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Regulation of Sulfotransferase Enzymes by Prototypical Microsomal Enzyme Inducers in Mice

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ABSTRACT

In the present study, the regulation of the mRNA of 11 sulfotransferases (Sults) and two 3′-phosphoadenosine 5′-phosphosulfate synthase (PAPSs) isozymes by 15 microsomal enzyme inducers (MEI) in livers of male mice and five MEIs in livers of female mice was examined. These MEIs represent the transcriptionally mediated pathways: aryl hydrocarbon receptor (AhR), pregnane X receptor (PXR), constitutive androstane receptor (CAR), peroxisomal proliferator-activated receptor α (PPARα), and NF-E2-related factor 2 (Nrf2). AhR ligands suppress the expression of Sults, especially the Sult1 isoenzymes in female mice. CAR activators up-regulate several Sults and PAPSs2 in female but not in male mice. PXR ligands cause marked induction of Sult1e1 in male, Sult2a1/2a2 in female, and PAPSs2 in both male and female mice. PPARα ligands do not have a marked effect on Sult expression in males, but they tend to suppress the expression of several Sult isoforms in female mice. Nrf2 activators appear to induce the mRNA expression of Sults in male and have mixed effects in female mice. In silico analysis indicated the presence of putative binding sites for all five transcription factors in the promoter region of many Sult and PAPSs isoforms. In conclusion, induction of Sults by typical MEIs is not as marked as the induction of P450 enzymes in mice. In addition to gender differences in basal expression of Sults, there is also a marked gender difference in the inducibility of various Sult isoenzymes in mice by MEIs.

Sulfotransferases (Sults) comprise a group of phase-II biotransformation enzymes that are responsible for the sulfonation of a wide range of molecules, ranging from endogenous neurotransmitters and hormones to xenobiotics. Sulfonation is a conjugation reaction that transfers a sulfonate group (SO_3^-) from the universal sulfonate donor 3′-phosphoadenosine,5′-phosphosulfate (PAPS) to a substrate. Sult proteins have two active sites: one for the sulfate donor (PAPS) and the other for the acceptor substrate (Chapman et al., 2004). PAPS is formed from dietary inorganic sulfate and ATP by the action of two enzymes, ATP-sulfurylase and adenosine-5′-phosphosulfate kinase. In mammals, the two enzyme activities are contained within one bifunctional protein termed PAPS synthase (PAPSs). Two PAPSs isoenzymes have been cloned from humans (ul Haque et al., 1998) and mice (Kurima et al., 1998), namely PAPSs1 and PAPSs2. The two isoenzymes differ in their tissue distribution and catalytic activity (Rosenthal and Leustek, 1995; Strott, 2002). Sulfonation markedly increases the water solubility of compounds and, therefore, accelerates their urinary and biliary excretion (detoxification). However, the formation of unstable sulfoconjugates can yield electrophilic cations that react with DNA and other cellular nucleophiles (Glatt, 2000). This is responsible for the bioactivation of some carcinogens and mutagens (Watabe et al., 1987; Wu et al., 2001).

Based on their subcellular localization, sulfotransferases can be classified into two main classes: cytosolic that exist as free proteins in the cytosol and membrane-associated proteins that are bound to walls of the Golgi apparatus. Membrane-associated sulfotransferases are involved in post-translational modification of macromolecules, such as carbohydrates, lipids, and proteins. Cytosolic sulfotransferases are responsible for the sulfonation of small endogenous and exogenous compounds. Therefore, the cytosolic sulfotransferases represent the class relevant to xenobiotic metabolism and disposition (Matsui and Homma, 1994). Cytosolic Sults are divided into five families

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ABBREVIATIONS: Sult, sulfotransferase; bDNA, branched DNA signal amplification assay; PAPS, 3′-phosphoadenosine,5′phosphosulfate; PAPSs, PAPS synthase; RLU, relative light unit(s); AhR, hydrocarbon receptor; CAR, constitutive androstane receptor; PXR, pregnane X receptor; PPARα, peroxisome proliferator-activated receptor α; Nrf2, NF-E2-related factor 2; BNF, β-naphthoflavone; MEI, microsomal enzyme inducer; TCDD, 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin; OPZ, oltipraz; PCB126, polychlorinated biphenyl 126; DAS, diallyl sulfide; CLOF, clofibric acid; DEX, dexamethasone; EXQ, ethoxyquin; PCN, pregnenolone-16α-carbonitrile; BHA, butylated hydroxyanisole; SPR, spironolactone; TCPOBOP, 1,4-bis[2-(3,5-dichloropuridyloxy)]benzene; 3MC, 3-methylcholanthrene; LCA, lithocholic acid.

with ~40% similarity in amino acid sequences: Sult1, 2, 3, 4, and 5, with subfamilies within each family, according to their DNA sequence resemblance. The Sult1 and 2 families have substrate specificity to phenolic and hydroxysteroid compounds, respectively (Strott, 2002). The Sult3 enzymes catalyze the formation of sulfamates, whereas substrates for Sult4 and 5 families have not been characterized adequately (Strott, 2002).

Because of the importance of Sults to xenobiotic metabolism, carcinogenesis, and hormone regulation, understanding the mechanisms responsible for their gene regulation by microsomal enzyme inducers (MEIs) is of clinical significance. MEIs exert their effects on target genes through direct binding to, or indirect activation of, transcription factors. Upon activation, transcription factors cause transcriptional induction/suppression of target proteins by binding to response elements in the promoter region of their target genes, leading to homeostatic adjustment of specific metabolic pathways. Transcription factors play a central role in drug metabolism by regulating the basal and induced expression of the phase I and II enzymes, as well as transporter proteins (Handschin and Meyer, 2003). The intensive studies on the regulation of cytochrome P450 enzymes have led to the discovery of several ligand-activated transcription factor pathways involved in the induction of P450 (Wang and Negishi, 2003). Examples of these transcription factors are aromatic hydrocarbon receptor (AhR), constitutive androstane receptor (CAR), pregnane X receptor (PXR), peroxisome proliferator-activated receptor α (PPARα), and NF-E2-related factor 2 (Nrf2). These transcription factors may overlap in their target genes and their activating ligands (Handschin and Meyer, 2003; Numazawa and Yoshida, 2004).

Several reports have addressed the regulation of Sult expression by various transcription factors and/or their activators. Expression of Sults has been reported to be regulated through CAR (Saini et al., 2004), PXR (Sonoda et al., 2002), PPARα (Fan et al., 2004), vitamin D receptor (Echchgadda et al., 2004b), farnesoid X receptor (Song et al., 2001), and glucocorticoid receptors (Fang et al., 2003). In many occasions, these reports present controversial results regarding the regulation of Sult expression by various transcription factors and their activators.

The goal of the present study is to examine the regulation of 11 Sults and two PAPSs enzymes by five groups of prototypical MEIs in male and female mice. These five groups represent common ligand-activated transcription factor pathways known to be involved in the induction of P450 enzymes and comprise AhR, PXR, CAR, PPARα, and Nrf2 activators.

Materials and Methods

Chemicals. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) was a gift from Dr. Karl Rozman (University of Kansas Medical Center, Kansas City, KS). Oltipraz (OPZ) was a gift from Dr. R. Lubet (National Cancer Institute, Bethesda, MD). Polychlorinated biphenyl 126 (PCB126) was obtained from AccuStandard (New Haven, CT). β-Naphthoflavone (BNF), diallyl sulfide (DAS), clofibric acid (CLOF), di-(2-ethylhexyl)-phthalate, ethoxyquin (EXQ), dexamethasone (DEX), pregnenolone-16α-carbonitrile (PCN), ciprofibrate, butylated hydroxyanisole (BHA), spironolactone (SPR), phenobarbital, and 1,4-bis[2-(3,5-dichloropuridyloxy)]benzene (TCPOBOP) were purchased from Sigma-Aldrich Co. (St. Louis, MO). RNA Bee was obtained from TelTest Inc. (Friendswood, TX).

Animals and Treatments. Approximately 8-week-old male and female C57BL/6 mice were purchased from Jackson Laboratories (Bar Harbor, ME) and were dosed with microsomal enzyme inducers (n = 5/treatment). Mice were dosed with five groups of prototypical microsomal enzymes inducers. Male mice were dosed with three inducers per group. The chemicals, route of administration, dosing regimen, and vehicles are included in Table 1. Female C57BL/6 mice were dosed with one inducer from each class. The female mice were treated with TCDD (AhR ligand), TCPOBOP (CAR activator), PCN (PXR ligand), CLOF (PPARα ligand), and OPZ (Nrf2 activator). After dosing each day for 4 days with the microsomal enzyme inducers, livers were removed on day 5, snap-frozen in liquid nitrogen, and stored at −80°C. The dose and dosing regimen of these compounds were previously shown to induce the corresponding target genes in mice, namely Cyp1A1 for AhR ligands, Cyp2B10 for CAR activators, Cyp3A11 for PXR ligands, Cyp4A14 for PPARα ligands, and Nqo1 for Nrf2 activators (Maher et al., 2005). Control female and male animals were treated with corn oil i.p.

Total RNA Isolation. Tissues were homogenized, and total RNA was isolated using RNA-Bee reagent (TelTest, Inc.) according to the manufacturer's protocol. Total RNA concentrations were determined spectrophotometrically at 260 nm. One microgram per microliter solutions were prepared from the stock RNA solution by dilution with diethyl pyrocarbonate-treated deionized water. Integrity of RNA samples was evaluated using formaldehyde-agarose gel electrophoresis. Samples were then visualized under ultraviolet light by ethidium bromide fluorescence.

Branched DNA Signal Amplification Analysis. mRNA was quantified using the branched DNA (bDNA) assay (Quantigene bDNA signal amplification kit; Panomics Inc., Fremont, CA) with modifications. Gene sequences of interest were accessed from GenBank. Target sequences were analyzed using ProbeDesigner software version 1.0 (Bayer Corp., Emerville, CA) to design oligonucleotide probe sets (capture, label, and blocker probes). All probes were designed with a melting temperature of 63°C, enabling hybridization conditions to be held constant (i.e., 53°C) during each hybridization step. Each designed probe was submitted to the National Center of Biotechnology Information (NCBI, Bethesda, MD) by basic local alignment search tool (BLAST) to ensure minimal cross-reactivity with other known mouse sequences. Oligonucleotides with a high degree of similarity (>80%) to other mouse gene transcripts were eliminated from the design. The accession numbers of all genes, the sequences, and functions of the probe sets have been published previously (Alnouti and Klaassen, 2006).

The Sult2a1 and 2a2 isoforms are 96% similar; therefore, the

TABLE 1
List of chemicals, dosing regimen, and known target transcription factors

Compound	Dose	Vehicle	Route
AhR ligands			
TCDD	34 μg/kg	Corn oil	i.p.
PCB126	300 μg/kg	Corn oil	i.p.
BNF	200 mg/kg	Corn oil	i.p.
CAR activators			
TCPOBOP	300 μg/kg	Corn oil	i.p.
DAS	200 mg/kg	Corn oil	i.p.
Phenobarbital	100 mg/kg	Saline	i.p.
PXR ligands			
PCN	200 mg/kg	Corn oil	i.p.
SPR	200 mg/kg	Corn oil	i.p.
DEX	75 mg/kg	Corn oil	i.p.
PPARα ligands			
CLOF	500 mg/kg	Corn oil	i.p.
Ciprofibrate	40 mg/kg	Corn oil	i.p.
Diethylhexylphthalate	1000 mg/kg	Corn oil	p.o.
Nrf2 activators			
OPZ	150 mg/kg	Corn oil	p.o.
EXQ	250 mg/kg	Corn oil	p.o.
BHA	350 mg/kg	Corn oil	i.p.

probes designed do not differentiate between the two isoforms. It has been suggested that mouse Sult2a1 and 2a2 are alleles of the same gene (Rikke and Roy, 1996). Therefore, our bDNA probe is referred to as Sult2a1/2a2. Sult2a2 is rarely referred to in the literature. As a matter of fact, we found many reports addressing Sult2a1 expression regulation using nonspecific primers or probes, which detect both Sult2a1 and 2a2 (Assem et al., 2004; Kim et al., 2004), or using Sult2a2 primers and refer to it as Sult2a1 (Echchgadda et al., 2004a).

Total RNA (1 μg/μl; 10 μl/well) was added to each well of a 96-well plate containing 100 μl of each diluted probe set. RNA was allowed to hybridize with the probe sets overnight at 53°C. Subsequent hybridization steps were carried out according to the manufacturer's protocol, and luminescence was quantified with a Quantiplex 320 bDNA luminometer interfaced with Quantiplex Data Management software version 5.02. Data are presented as relative light units (RLU) per 10 μg of total RNA.

In Silico Analysis. We analyzed 10-kb upstream of the start codon for each gene to search for putative binding sites of the five transcription factors. Putative binding sites specific for each transcription factor are listed in Table 2. These potential binding sites were identified using NHR-scan (Sandelin and Wasserman, 2005). The sequences "RTGAYnnnGC" and "GCGTGMS" were used for the xenobiotic response element and antioxidant-response element, respectively, and were identified using manual sequence alignment.

Statistical Analysis. Data were analyzed by analysis of variance followed by Duncan's post-hoc test. Gender differences were determined using student's t test. Statistical significance was set at $p \leq 0.05$. Bars represent mean ± S.E.M.

Results

The regulation of Sult1a1, 1b1, and 1c1 mRNA by the five groups of MEIs in male mice is shown in Fig. 1. In general, Sult1a1, 1b1, and 1c1 mRNA expression is not affected by all three inducers in any of the five classes. Sult1a1 mRNA is markedly suppressed by one of the AhR ligands (TCDD) and induced by one of the Nrf2 activators (BHA). Sult1b1 mRNA is slightly induced by one PXR ligand (SPR) and two of the Nrf2 activators (OPZ and EXQ). Sult1c1 is only induced by the Nrf2 activator (BHA).

The regulation of Sult1c2, 1d1, and 1e1 mRNA by the five groups of microsomal inducers in male mice is shown in Fig. 2. Basal expression of Sult1c2 mRNA is not detected in livers of male mice. However, Sult1c2 mRNA seems to be induced by one Nrf2 activator (EXQ). Sult1d1 mRNA is induced by one PXR (DEX), CAR (TCPOBOP), and Nrf2 (BHA) activator. The basal expression of Sult1e1 mRNA in liver is very low in both male and female mice. However, the mRNA expression of Sult1e1 is induced by two PXR and one Nrf2 activators, but most markedly (more than 100-fold) by DEX (PXR ligand).

Sult2a1/2a2, 2b1, and 3a1 mRNA regulation by MEIs in male mice is shown in Fig. 3. The basal expression of all three isozymes is very low in liver of male mice. Neither Sult2a1/2a2 nor 2b1 mRNA was affected by any of the microsomal enzyme inducers. However, Sult3a1 mRNA is induced by one CAR activator (DAS), one PXR ligand (SPR), and two Nrf2 activators (EXQ and BHA).

TABLE 2
List of putative binding sites for each transcription factor

Transcription Factor	Binding Sites	References
AhR	Xenobiotic response element	Lusska et al., 1993
CAR	Dr-3, Dr-4, D4–5, ER-6	Nakata et al., 2006
PXR	Dr-3, Dr-4, D4–5, ER-6, ER-8	Nakata et al., 2006
PPARα	DR-1	Nakata et al., 2006
Nrf2	Antioxidant-response element	Wasserman and Fahl, 1997

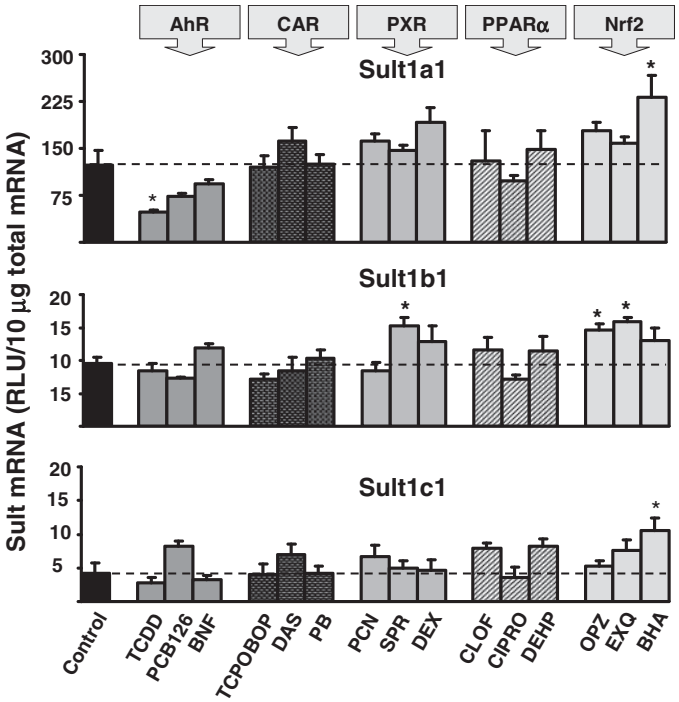

Fig. 1. Expression of Sult1a1, 1b1, and 1c1 in liver after treatment with 15 prototypical drug-metabolizing enzyme inducers. Total RNA from liver of treated male mice (n = 5/treatment) was analyzed by the bDNA signal amplification assay. The data are presented as mean RLU ± S.E.M. (n = 5). *, a statistically significant difference ($p \leq 0.05$) between treated and control mice. †, a statistically significant difference ($p \leq 0.05$) between male and female mice.

Figure 4 shows the regulation of Sult4a1 and 5a1 mRNA in male mice by MEIs. The basal expression of Sult4a1 mRNA was very low in livers of male mice, which is known (Sakakibara et al., 2002), and was not affected by any of the inducers. In contrast, Sult5a1 mRNA expression was induced by all AhR ligands (TCDD, PCB126, and BNF), one PXR ligand (PCN), and two Nrf2 activators (OPZ, EXQ). Regulation of PAPSs1 and 2 mRNAs in male mice is shown in Fig. 5. PAPSs1 mRNA was not changed by any of the inducers, whereas PAPSs2 mRNA was induced by two PXR ligands (PCN and SPR), one PPARα ligand (CLOF), and one Nrf2 ligand (BHA).

In female mice, the mRNA of the Sult and PAPSs isoenzymes shows the same general trend of relative insensitivity to induction by the prototypical microsomal inducers. The regulation of Sult1a1, 1b1, and 1c1 mRNA by the five groups of microsomal inducers in males and females is shown in Fig. 6. Sult1a1 mRNA expression was suppressed by the AhR ligand (TCDD) in both male and female mice. Similar to Sult1a1, Sult1b1 mRNA expression was suppressed by TCDD in female mice. In contrast, Sult1b1 mRNA expression in male mice was not altered by TCDD but induced by the Nrf2 activators (OPZ and EXQ). Sult1c1 mRNA expression was suppressed by TCDD, CLOF, and OPZ in female mice.

The regulation of Sult1c2, 1d1, and 1e1 mRNA by the prototypes of the five groups of MEIs in male and female mice is shown in Fig. 7. Sult1c2 mRNA expression was suppressed by TCDD, PCN, CLOF, and OPZ and induced by TCPOBOP

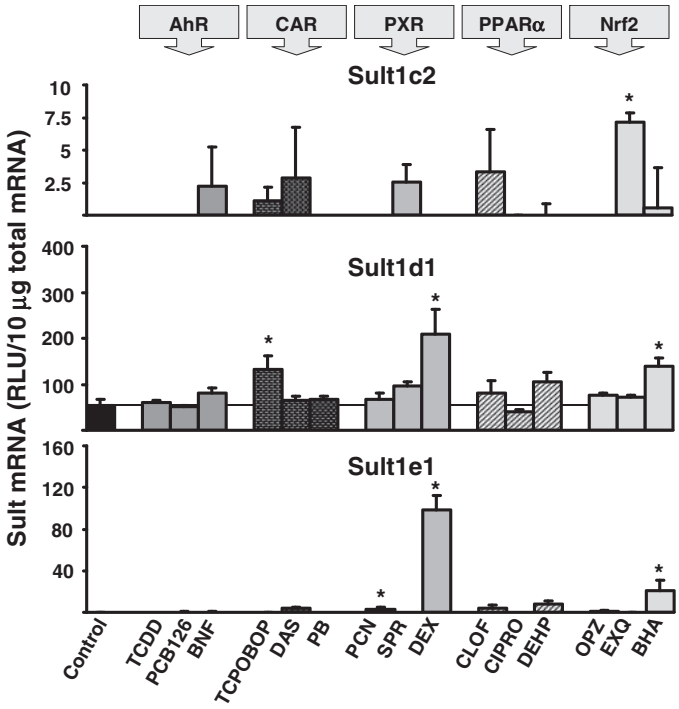

Fig. 2. Expression of Sult1c2, 1d1, and 1e1 in liver after treatment with 15 prototypical drug-metabolizing enzyme inducers. Total RNA from liver of treated male mice (n = 5/treatment) was analyzed by the bDNA signal amplification assay. The data are presented as mean RLU ± S.E.M. (n = 5). ∗, represents a statistically significant difference ($p \leq 0.05$) between treated and control mice. †, a statistically significant difference ($p \leq 0.05$) between male and female mice.

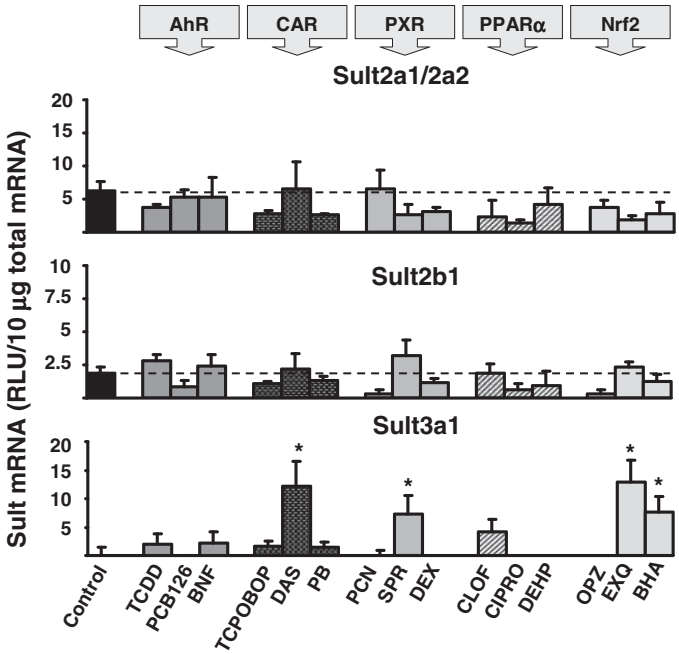

Fig. 3. Expression of Sult2a1/2a1, 2b1, and 3a1 in liver after treatment with 15 prototypical drug-metabolizing enzyme inducers. Total RNA from liver of treated male mice (n = 5/treatment) was analyzed by the bDNA signal amplification assay. The data are presented as mean RLU ± S.E.M. (n = 5). ∗, a statistically significant difference ($p \leq 0.05$) between treated and control mice. †, a statistically significant difference ($p \leq 0.05$) between male and female mice.

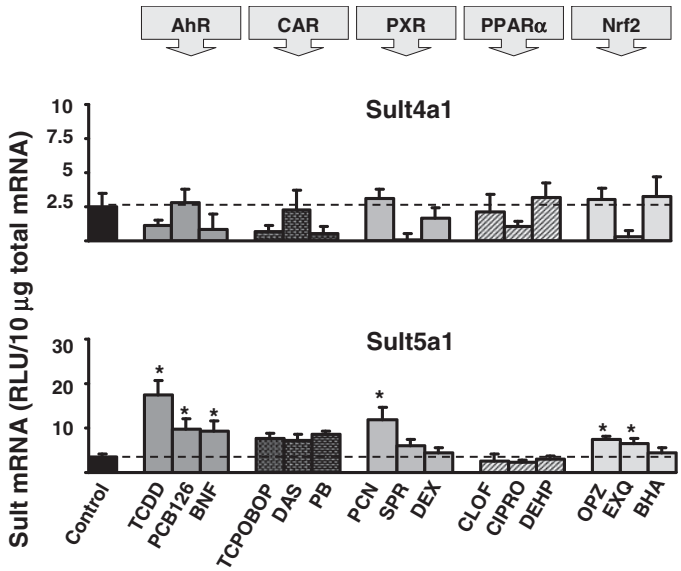

Fig. 4. Expression of Sult4a1, and 5a1 in liver after treatment with 15 prototypical drug-metabolizing enzyme inducers. Total RNA from liver of treated male mice (n = 5/treatment) was analyzed by the bDNA signal amplification assay. The data are presented as mean RLU ± S.E.M. (n = 5). ∗, a statistically significant difference ($p \leq 0.05$) between treated and control mice. †, a statistically significant difference ($p \leq 0.05$) between male and female mice.

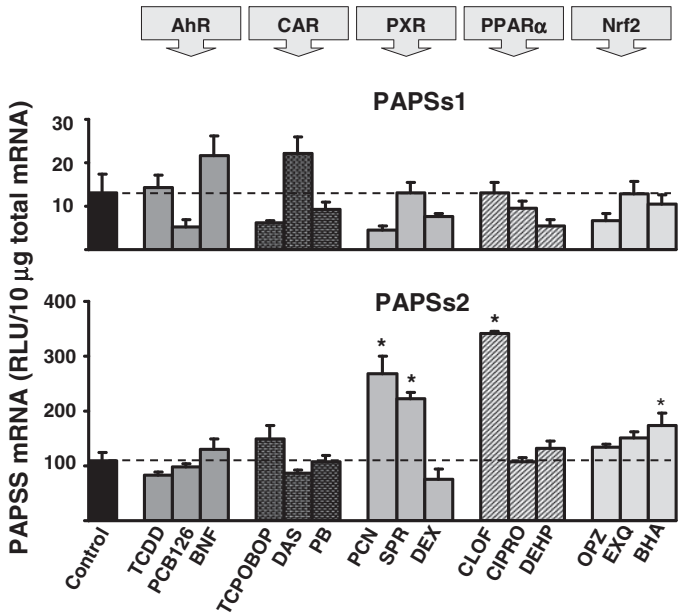

Fig. 5. Expression of PAPSS1 and PAPSS2 in liver after treatment with 15 prototypical drug-metabolizing enzyme inducers. Total RNA from liver of treated male mice (n = 5/treatment) was analyzed by the bDNA signal amplification assay. The data are presented as mean RLU ± S.E.M. (n = 5). ∗, a statistically significant difference ($p \leq 0.05$) between treated and control mice. †, a statistically significant difference ($p \leq 0.05$) between male and female mice.

in female mice, whereas it was not affected by any of the five inducers in male mice. Sult1d1 mRNA expression is suppressed by TCDD and induced by TCPOBOP and OPZ in female mice, whereas it was only induced by TCPOBOP in male mice. Sult1e1 mRNA expression was suppressed by TCDD and CLOF and induced by TCPOBOP in female mice.

Figure 8 shows the regulation of Sult2a1/2a2, 2b1, and 3a1

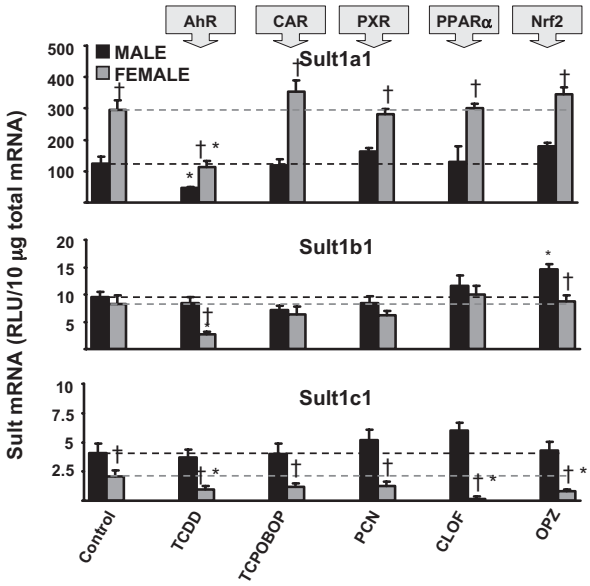

Fig. 6. Expression of Sult1a1, 1b1, and 1c1 in liver after treatment with five prototypical drug-metabolizing enzyme inducers. Total RNA from liver of treated female mice (n = 5/treatment) was analyzed by the bDNA signal amplification assay. Data from male mice treatment with the same inducers from the corresponding enzymes (Fig. 1) are also shown. The data are presented as mean RLU ± S.E.M. (n = 5). ∗, a statistically significant difference ($p \leq 0.05$) between treated and control mice. †, a statistically significant difference ($p \leq 0.05$) between male and female mice.

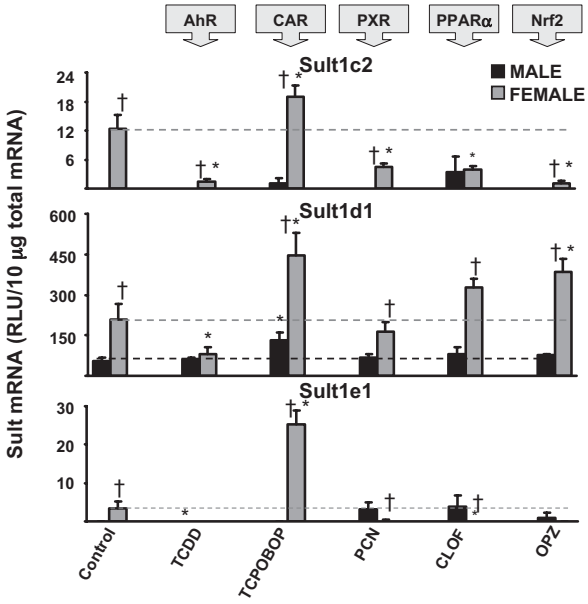

Fig. 7. Expression of Sult1c2, 1d1, and 1e1 in liver after treatment with five prototypical drug-metabolizing enzyme inducers. Total RNA from liver of treated female mice (n = 5/treatment) was analyzed by the bDNA signal amplification assay. Data from male mice treatment with the same inducers from the corresponding enzymes (Fig. 2) are also shown. The data are presented as mean RLU ± S.E.M. (n = 5). ∗, a statistically significant difference ($p \leq 0.05$) between treated and control mice. †, a statistically significant difference ($p \leq 0.05$) between male and female mice.

mRNA expression in male and female mice. Sult2a1/2a2 mRNA expression was induced by TCPOBOP and PCN in female mice, whereas it was not affected by any of the five inducers in male mice. Sult2b1 mRNA expression was induced by the CAR activator TCPOBOP female mice, but was

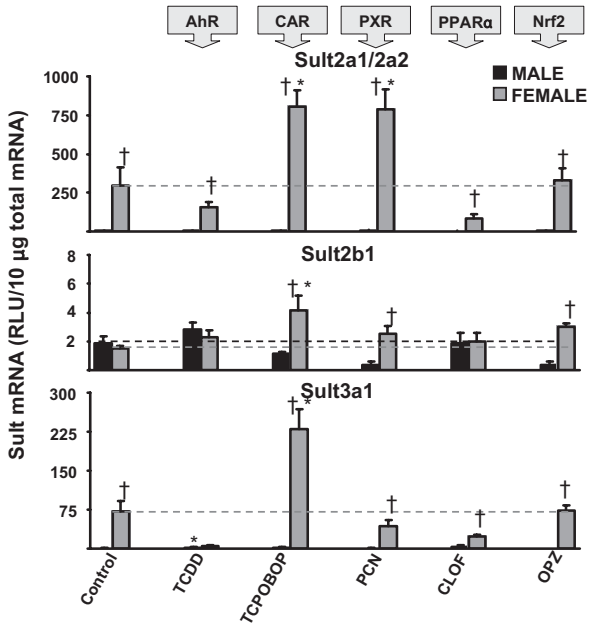

Fig. 8. Expression of Sult2a1/2a1, 2b1, and 3a1 in liver after treatment with five prototypical drug-metabolizing enzyme inducers. Total RNA from liver of treated female mice (n = 5/treatment) was analyzed by the bDNA signal amplification assay. Data from male mice treatment with the same inducers from the corresponding enzymes (Fig. 3) are also shown. The data are presented as mean RLU ± S.E.M. (n = 5). ∗, a statistically significant difference ($p \leq 0.05$) between treated and control mice. †, a statistically significant difference ($p \leq 0.05$) between male and female mice.

not affected by any inducer in males. In female mice, Sult3a1 mRNA was suppressed by TCDD, whereas it was induced by TCPOBOP.

The mRNA regulation of Sult4a1 and 5a1 is shown in Fig. 9. The basal mRNA expression of Sult4a1 was higher in male mice but was not induced by any of the five classes of inducers. Sult4a1 mRNA expression was induced by TCPOBOP, PCN, CLOF, and OPZ in female mice from essentially undetectable levels to low levels. Sult5a1 mRNA expression was suppressed by PCN, CLOF, and OPZ in female mice (Fig. 9),

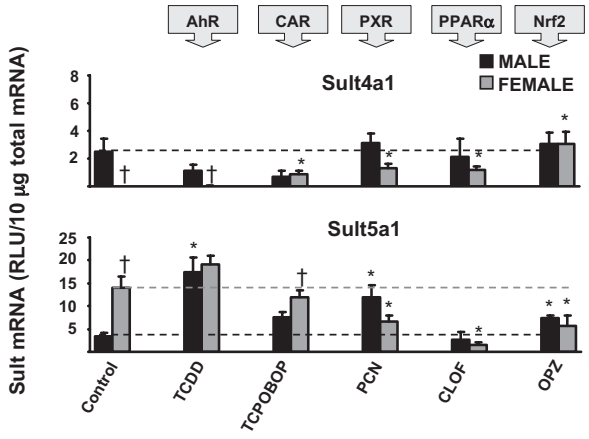

Fig. 9. Expression of Sult4a1 and 5a1 in liver after treatment with five prototypical drug-metabolizing enzyme inducers. Total RNA from liver of treated female mice (n = 5/treatment) was analyzed by the bDNA signal amplification assay. Data from male mice treatment with the same inducers from the corresponding enzymes (Fig. 4) are also shown. The data are presented as mean RLU ± S.E.M. (n = 5). ∗, a statistically significant difference ($p \leq 0.05$) between treated and control mice. †, a statistically significant difference ($p \leq 0.05$) between male and female mice.

whereas it was induced by all AhR ligands (TCDD, PCB126, and BNF), one PXR ligand (PCN), and two Nrf2 activators (OPZ and EXQ) (Fig. 4).

Figure 10 illustrates the regulation of PAPSs1 and 2 mRNA expression by MEIs in male and female mice. PAPSs1 mRNA expression was insensitive to any of the five inducers in both male and female mice. PAPSs2 mRNA in female mice was decreased by TCDD and increased by TCPOBOP and PCN, whereas it was induced by PCN and CLOF in male mice. Table 3 summarizes the effect of all 15 microsomal inducers on all 11 Sult and 2 PAPSs isoenzymes in both male and female mice.

Table 4 shows that Sult genes have putative binding consensuses for some of the transcription factors. This provides further evidence of the possible regulation of these Sult isoforms by the various transcription factors.

Discussion

Induction of phase-I metabolizing enzymes, especially P450s, and the resulting metabolic activation/deactivation of xenobiotics has been well characterized. The unfavorable effects of metabolic activation of xenobiotics are often neutralized by conjugation during phase-II metabolism. Therefore, it is important to understand the effect of typical phase-I enzyme inducers on phase-II enzymes. Sults represent a large group of phase-II metabolizing enzymes.

Therefore, we studied the influence of 15 prototypical microsomal metabolizing enzyme inducers, which are known to induce phase-I enzymes by activating five distinct transcription factors (three inducers in each group) on the mRNA expression of Sults (11 isoenzymes) and PAPSs (two isoenzymes) in male mice. In addition, the influence of one representative microsomal metabolizing enzyme inducer, which belongs to each transcription pathway, on the mRNA expression of the same enzymes in female mice was examined.

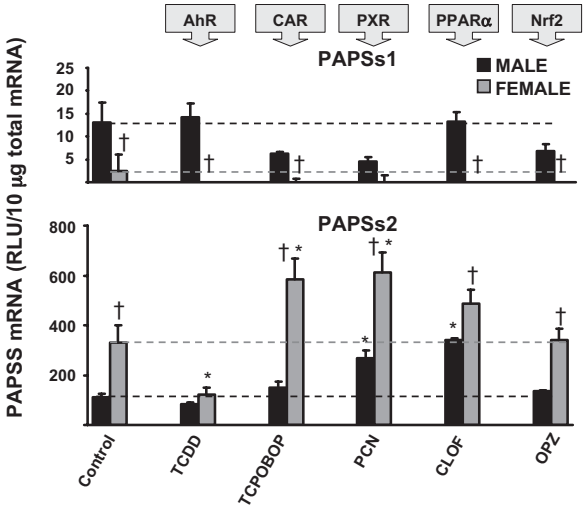

Fig. 10. Expression of PAPSS1 and PAPSS2 in liver after treatment with five prototypical drug-metabolizing enzyme inducers. Total RNA from liver of treated female mice (n = 5/treatment) was analyzed by the bDNA signal amplification assay. Data from male mice treatment with the same inducers from the corresponding enzymes (Fig. 5) are also shown. The data are presented as mean RLU ± S.E.M. (n = 5). *, a statistically significant difference ($p \leq 0.05$) between treated and control mice. †, a statistically significant difference ($p \leq 0.05$) between male and female mice.

Table 3 summarizes the effect of the various MEIs on the mRNA expression of Sults and PAPSs in male and female mice.

There are marked gender differences in the expression of Sults in mice. Most of the Sults have a higher expression in liver of female than in male mice (Alnouti and Klaassen, 2006). As summarized in Table 3, four of the six members of Sult1 family, Sult2a1/2a2, Sult5a1, and PAPSs2, have higher expression in female than male mice. The only enzymes that have higher expression in males are Sult1c1, Sult4a1, and PAPSs1, which all have very low expression in liver.

AhR ligands appear to suppress the mRNA expression of many Sults in mice (Table 3). In female mice, TCDD suppressed the mRNA expression of all Sult1 enzymes (a1, b1, c1, c2, d1, and e1) (Figs. 6 and 7), Sult3a1 (Fig. 8), and PAPSs2 (Fig. 10). Sult5a1 is the only Sult induced by all AhR ligands in male mice (Fig. 4). In silico analysis revealed the presence of two potential xenobiotic response element sites in the promoter region of Sult5a1. It was previously reported that the expression of Sult1a1 and Sult2a1 is suppressed by AhR agonists, 3-methylcholanthrene (3MC) (Runge-Morris and Wilusz, 1994) and TCDD (Runge-Morris, 1998), in cultured rat hepatocytes. However, another study reported no influence of 3MC on rat Sult1a1, 2a1, and 1e1 in rats in vivo (Rushmore and Kong, 2002). In cultured mouse hepatocytes, TCDD suppressed the expression of Sult1a1 but did not alter the expression of Sult2a1 (Runge-Morris and Kocarek, 2005). Therefore, it can be concluded that AhR ligands are more likely to decrease rather than increase expression of Sults in rodents.

CAR activators did not affect the mRNA expression of Sults and PAPSs in male mice (Table 3). However, Sult1d1 (Fig. 2) and 3a1 (Fig. 3) were induced by only one of the three CAR activators (TCPOBOP for Sult1d1; DAS for 3a1). In female mice, the CAR activator (TCPOBOP) induces the expression of Sult1c2, 1d1, 1e1 (Fig. 7), 2a1/2a2, 2b1, 3a1 (Fig. 8), 4a1 (Fig. 9), and PAPSs2 (Fig. 10). Therefore, CAR is more likely to play a role in the up-regulation of Sults and PAPSs in female but not in male mice. Table 4 provides further evidence that these genes might be regulated by CAR because they have at least one potential CAR binding site in their promoter regions.

CAR activators were reported to induce Sult1d1 in male rats (Runge-Morris et al., 1998); 1d1 in male mice (Garcia-Allan et al., 2000; Maglich et al., 2004) and human hepatocytes (Maglich et al., 2002); 1e1 in male rats (Hellriegel et al., 1996); Sult2a1 in male mice (Assem et al., 2004; Maglich et al., 2004; Saini et al., 2004; Zhang et al., 2004) and female mice (Assem et al., 2004; Saini et al., 2004); and PAPSs2 in male mice (Ueda et al., 2002; Saini et al., 2004). In contrast, CAR activators were previously reported to suppress or have no effect on 1a1 in male mice (Maglich et al., 2002, 2004) and human hepatocytes (Maglich et al., 2002); 1a1 in male rats (Runge-Morris et al., 1998); 1c1 in male rats (Runge-Morris et al., 1998); 1d1 in male mice (Maglich et al., 2002; Assem et al., 2004; Saini et al., 2004); 1e1 in male and female rats (Hellriegel et al., 1996; Runge-Morris et al., 1998; Rushmore and Kong, 2002); 2b1 in male mice (Maglich et al., 2002); 2a1 in male rats (Hellriegel et al., 1996; Runge-Morris et al., 1998; Rushmore and Kong, 2002); and all Sults in mice (Ueda et al., 2002). Therefore, these previous studies have reported different and sometimes contradictory results regarding reg-

TABLE 3
Summary of the number of inducers in each class that alter mRNA expression of Sults after treatment

Sult	F/M[a]	M/F[b]	Gender	AhR[c]	CAR[c]	PXR[c]	PPARα[c]	Nrf2[c]
Sult1a1	2.4		Male	−(1/3)				+(1/3)
			Female	−(1/1)				
Sult1b1	1	1	Male			+(1/3)		+(2/3)
			Female	−(1/1)				
Sult1c1		2	Male					+(1/3)
			Female	−(1/1)			−(1/1)	−(1/1)
Sult1c2	12		Male					+(1/3)
			Female	−(1/1)	+(1/1)	−(1/1)	−(1/1)	−(1/1)
Sult1d1	4		Male		+(1/3)	+(1/3)		+(1/3)
			Female	−(1/1)	+(1/1)			+(1/1)
Sult1e1	4		Male			+(2/3)		+(1/3)
			Female	−(1/1)	+(1/1)		−(1/1)	
Sult2a1/2a2	50		Male					
			Female		+(1/1)	+(1/1)		
Sult2b1	1	1	Male					
			Female					
Sult3a1	72		Male		+(1/3)	+(1/3)		+(2/3)
			Female	−(1/1)	+(1/1)		−(1/1)	
Sult4a1		2	Male					
			Female		+(1/1)	+(1/1)	+(1/1)	+(1/1)
Sult5a1	4		Male	+(3/3)		+(1/3)		+(2/3)
			Female			−(1/1)	−(1/1)	−(1/1)
PAPSs1		5.5	Male					
			Female					
PAPSs2	3.8		Male			+(2/3)	+(1/3)	+(1/3)
			Female	−(1/1)	+(1/1)	+(1/1)		

[a] F/M represents female/male ratio of mRNA of female-predominant isoenzymes.
[b] M/F represents male/female ratio of mRNA of male-predominant isoenzymes.
[c] + indicates up-regulation of a particular Sult or PAPSs mRNA expression; − indicates down-regulation of mRNA expression; blank indicates no influence on mRNA expression; and the number in parenthesis (for male mice only) indicates the number of inducers among the three in each group that exerted a certain influence on the mRNA expression of a particular enzyme.

ulation of Sults by the transcription factor CAR. It was previously shown that the basal expression of CAR is higher in female than male mice (Kawamoto et al., 2000). Furthermore, the CAR activator (TCPOBOP) resulted in higher induction of the mRNA expression of Cyp2B10 (CAR target gene) in female than male rats (Ledda-Columbano et al., 2003). This might explain the finding in this article that CAR activators do not appear to be strong inducers of Sults, especially in male mice, but are better inducers of Sults in female mice.

PXR appears to play a role in the induction of Sult1e1 (Fig. 2) and PAPSs2 (Fig. 5) in male mice. The strongest induction of all Sults by all 15 used MEIs was the induction of Sult1e1 by DEX. Because other PXR ligands do not produce similar effects, Sult1e1 induction by DEX may be mediated via other pathways, such as the glucocorticoid receptor. In female mice, the PXR ligand PCN induced the mRNA expression of Sult2a1/2a2 (Fig. 8), 4a1 (Fig. 9), and PAPSs2 (Fig. 10), whereas it suppressed the mRNA expression of Sult1c2 (Fig. 7) and Sult5a1 (Fig. 9). PXR ligands have been reported to induce the expression of Sult1a1 in female rats (Hartley et al., 2004), male rats (Liu and Klaassen, 1996; Rushmore and Kong, 2002), 1b1 in female rats (Hartley et al., 2004); 1d1 and 1e1 in male mice (Sonoda et al., 2002); 2a1 in male rats (Liu and Klaassen, 1996; Runge-Morris et al., 1996; Rushmore and Kong, 2002), male mice (Sonoda et al., 2002; Echchgadda et al., 2004a; Kim et al., 2004), female mice (Sonoda et al., 2002), and human hepatocytes (Duanmu et al., 2002); and PAPSs2 in male mice (Sonoda et al., 2002). In contrast, PXR ligands were reported to have no influence on the expression of Sult1a1 in male mice (Maglich et al., 2002), human hepatocytes (Duanmu et al., 2002), male rats (Liu and Klaassen, 1996; Duanmu et al., 2001), and female rats (Liu and Klaassen, 1996); 1c1 in male and female rats (Liu and Klaassen, 1996); 1d1 in male mice (Maglich et al., 2002); 1e1 in male and female rats (Liu and Klaassen, 1996); 2a1 in mice (Saini et al., 2004), and male and female rats (Liu and Klaassen, 1996); and 2b1 in male mice (Maglich et al., 2002). Thus, PXR ligands in general are not consistent inducers of Sults. However, they cause marked induction of Sult1e1 in male, Sult2a1/2a2 in female, and PAPSs2 in both male and female mice. Table 4 provides further evidence of the regulation of these genes by PXR because they have at least one potential CAR binding site in their promoter regions.

PPARα ligands, in general, do not have marked effects on Sult enzymes; they tend to have no effects on the mRNA expression of Sults in male but tend to suppress the expression of some Sult isoenzymes in female mice (Table 3). The PPARα activator (ciprofibrate) was reported to induce the expression of Sult2a1 in human hepatocytes but not in rat hepatocytes (Fang et al., 2005). However, PPARα was previously reported to suppress the expression of Sult1e1 in male and female mice (Fan et al., 2004). In contrast, CLOF was reported to have no effect on the mRNA expression of Sult1a1, 1e1, and 2a1 in male rats (Rushmore and Kong, 2002). Therefore, PPARα activators do not appear to produce marked effects on the expression of Sults in rodents.

At least one Nrf2 activator induced the mRNA expression of most Sult1 family isoenzymes in male mice (Table 3). In female mice, the Nrf2 activator (OPZ) induced the mRNA expression of Sult1d1 (Fig. 7) and 4a1 (Fig. 9), whereas it suppressed the mRNA expression of Sult1c1 (Fig. 6), 1c2 (Fig. 7), and 5a1 (Fig. 9). The regulation of Sults by Nrf2 activators has not been reported previously in any species.

TABLE 4
In silico analysis of Sults
Locations of binding sites are described as the distance from the start codon.

	AhR	CAR/PXR				PXR	PPARα	Nrf2
	XRE	Dr-3	Dr-4	Dr-5	ER-6	ER-8	Dr-1	ARE
Sult1a1	3982 6431	792 3526	2351		5424 6102 7425	1786	1138 1829 2826 5006 8632	9018
Sult1b1	5518		3815 4588		5777 6001 7183		4657	3178
Sult1c1		8489 9811	9403		1153 2612 5145	2413	2434	25,150 25,666
Sult1c2			3760 5722 6413 7449		5318 6843		5929	15,976
Sult1d1		7413	1336 1375	7572	10,300	386	5491	10,505
Sult1e1			339		670 2816	5233 9758	5309 8118	519
Sult2a1					5528 9856		9646	
Sult2b1	4329					584 3516 9160		
Sult3a1					1673 4022 5806	5423 7861	5580 6694	3901
Sult4a1	96	5725	8236 9666		4104 7499	1366	1464 2061 3740	8465 9775 9867
Sult5a1	648 4376	5871 6124 9503	1478 3320 5351 5548	8816	6788		8627 8739	3300
PAPSs1		9415	1471		2461 2656 5244 8196	8609	4276 6798	
PAPSs2	2569	8527			6733 6847	4690	5587 7204 7409 8251	

Table 4 demonstrates the presence of potential Nrf2 binding sites (antioxidant-response element) in the promoter region of several Sults. Overall, Nrf2 activators appear to induce the mRNA expression of Sults in male and have mixed effects in female mice.

The Sult1 family conjugates phenolic compounds with overlapping substrate specificity between the individual isoenzymes. Sult1 substrates include thyroid hormones (T3 and T4) catecholamines, estrogens, and androgens (Strott, 2002). In this current study, members of the Sult1 family were generally resistant to induction by activators of all five transcription pathways, with the exception of Sult1e1, which is induced in male but not female mice, by PXR ligands. Sulfonation is a major metabolic pathway for steroidal hormone deactivation, where 40 to 70% of circulating steroids are in the sulfated form. Sult1e1 particularly catalyzes the sulfonation of estrogens with K_m values in the low nanomolar range (Falany et al., 1994). Therefore, the induction of Sult1e1 reported here, especially the ~100-fold induction by the PXR ligand (DEX), might increase endogenous and exogenous steroid detoxification.

The Sult2 family mainly conjugates hydroxysteroid-like compounds, including androsterone, pregnenolone, dehydroepiandrosterone, and bile acids (Strott, 2002). In the Sult2 family, Sult2b1 mRNA expression is not affected by any inducer. The basal level of Sult2a1/2a2 mRNA expression in livers of male mice is almost undetectable, in contrast to female mice where Sult2a1/2a2 mRNA is the highest expressed Sult isoenzyme. Female rats also have higher expression of Sult2a1 than male rats (Dunn and Klaassen, 1998). This is in agreement with Runge-Morris and co-workers report, where Sult2a1 mRNA was not detected in different mice strains, including the one used for this study (C57BL/6) by Northern blotting (Wu et al., 2001). In the same report, the induction of Sult2a1 by DEX was observed in female but not in male mice; however, induction occurred in both genders in rats. In this current study, we report a similar phenomenon, where the CAR activator (TCPOBOP) and the PXR ligand (PCN) induce Sult2a1/2a2 mRNA expression in female but not in male mice. Both mice and rats exhibit gender bias in basal expression of Sult2a1 toward females, but the extent of this gender difference is more prominent in mice (Runge-Morris and Wilusz, 1991; Wu et al., 2001). Furthermore, Sult2a1 expression is inducible by PXR activators in both

male and female rats, whereas in mice it is induced only in females (Wu et al., 2001).

Regulation of Sult2a1 expression is the most studied Sult isoenzyme in different species because of its important role in detoxification of bile acids. Sult2a1 expression was reported to be activated by CAR, PXR, and VDR transcription pathways (Sonoda et al., 2002; Echchgadda et al., 2004b; Saini et al., 2004). We also report the induction of Sult2a1/2a2 mRNA by CAR and PXR activators. Sulfation is the major metabolic pathway for the detoxification of the toxic secondary bile acid, lithocholic acid (LCA), and Sult2a1 is the exclusive enzyme for LCA sulfoconjugation (Radominska et al., 1990). Accumulation of secondary bile acids, especially LCA, is a risk factor for cholestatic liver injury and colorectal cancer (Sonoda et al., 2002). Induction of Sult2a1 has been suggested to protect against the toxicity of LCA and might represent a target strategy for the treatment of cholestasis and colon cancer (Sonoda et al., 2002). In fact, rifampicin, a PXR ligand in humans, has been reported to be useful in treating cholestasis, which might be in part due to Sult2a1 induction (Bachs et al., 1989; Cancado et al., 1998; Sonoda et al., 2002). The higher expression of Sult2a1 in female than male mice is thought to contribute to the fact that female farnesoid X receptor-null mice are less susceptible to lithocholic acid toxicity than male mice (Sinal et al., 2000; Kitada et al., 2003; Zhang et al., 2004). The present data indicate that not only is Sult2a1/2a2 mRNA expression higher in female mice, CAR and PXR activators induce Sult2a1/2a2 in female mice only. This might imply an important role of CAR and PXR activators in further protecting female mice from secondary bile acid toxicity compared to males.

Very little is known about the regulation of Sult3, 4, and 5 families. The present study is the first to report the regulation of these Sult families by MEIs in mice. The basal expression of these Sults is very low in mouse liver, with the exception of Sult3a1, which is highly expressed in female livers. In male mice, the mRNA expression of Sult5a1 was induced by all AhR, one PXR, and two Nrf2 activators, whereas it was suppressed by PXR, PPARα, and Nrf2 activators in female mice.

PAPSs is responsible for the bioactivation of inorganic sulfate into PAPS, which is utilized by Sults to catalyze sulfonation of target compounds. Two PAPSs isoenzymes have been cloned from humans (ul Haque et al., 1998) and mice (Kurima et al., 1998), namely PAPSs1 and PAPSs2. The two isoenzymes differ in their tissue distribution and catalytic activity (Fuda et al., 2002). Another difference in PAPSs isoenzymes is reported in this study. PAPSs1 mRNA expression is resistant to all 15 MEIs in both male and female mice. In contrast, PAPSs2 mRNA expression is induced by PXR and PPARα ligands in male mice and PXR and CAR activators in female mice. This is in agreement with previous reports of PAPSs2 induction by CAR (Saini et al., 2004) and PXR (Sonoda et al., 2002) activators in mice.

The current data indicate fundamental gender differences not only in the basal mRNA expression of Sults but also in their inducible mRNA expression. Many Sult isoforms were induced in one gender; whereas to a lesser extent (for example Sult1d1 by TCPOBOP and PAPSs2), they were either not affected (for example, Sult1e1 by TCPOBOP, and Sult2a1/2a2 by TCPOBOP and PCN) or were suppressed (for example, Sult1c1 by CLOF, and Sult5a1 by PCN) in the other gender by the same inducer. This gender difference in the inducible expression of Sults was previously reported in the case of the Sult2a1 isoform (Wu et al., 2001), and according to the data in the present study, this applies for other Sult isoenzymes as well. This might explain part of the contradiction on the regulation of Sult expression in the literature, where male animals are usually used as in vivo models to study the regulation of enzyme expression.

Historically, Sults have not been considered as xenobiotic-inducible enzymes (Thompson et al., 1982; Runge-Morris et al., 1998). Yet, many studies have reported the regulation of different Sult isoenzymes via different transcriptional pathways. These studies have reported contradictory results in many occasions, as mentioned above. This can be attributed to the fact that i) different activators were used with different doses for the same transcriptional pathway; ii) different methodologies were used ranging from in vitro incubation, in vivo animal dosing, and reporter construct genes cotransfected with the transcription factor gene; iii) induction of Sults was studied at different levels [i.e., mRNA expression with different techniques (reverse transcription-polymerase chain reaction, Northern blotting), protein expression with Western blotting, and Sults activity with various substrates]; iv) Sults were induced in various species, mainly, mice, rats, and humans, providing different results due to species variations; and v) most of these studies considered one gender as a model. The present data indicate that Sults gene regulation and induction is clearly gender-dependent. In the present study, the mRNA regulation of Sults and PAPSs enzymes by microsomal enzyme inducers in both genders of one species (mice), and using one technique (bDNA), was studied. Based on the Sult literature and on this current study, it can be concluded that most typical microsomal inducers do not have as marked effect on Sults expression as they have on phase-I metabolic enzymes. Furthermore, the current data indicate fundamental gender differences not only on the basal mRNA expression of Sults but also their inducible mRNA expression.

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