaaggcagttTTTTTaggcataggacccgtgtct
1418-1441	LE	gcaagtttcatattcatgagagcaTTTTTaggcataggacccgtgtct
1541-1563	LE	gtggcacaacctttagaacaatgTTTTTaggcataggacccgtgtct
1564-1585	LE	gctccagttatgactgcatcccTTTTTaggcataggacccgtgtct

Cyp4A14 (# NM_007822)

162-181	CE	gggaaatgctggagggtcttTTTTTctcttggaaagaaagt
220-240	CE	tctgctggagctccttgtcctTTTTTctcttggaaagaaagt
290-311	CE	caggactcgtatattgctccccTTTTTctcttggaaagaaagt
359-382	CE	aattgataaattccagaagcctttTTTTTctcttggaaagaaagt
143-161	LE	gagcagccactgccttcgtTTTTTaggcataggacccgtgtct
201-219	LE	tcagatggtgcccccaaagTTTTTaggcataggacccgtgtct
266-289	LE	gagagacactgtaagcaggcacttTTTTTaggcataggacccgtgtct
312-337	LE	accttcacatagtcaggatcataaagTTTTTaggcataggacccgtgtct
338-358	LE	ggatctgatctccccagaaccTTTTTaggcataggacccgtgtct
383-402	LE	aaccaatccagggagcaaagTTTTTaggcataggacccgtgtct
403-424	LE	ccattcaacaggagcaaaccatTTTTTaggcataggacccgtgtct
182-200	BL	ccagtgggaaggcatgcat
241-265	BL	gggaatttctctacccatataagaa

Nqo1 (#S75951)

60-78	CE	gccttggggtatgggctgaTTTTTctcttggaaagaaagt
103-123	CE	gagagatccttagggctggctTTTTTctcttggaaagaaagt
208-225	CE	ggctcttctcgccgccatTTTTTctcttggaaagaaagt
272-292	CE	cctctacagcagcctccttcaTTTTTctcttggaaagaaagt
842-860	CE	tcctcccagacggtttccaTTTTTctcttggaaagaaagt
929-951	CE	cttctgctcctcttgaacttcctTTTTTctcttggaaagaaagt
952-974	CE	acagagaggccaaacttgttcttTTTTTctcttggaaagaaagt
36-59	LE	gcgaaggctttatatcctgcttatTTTTTaggcataggacccgtgtct
79-102	LE	acaggctaggctagtaagagctgaTTTTTaggcataggacccgtgtct
146-164	LE	ggctgagcagctggttgccTTTTTaggcataggacccgtgtct
165-186	LE	atcgtaataccgaacgctgattTTTTTaggcataggacccgtgtct
187-207	LE	ggctccagatgttgagggaggTTTTTaggcataggacccgtgtct

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226-247	LE	ctgaatgggccagtacaatcagTTTTTaggcataggacccgtgtct
248-271	LE	tggcgtagttgaatgatgtcttctTTTTTaggcataggacccgtgtct
801-819	LE	gatctgcatgcgggcatctTTTTTaggcataggacccgtgtct
820-841	LE	gacgtttcttccatccttccagTTTTTaggcataggacccgtgtct
883-905	LE	aagtttaggtcaaacaggctgctTTTTTaggcataggacccgtgtct
906-928	LE	ttttcattaagaatcctgcctgaTTTTTaggcataggacccgtgtct
975-991	LE	tgcccaggtgatggcccTTTTTaggcataggacccgtgtct
124-145	BL	aagacgaccctaaagctcttcg
861-882	BL	tggagcaaaatagagtggggtc

CE, capture extender; LE, label extender; BL, blocker

[a]Target sequence refers to the region of the mRNA transcript to which a given probe is complementary, GenBank accession numbers for each transcript are given in parenthesis next to the gene name

[b]Function refers to the type of bDNA oligonucleotide probe represented by each sequence

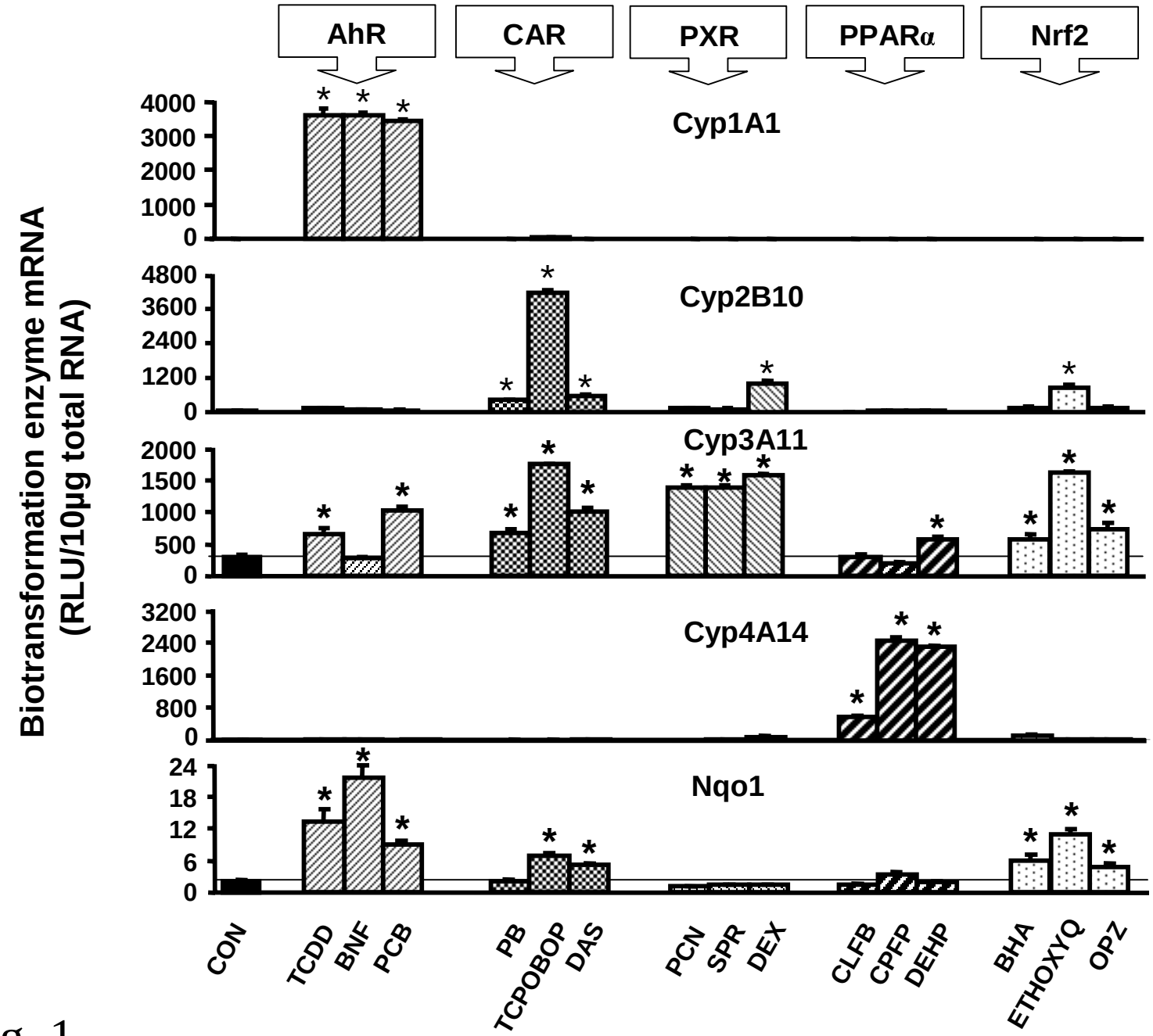

Fig. 1

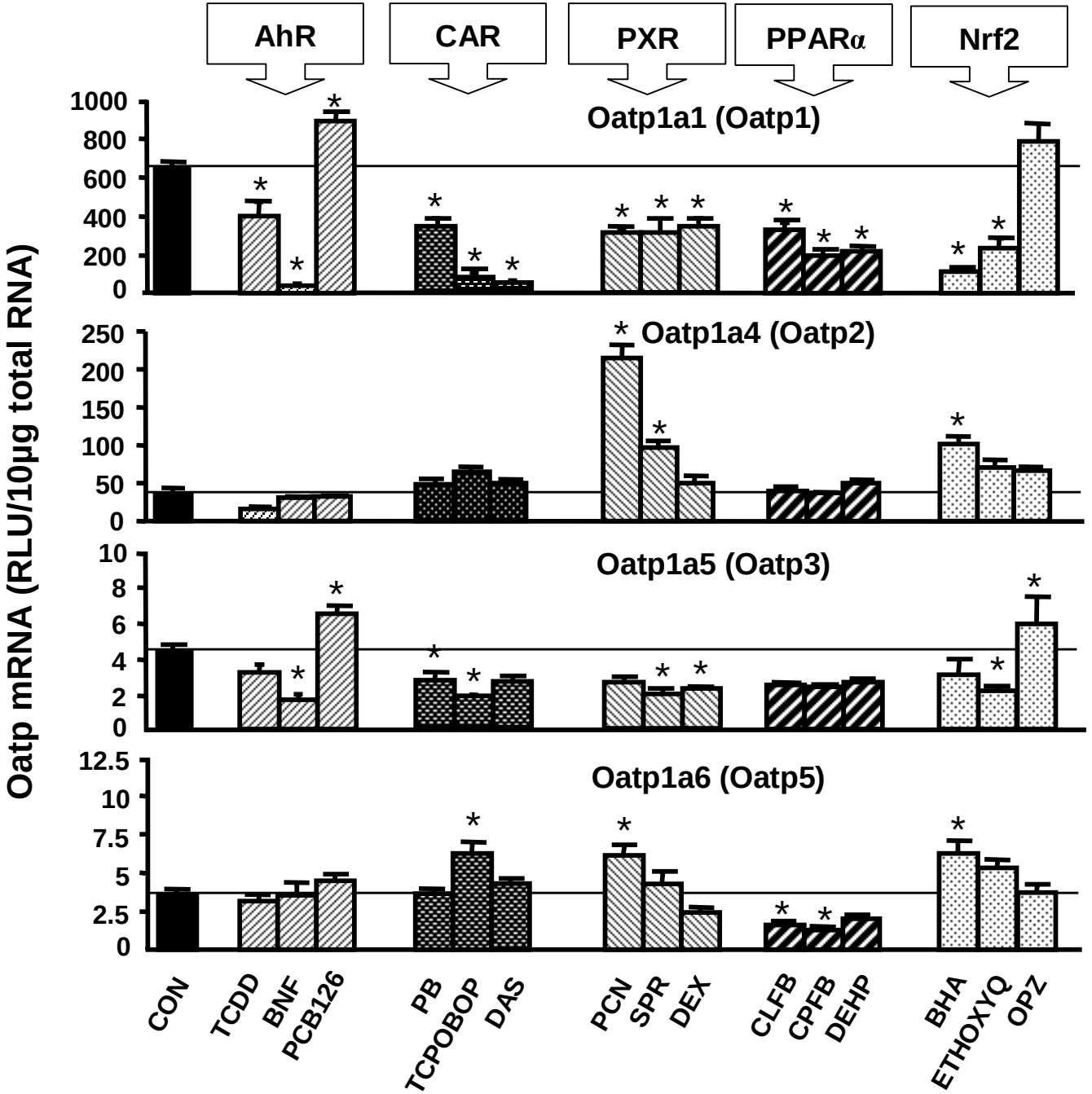

Fig. 2

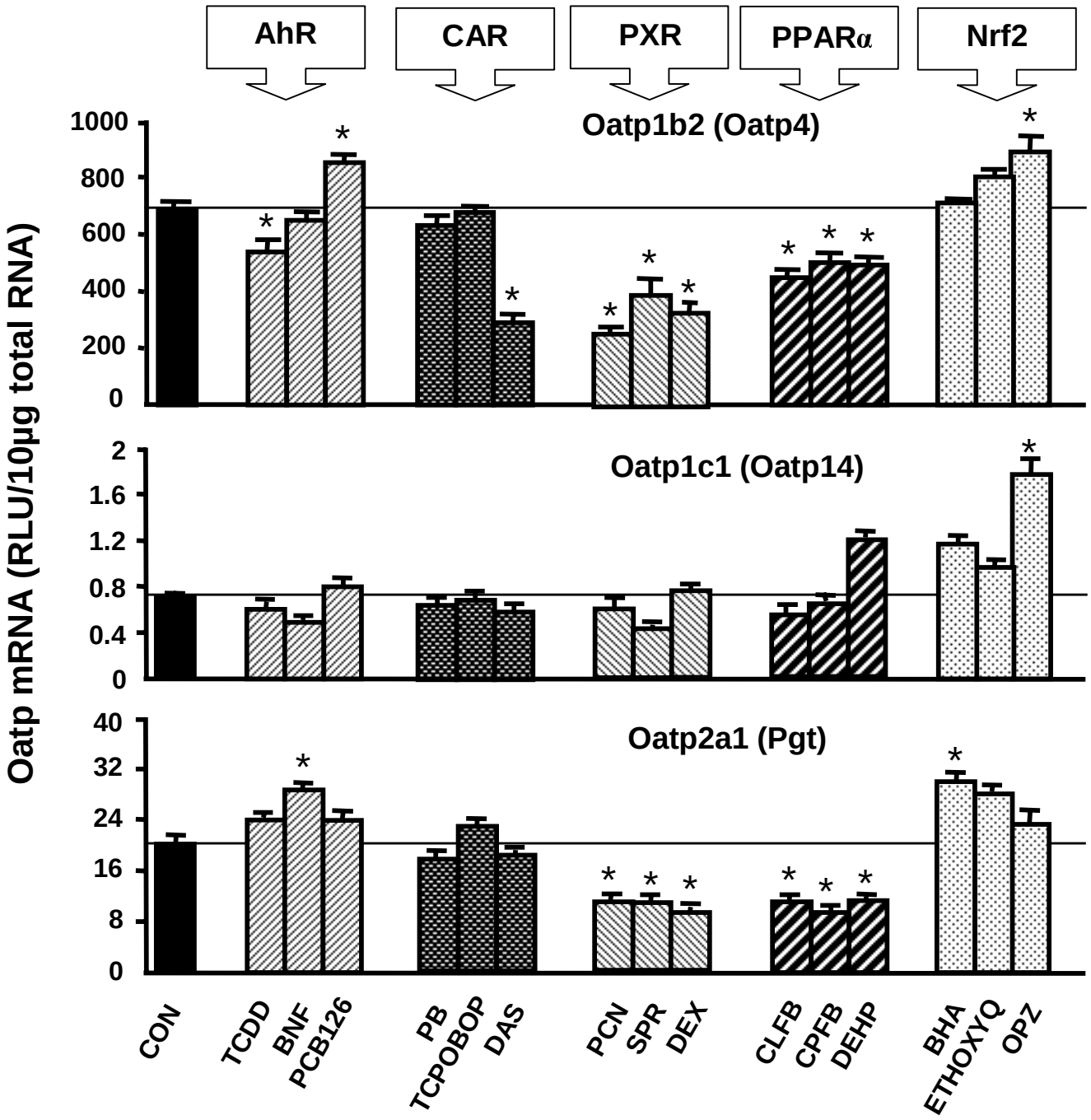

Fig. 3

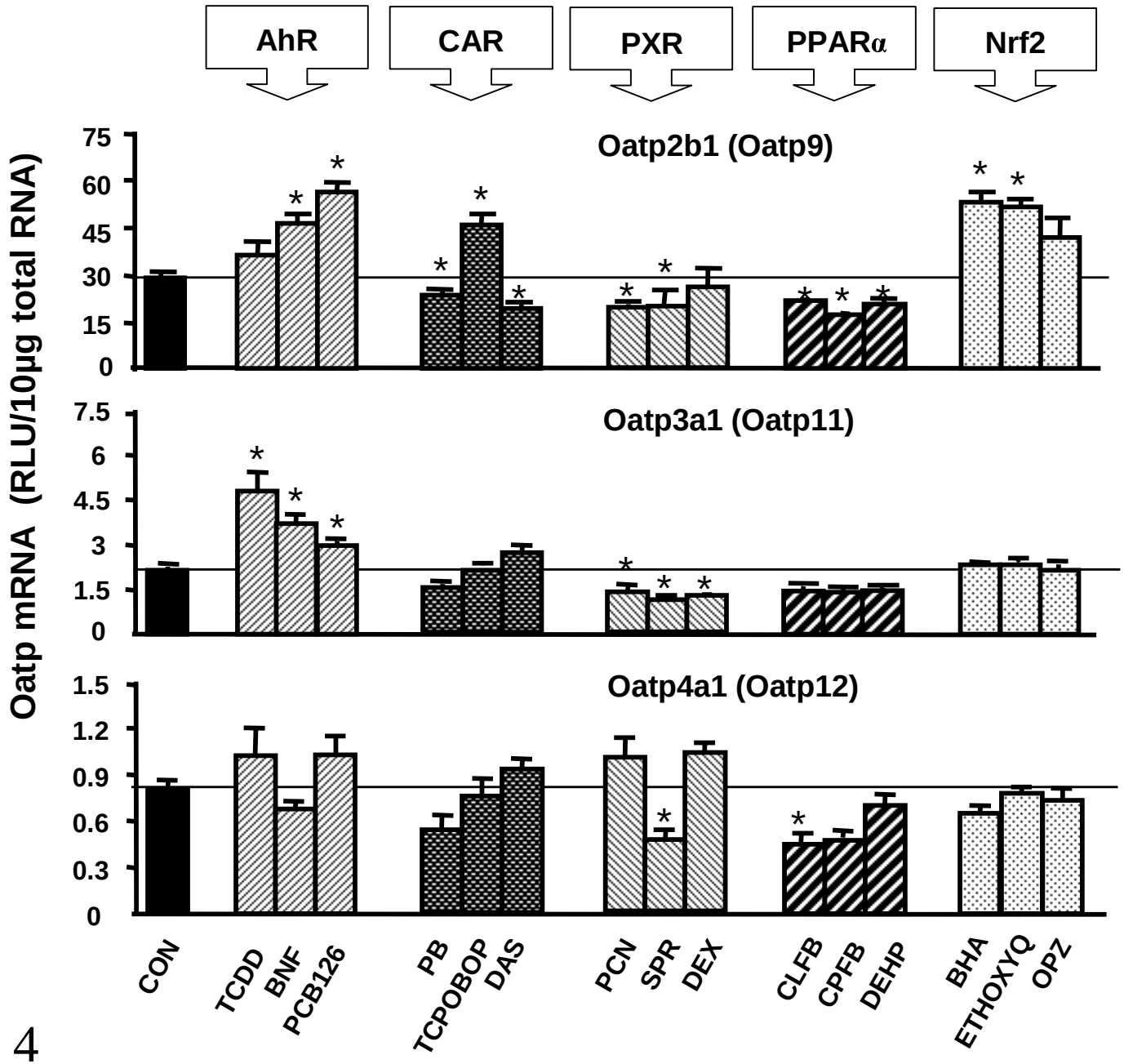

Fig. 4