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Importance of Hepatic Induction of Constitutive Androstane Receptor (CAR) and Other Transcription Factors That Regulate Xenobiotic Metabolism and Transport

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Abbreviations: AhR, aryl hydrocarbon receptor; ARE, antioxidant response element; BHA, butylated hydroxyanisole; CAR, constitutive androstane receptor; DEHP, Di (2-ethylhexyl) phthalate; MEI, microsomal enzyme inducer; MRP, multidrug resistance associated protein; Nqo1, NAD(P)H quinine oxidoreductase 1; Nrf2, nuclear factor E2 related factor 2; PBREM, Phenobarbital response element module; PCB-126; polychlorinated biphenyl 126; PCN, Pregnenolone-16α-carbonitrile; PPARα, peroxisome proliferator activated receptor alpha; PXR, pregnane X receptor; PXRE, PXR response element; TCPOBOP, 1,4-bis-[2-(3,5idichloropyridyloxy)] benzene.

Abstract

Aryl hydrocarbon receptor (AhR), constitutive androstane receptor (CAR), pregnane X receptor (PXR), peroxisome proliferator-activated receptor alpha (PPARα), and nuclear factor E2 related factor 2 (Nrf2) are transcription factors that mediate xenobiotic induction of biotransformation enzymes and transporters. The purpose of this study was to determine the tissue distribution and xenobiotic induction of these transcription factors and their associated target genes in mice. Many of these transcription factors were most highly expressed in extrahepatic tissues. CAR expression in female liver was twice that of male liver. This corresponded with greater induction of the CAR target genes Cyp2b10 and multidrug resistance associated protein (Mrp) 4 by the CAR activator TCPOBOP in female liver than in male liver. Mice were treated with xenobiotic activators of AhR, CAR, PXR, PPARα, or Nrf2 and their associated marker genes were highly induced in liver by these xenobiotic activators. Transcription factor target gene induction occurred with minimal induction of their associated transcription factors. CAR expression was induced the AhR ligand TCDD, leading to increased basal expression of Cyp2b10 mRNA and enhanced induction of Cyp2b10 by TCPOBOP. Mrp2, 3, and 4 induction was augmented by co-treatment with TCDD and TCPOBOP compared to either compound alone. These studies illustrate CAR induction by TCDD in mice, indicating that AhR may transcriptionally regulate CAR and thus enhance induction of key metabolism and transporter genes by the CAR activator TCPOBOP. Collectively, these studies illustrate that some xenobiotic inducers may elicit their response through mechanisms involving transcription factor regulation.

Introduction

Emerging evidence illustrates that physiological regulation of transporters and biotransformation enzymes is under control of transcription factors that are responsive to an array of xenobiotics and endogenous activators (Xie and Evans, 2001; Staudinger *et al.*, 2003; Klaassen and Slitt, 2005). Specifically, the aryl hydrocarbon receptor (AhR), constitutive androstane receptor (CAR), pregnane-X receptor (PXR), peroxisome proliferator-activated receptor alpha (PPARα), and nuclear factor-E2 related factor 2 (Nrf2), are transcription factors through which classical microsomal enzyme inducers (MEIs) and other chemicals increase the expression of transporters and biotransformation enzymes (Klaassen and Slitt, 2005).

The AhR is activated by dioxin and other aromatic hydrocarbons leading to activation of target gene transcription through binding to xenobiotic response elements found within their promoter regions (Reyes *et al.*, 1992; Harper *et al.*, 2006). AhR activation markedly induces mRNA expression and enzyme activity of cytochrome P450 (CYP) 1A1 and CYP1A2 in rats (Kedderis *et al.*, 1991) and Cyp1a1 and Cyp1a2 in mice (Beebe *et al.*, 1990). Furthermore, AhR is also important in regulating inducible expression of xenobiotic metabolizing enzymes including NAD(P)H quinone oxidoreductase 1 (Nqo1), aldehyde dehydrogenase 3a1, UDP glucuronosyl transferase (Ugt) 1a6, and glutathione *S*-transferase (Gst) α1 (Nebert *et al.*, 2000).

The orphan nuclear receptor CAR mediates Cyp2b10 induction in response to phenobarbital (PB) and 1,4-bis-[2-(3,5idichloropyridyloxy)] benzene (TCPOBOP) in

mice (Wei *et al.*, 2000). CAR is also associated with xenobiotic-induced transactivation of human CYP2B6 and CYP3A4 (Kawamoto *et al.*, 1999). Target gene activation by CAR is mediated through binding to phenobarbital response element modules (PBREM) found within target gene promoter regions (Honkakoski and Negishi, 1997). Although CAR is a critical transcription factor mediating Cyp induction, CAR also mediates hepatic induction of some phase II xenobiotic metabolizing enzymes such as Ugt1a1, Gstα1, Gstα2, and sulfotransferase 2a1 (Huang *et al.*, 2003; Assem *et al.*, 2004). CAR has also been implicated in the induction of canalicular export transporters in liver, namely the multidrug resistance associated proteins (Mrp) Mrp2, Mrp3, and Mrp4 (Cherrington et al., 2002; Johnson and Klaassen, 2002; Cherrington et al., 2003; Slitt et al., 2003; Assem et al., 2004; Maher et al., 2005a). Furthermore, CAR activation by TCPOBOP augmented cholic acid induction of mouse biotransformation enzymes and uptake transporters (Guo *et al.*, 2003). Thus, xenobiotics that activate CAR can regulate both xenobiotic metabolism and transport.

PXR is an orphan nuclear receptor that facilitates hepatic and intestinal induction of Cyp3a expression by xenobiotics, synthetic glucocorticoids, and naturally occurring steroids (Kliewer *et al.*, 1998), a function mediated through PXR response element (PXRE) binding. The pivotal role of PXR in regulating induction of Cyp3a11 and organic anion transporting polypeptide (Oatp) 2 (new nomenclature, Oatp1a4) in mice by the PXR agonist pregnenolone-16α-carbonitrile (PCN) and by lithocholic acid (a bile acid), was established using PXR-null mice (Staudinger *et al.*, 2001).

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The nuclear receptor PPARα regulates target gene transactivation through binding to peroxisome proliferator response elements (reviewed by Kliewer *et al.*, 1994). Mouse PPARα mediates peroxisome proliferation and hepatomegaly in response to the classical peroxisomal proliferators Wy-14,643 and clofibrate (Lee *et al.*, 1995). As evidenced by studies in PPARα-null mice, PPARα also mediates hepatic mRNA induction of fatty acyl-CoA oxidase, and the fatty acid β-oxidation and ω-oxidation genes, Cyp4a10 and Cyp4a14, respectively (Lee *et al.*, 1995; Barclay *et al.*, 1999).

Nrf2 is an oxidative stress and antioxidant inducible transcription factor that mediates target gene transactivation through antioxidant response element (ARE) binding (McMahon *et al.*, 2001). Nrf2 is thought to regulate expression of a battery of antioxidant genes including its classical target gene Nqo1 (Venugopal and Jaiswal, 1996), GSTs and the glutamate cysteine ligase catalytic and modifier subunits (McMahon *et al.*, 2001). Furthermore, Nrf2 has been implicated in regulation of inducible expression of the Mrp1 (Hayashi *et al.*, 2003) and Mrp2 (Vollrath *et al.*, 2006) transporters in mice. Nrf2 is thus an important regulator of both xenobiotic metabolism and transport.

AhR, CAR, PXR, PPARα, and Nrf2 are important molecular mediators that regulate expression of xenobiotic metabolizing enzymes and transporters. In the present study, the tissue distribution and induction of these key transcription factors and their associated target genes was investigated in mice in order to better understand the expression of these genes in vivo. The present studies illustrate transcription factor induction by xenobiotics that are known to induce biotransformation enzymes and

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transporters and thus implicate an important role of transcription factors in xenobiotic induction of drug metabolism and disposition.

Methods

Animals. Male and female C57BL/6 mice (age 7 weeks, 18-20g) were obtained from Charles River Laboratories, Inc. (Wilmington, MA) and were housed in an American Animal Association for Laboratory Animal Care accredited facility. Mice were given access to Teklad 8064 rodent chow (Harlan Labs, Madison, WI) and water ad libitum. Mice were acclimated to the housing facility for 1 week prior to experiments (4 mice per cage, 50 % relative humidity, 12-h light/dark cycle).

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 was a gift from Dr. Steve Safe (Texas A&M University, College Station, TX). 3,3',4,4',5-Pentachlorobiphenyl (PCB-126) was purchased from AccuStandard (New Haven, CT). All other chemicals were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO). Names, designated abbreviations, and CAS Registry numbers for all chemicals are cited in Table 1.

Transcription Factor Tissue Distribution. Eight-week-old male and female C57BL/6 mice were euthanized and the following organs collected: liver, kidney, lung, stomach, duodenum, jejunum, ileum, large intestine, brain, heart, testes, ovaries, and uterus. Placentae were isolated from pregnant C57BL/6 female mice 2 days before parturition. The contents of the stomach and intestinal segments were removed by cutting these

tissues open and washing them in 0.9 % saline. All tissues were snap frozen in liquid nitrogen and stored at -80ºC prior to RNA isolation. All data represent n=5 per tissue. Heart and lung data represent an n=5 of three pooled organs each. Placenta data represent an n=5 of five pooled organs each. Ovary data represent an n=5 of twenty-five pairs of pooled organs each.

Transcription Factor, Cyp and Mrp Induction Studies. Eight-week-old male and female C57BL/6 mice were treated with microsomal enzyme inducers of AhR, CAR, PXR, PPARα, and Nrf2. The microsomal enzyme inducer compounds, dosing regimens, target receptors, and marker genes are given in Table 1. Control animals were dosed with either corn oil or saline vehicle. Mice were treated for 4 days using an injection volume of 5 ml/kg (n=5 per treatment). Livers were removed from animals on day 5, snap frozen in liquid nitrogen, and stored at -80ºC.

TCDD and TCPOBOP Co-exposure Studies. Eight-week-old male C57BL/6 mice were treated with TCDD (AhR ligand) and/or TCPOBOP (CAR activator) over the course of four days. Treatment groups were as follows: 1) corn oil; corn oil ip (5 ml/kg) 1x/day for four days, and 1 corn oil ip injection (5 ml/kg) 12 h before organ harvesting. 2) TCDD; 37 μg/kg/day TCDD ip for 4 days and 1 corn oil ip injection (5 ml/kg) 12 h before organ harvesting. 3) TCPOBOP; corn oil ip for four days, and a single 3 mg/kg ip TCPOBOP dose 12 h before organ harvesting. 4) TCDD plus TCPOBOP; 37 μg/kg/day TCDD ip for 4 days, and a single 3 mg/kg ip TCPOBOP dose 12 h before harvesting. All treatments were given using an injection volume of 5 ml/kg (n=5 per treatment). Corn oil

was used as a vehicle for all treatment groups. All mice were treated once a day for four days and were given a final treatment 12 h before organ harvesting. Livers were removed and snap frozen in liquid nitrogen, and stored at -80ºC.

Total RNA Isolation. Total RNA was isolated from frozen mouse tissues using the RNA-Bee RNA isolation reagent (Tel-Test Inc., Friendswood, Texas), as per the manufacturer's protocol. RNA pellets were resuspended in diethylpyrocarbonate-treated deionized water. RNA concentration was evaluated spectrophotometrically by its absorbance at 260 nm. Agarose gel electrophoresis was used to evaluate RNA integrity by observation of 18S and 28S ribosomal RNA bands visualized via ethidium bromide fluorescence under ultraviolet illumination.

Branched-DNA (bDNA) Analysis of Gene Expression. The bDNA method has been utilized extensively in our laboratory and the methods used have been described in detail (Maher et al., 2005b). Multiple sequence specific probes were developed to each mRNA transcript of interest (Table 2). Probe sequences for Mrp2, Mrp3, and Mrp4 were published previously (Maher et al., 2005b),as were the probe sequences for Cyp1a1, Cyp2b10, Cyp3a11, Cyp4a14, and Nqo1 (Cheng et al., 2005). Gene expression analysis was performed using the QuantiGene Screen bDNA signal amplification kit (Genospectra, Fremont, CA) and luminescence was quantified using a Quantiplex® 320 bDNA luminometer interfaced with Quantiplex® data management software version 5.02

(Bayer Corporation Diagnostics Division, Tarrytown, NY). All data are expressed as relative luminescence units (RLU) per 10 μg total RNA.

Statistics. Data from male and female mice were compared in the tissue distribution study using Student's t-test. CAR target gene analysis between males and females or between control and treated animals were also analyzed using Student's t-tests. Control groups (corn oil po, corn oil ip, and saline ip) for the transcription factor inducer study were analyzed by one-way ANOVA, followed by Bonferroni's post-hoc test. This analysis indicated no statistical difference between corn oil or saline controls, regardless of route of exposure. Controls (n=13) were thus pooled for remaining analyses. Inducer data were evaluated for statistical significance using ANOVA, followed by Duncan's multiple range post hoc test. All statistical flags represent a statistically significant difference ($p<0.05$), as described in the figure legends.

Results

Tissue Distribution of Transcription Factors in Mice. AhR mRNA was ubiquitously expressed in the tissues analyzed in this study, with the highest expression observed in the lung (Fig. 1). Expression of AhR mRNA did not exhibit gender differences in any of the tissues examined. CAR mRNA expression was highest in liver, followed by duodenum, jejunum, lung, heart, and placenta (Fig 1). CAR expression was female-predominant in liver and male-predominant in brain and heart. Expression of PXR mRNA was highest in the intestinal tract and was moderately expressed in liver (Fig. 1). PXR expression exhibited a slight, but statistically significant female predominance in liver and brain. PPARα mRNA expression was highest in liver, kidney, and heart (Fig. 1). PPARα was moderately expressed in the intestinal tract, lung, and gonads, and exhibited slight female-predominance in lung and duodenum (Fig. 1). Expression of Nrf2 mRNA was highest in the stomach, followed by the intestinal tract and lung (Fig 1). Nrf2 was moderately expressed in liver, kidney, heart, ovary, uterus, and placenta. Nrf2 exhibited female predominance in the lung and in gonad, as expression was higher in the ovary than in testes.

Induction of Transcription Factor and Marker Gene mRNA in Liver by Microsomal Enzyme Inducers. AhR was not consistently induced by any class of transcriptional inducers. However, AhR mRNA expression in liver was induced 250% by the AhR activator β-naphthoflavone (BNF), but not by other AhR activators (Fig. 2). AhR was induced 170% by both the PXR activator spironolactone (SPR) and the PPARα

activator clofibrate (CLOF), and was induced 300% by the Nrf2 activator butylated hydroxyanisole (BHA) (Fig. 2). The AhR target gene Cyp1a1 was highly induced (up to 1200 fold) by AhR activators, but not by any activators of CAR, PXR, PPARα, or Nrf2 (Fig. 2).

Hepatic expression of CAR mRNA was induced by all three AhR activators: 160% by TCDD, 120% by PCB-126, and 60% by BNF (Fig. 3). CAR expression was doubled by the PPARα activator 2-diethylhexyl phthalate (DEHP) and was modestly induced by the Nrf2 activator BHA (Fig. 3). CAR expression, however, was not induced by activators of CAR or PXR. The CAR marker gene Cyp2b10 was highly induced by CAR activating compounds: 118 fold by TCPOBOP, 13 fold by diallyl sulfide (DAS), and 17 fold by phenobarbital (PB) (Fig. 3). The AhR ligand TCDD modestly induced expression of Cyp2b10 (Fig. 3 and Fig. 8). The PXR activators pregnenolone 16α-carbonitrile (PCN) and DEX induced Cyp2b10 mRNA expression by 9.4 and 25.7 fold, respectively. The PPARα activators CLOF and DEHP induced Cyp2b10 mRNA expression 3.5 and 2.8 fold, respectively. Furthermore, the Nrf2 activators oltipraz (OPZ), ethoxyquin (EXQ), and BHA induced expression of Cyp2b10 by 8.9, 9.1, and 4.9 fold, respectively (Fig. 3).

Expression of PXR in liver was modestly induced by the PXR activator SPR, by the PPARα activators CLOF and DEHP, and by the Nrf2 activator BHA (Fig. 4). Neither AhR nor CAR activators altered expression of PXR (Fig. 4). Expression of the PXR target gene Cyp3a11 was reduced 41% by the AhR ligand BNF (Fig. 4). The CAR

activators TCPOBOP, DAS, and PB induced Cyp3a11 expression 190, 140, and 110%, respectively (Fig. 4). Cyp3a11 was induced markedly by PXR activators: 320% by PCN, 200% by SPR, and 310% by DEX (Fig. 4). Expression of Cyp3a11 was modestly induced by the PPARα activator CLOF.

Hepatic expression of PPARα was reduced 50% following treatment with the CAR activator TCPOBOP (Fig. 5). PPARα expression was slightly induced by the PXR activator SPR, and by the PPARα activators CLOF and CPFB (Fig. 5). Expression of PPARα was not altered in response to any AhR or Nrf2 activator. Hepatic expression of the PPARα marker gene Cyp4a14 was downregulated by treatment with activators of AhR and CAR (Fig. 5). The PXR activators PCN and SPR decreased expression of Cyp4a14, whereas DEX induced Cyp4a14 expression 170% (Fig. 5). The PPARα activators CLOF, CPFB, and DEHP induced Cyp4a14 expression 29, 30, and 25 fold, respectively (Fig. 5). Cyp4a14 was also modestly induced by the Nrf2 activator BHA.

Nrf2 expression was induced 230% by the AhR activator TCDD (Fig. 6). The PXR activator dexamethasone (DEX) reduced expression of Nrf2 by 47% (Fig. 6). Activators of CAR, PPARα, and Nrf2 did not alter expression of Nrf2 mRNA. Expression of the Nrf2 target gene Nqo1 in mouse liver was induced 370, 160, and 470% by the AhR activators TCDD, PCB-126, and BNF, respectively (Fig. 6). Hepatic expression of Nqo1 was induced 300% by the CAR activator DAS, and 110% by the PXR activator PCN (Fig. 6). Nqo1 expression was induced 150% by the PPARα activators CLOF and

DEHP. Furthermore, Nqo1 induction was observed in response to the Nrf2 activators OPZ, EXQ, and BHA (350, 280, and 680%, respectively, Fig. 6).

Gender-Divergent Induction of CAR Target Genes in Liver by the CAR ligand TCPOBOP. Basal expression of CAR mRNA was significantly higher (260%) in liver of female C57BL/6 mice treated with corn oil than in males (Fig. 7). CAR expression was not inducible by TCPOBOP treatment in males or females. TCPOBOP reduced basal expression of CAR in females. The CAR target gene Cyp2b10 exhibited 14-fold higher basal expression in females than in males. Cyp2b10 was highly induced by TCPOBOP in male and female mouse liver, however, Cyp2b10 was induced to a higher level in females. The fold induction of Cyp2b10 by TCPOBOP in males was greater than that observed in females. Basal expression of Mrp2 and Mrp3 in female liver was double that observed in males, and this same trend was observed for Mrp4 (though not statistically significant, Fig. 7). The fold induction of Mrp2 and Mrp3 by TCPOBOP did not exhibit a gender difference, although the magnitude of the expression of these transporters was induced to a greater extent in females than in males. Mrp4 induction by TCPOBOP in female liver was twice than observed in males.

Hepatic Induction of CAR and Cyp2b10 mRNA by AhR Ligands in Mice. CAR expression in mouse liver was induced 360% by treatment with TCDD for 4 days (Fig. 8). CAR expression was not induced in liver following a 12-h treatment with TCPOBOP, although a four-day TCDD treatment followed by a 12-h TCPOBOP treatment induced

CAR expression by 250% over corn oil control and by 320% compared to treatment with TCPOBOP alone. Cyp2b10 expression was induced 330% by treatment with TCDD alone, and was markedly induced (95-fold) by TCPOBOP alone (Fig. 8). Pre-treatment with TCDD for four days, followed by a 12-h TCPOBOP treatment induced Cyp2b10 by 180 fold, nearly twice the induction observed in animals treated with TCPOBOP alone.

Hepatic Induction of Mrp2, Mrp3, and Mrp4 in Mice. Mrp2 mRNA expression was not significantly induced by TCDD treatment alone, however, Mrp2 was induced 190% by TCPOBOP treatment alone. Pre-treatment with TCDD for four days, followed by a 12-h TCPOBOP treatment induced Mrp2 expression by 285% over control, by 190% over TCDD alone, and by 150% over TCPOBOP alone (Fig. 9). Mrp3 mRNA expression was induced 265% over corn oil controls in mice pre-treated with TCDD and subsequently given TCPOBOP, but not by treatment with either compound alone (Fig. 9). Mrp4 mRNA expression was not induced by treatment with TCDD (four days) or TCPOBOP (12 hrs) alone. Although a statistically significant reduction in Mrp4 mRNA expression was also observed after 12 hours of TCDD treatment, the extremely low level of expression of Mrp4 at levels near the limit of detection call into question any potential biological significance for this observation. However, in mice pre-treated with TCDD, followed by TCPOBOP treatment, Mrp4 mRNA was induced to a significant extent relative to those animals administered corn oil vehicle or either compound alone.

Discussion

The present studies examined tissue distribution, gender divergent expression, and hepatic induction of AhR, CAR, PXR, PPARα, and Nrf2 mRNA in mice. Gender divergent expression and hepatic induction of CAR by TCDD produced concomitant alterations in expression of its associated target genes. Data from the present studies show that in some cases, AhR, CAR, PXR, PPARα, and Nrf2 were modestly induced by chemical activators that markedly induce expression of their associated target genes. Collectively, these data indicate that inducible expression of several of these transcription factors may play an important role in induction of their associated target genes.

AhR, CAR, PXR, PPARα, and Nrf2 were ubiquitously expressed in the observed tissues and typically exhibited higher expression levels in extrahepatic tissues. The tissue expression patterns of these transcription factors coincided with those reported in other mouse studies appearing in the literature (Kliewer *et al.*, 1994; Li *et al.*, 1994; Moi *et al.*, 1994; Choi *et al.*, 1997; Kliewer *et al.*, 1998; McMahon *et al.*, 2001; Bookout *et al.*, 2006). The present study is the first to quantitatively compare expression of Nrf2 in various segments of the small intestine, the large intestine, and in several portions of the female reproductive tract. Nrf2 expression was highest in stomach and intestinal tract, consistent with earlier studies noting that the highest expression of Nrf2 is in extrahepatic tissues (Moi *et al.*, 1994; McMahon *et al.*, 2001).

In the present studies, xenobiotic induction of AhR, CAR, PXR, PPARα, and Nrf2 was evaluated in the liver, along with induction of their associated target genes,

Cyp1a1, 2b10, 3a11, 4a14, and Nqo1, respectively. The xenobiotic compounds used in this study are classical microsomal enzyme inducers (MEIs) whose induction by xenobiotics is mediated by these transcription factors (Table 1). The specificity of this induction was evaluated by examining the induction of these transcription factors by MEIs and by observing corresponding induction of their associated target genes. In most cases, transcription factor induction was modest, whereas marked induction of their associated marker genes was observed. Xenobiotic inducers exhibited selective induction of the target genes, and minimal selectivity in induction of their associated transcription factors.

AhR ligands were potent inducers of Cyp1a1 mRNA expression and also modestly induced expression of the Nrf2 marker gene Nqo1. Induction of Nqo1 expression by AhR ligands requires Cyp1a1 expression and is thus likely to involve the AhR (Marchand *et al.*, 2004). Furthermore, Cyp1a1 oxidative metabolites may initiate ARE-dependent activation of Nqo1 through a Nrf2-dependent signaling pathway, as TCDD induction of Nqo1 expression requires the presence of Nrf2 (Ma *et al.*, 2004). Interestingly, the Nrf2 activator BHA significantly induced expression of AhR mRNA, indicating a further relationship between these two important transcription factor pathways. AhR mRNA expression was modestly induced in response to the AhR activator BNF, however, AhR was not induced significantly by other AhR activator or in response to more than one activator from any other class of microsomal enzyme inducer used in the present studies (Fig. 2).

CAR and PXR activators markedly enhanced hepatic Cyp2b10 and Cyp3a11 mRNA expression, as these transcription factors bind to both the PBREM and PXRE (Wang and Negishi, 2003). Induction of Cyp2b10 mRNA expression by PPARα and Nrf2 activators in the present study may also implicate these transcription factors in Cyp2b regulation. In the present studies, CAR mRNA expression was not induced by CAR activators (Fig. 3), however, expression of CAR in the liver was induced by all three AhR ligands and TCDD modestly induced basal expression of the CAR target gene Cyp2b10. Furthermore, induction of Cyp2b10 and Mrp2, 3, and 4 mRNA expression by TCPOBOP was also enhanced following CAR induction by TCDD (Fig. 8 and Fig. 9). The present studies thus present the novel finding that AhR can regulate expression of CAR and its downstream target genes. Because of the important role played by CAR in regulation of xenobiotic metabolism and transport, induction of CAR by TCDD implicates AhR as an important regulator of drug metabolism and disposition.

In the present studies, hepatic expression of PXR was modestly inducible by the PXR ligand SPR, and by the Nrf2 activator BHA. PXR expression was doubled by the PPARα agonists CLOF and DEHP in liver, findings that may merit further investigation. Only PPARα ligands markedly induced Cyp4a14 expression in liver, thus showing the high degree of selectivity of these ligands as Cyp4a14 inducers. Hepatic expression of PPARα mRNA was also moderately induced by the PPARα ligands CLOF and CPFB, making this the only transcription factor in the present study to be induced by its activator compounds.

Nrf2 activators induced hepatic Nqo1 expression up to three-fold, consistent with intestinal induction of Nqo1 by the Nrf2 activators BHA and OTZ (McMahon *et al.*, 2001). Nqo1 was markedly induced in liver by AhR ligands and Nqo1 induction was modest in response to CAR, PXR, and PPARα activators. The present data illustrate that marked induction of Nrf2 is not required for Nqo1 induction in vivo. Nrf2 expression in liver was not appreciably inducible by CAR, PXR, PPARα, or Nrf2 activators, however, Nrf2 expression was more than doubled by treatment with the AhR ligand TCDD. In rats, Nqo1 induction by AhR activators is dependent on CYP1A1 expression, thus implicating the AhR as a mediator of Nqo1 induction (Marchand *et al.*, 2004). This induction may also be mediated via ARE-dependent Nqo1 activation by Cyp1a1 oxidative metabolites as evidenced by Nrf2-dependent induction of Nqo1 by TCDD (Ma *et al.*, 2004).

The present studies, and numerous reports in the literature thus illustrate the complex nature of the interaction between xenobiotic activator compounds and transcription factors. Furthermore, the present studies also demonstrate the non-selectivity of these classical xenobiotic activator compounds. In addition to tissue-specific variations in transcription factor expression and xenobiotic-dependent induction of these genes and their associated target genes, a number of these transcription factors also exhibited gender-divergent expression in certain tissues. Most notably, CAR expression in female liver was found in the present study to be twice that in male liver.

A gender difference in mouse hepatic CAR expression has been previously noted (Kawamoto *et al.*, 2000; Ledda-Columbano *et al.*, 2003). Furthermore, basal and

inducible expression of Cyp2b10 mRNA in female liver is greater than that observed in males (Honkakoski *et al.*, 1992; Ledda-Columbano *et al.*, 2003). *In vitro* studies demonstrate that estrogen stimulates activation of mouse CAR (Kawamoto *et al.*, 2000). Further evidence that gender-divergent responses to CAR activators in mice are mediated by sex hormones, is demonstrated by suppression of TCPOBOP-mediated CAR and Cyp2b10 induction following treatment with androstanol (Ledda-Columbano *et al.*, 2003). In the present study, CAR was not induced by TCPOBOP in the livers of male mice, a finding that was reproduced in several independent experiments (Figs 2, 7, and 8), thus indicating an apparent strain difference between the C57BL6/J mouse used in the present study and the CD-1 mouse, in which CAR was induced by TCPOBOP in both male and female mice (Ledda-Columbano *et al.*, 2003). Furthermore, in the present studies, CAR expression was reduced following four days of TCPOBOP administration, indicating another possible strain difference between C57BL6/J and CD-1 mice.

In addition to regulation of Cyp2b10, CAR has been implicated in xenobiotic induction of the Mrp transporters Mrp2, 3, and 4 (Cherrington et al., 2002; Assem et al., 2004; Maher et al., 2005a). The present studies illustrate that basal expression of Mrp2, 3, and 4 is female-predominant. Although relative expression of Mrp2, 3, and 4 was higher in TCPOBOP-treated female mice than in TCPOBOP-treated male mice, only Mrp4 was induced to a greater extent in females than in males. Taken together, these data illustrate that elevated CAR expression in females produces elevated basal and inducible expression of the CAR target genes Cyp2b10, Mrp2, Mrp3, and Mrp4.

Despite marked induction of target genes by activators of AhR, CAR, PXR, PPARα, and Nrf2, these transcription factors were modestly inducible or not induced by their associated chemical activators. With the exception of CAR induction by AhR and its effect on CAR target genes, those compounds that significantly induce any of the selected transcription factors tended to have little or no effect on expression of their associated target genes. Additionally, the present studies show that these classical inducer compounds activate their targeted nuclear receptor pathways and that they may also selectively activate other transcription factor pathways and their associated target genes.

Collectively, the present data provide important insight into the tissue specific expression, gender divergent expression and xenobiotic regulation of AhR, CAR, PXR, PPARα, and Nrf2 mRNA and of their associated target genes. The present studies provide useful information that may enhance the understanding of the role of both gender and xenobiotics in the regulation of xenobiotic metabolizing enzymes and transporters. Understanding this gene regulation is critical, as dysregulation of transporters and drug metabolizing enzymes can produce significant variations in drug absorption, distribution, metabolism, and elimination, and can thus adversely impact clinical outcome of drug treatment.

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References

Assem M, Schuetz EG, Leggas M, Sun D, Yasuda K, Reid G, Zelcer N, Adachi M, Strom S, Evans RM, Moore DD, Borst P and Schuetz JD (2004) Interactions between hepatic Mrp4 and Sult2a as revealed by the constitutive androstane receptor and Mrp4 knockout mice. *J Biol Chem* **279:**22250-22257.

Barclay TB, Peters JM, Sewer MB, Ferrari L, Gonzalez FJ and Morgan ET (1999) Modulation of cytochrome P-450 gene expression in endotoxemic mice is tissue specific and peroxisome proliferator-activated receptor-alpha dependent. *J Pharmacol Exp Ther* **290:**1250-1257.

Beebe L, Park SS and Anderson LM (1990) Differential enzyme induction of mouse liver and lung following a single low or high dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). *J Biochem Toxicol* **5:**211-219.

Bookout AL, Jeong Y, Downes M, Yu RT, Evans RM and Mangelsdorf DJ (2006) Anatomical profiling of nuclear receptor expression reveals a hierarchical transcriptional network. *Cell* **126:**789-799.

Cheng X, Maher J, Dieter MZ and Klaassen CD (2005) Regulation of mouse organic anion-transporting polypeptides (Oatps) in liver by prototypical microsomal enzyme inducers that activate distinct transcription factor pathways. *Drug Metab Dispos* **33:**1276-1282.

Cherrington NJ, Hartley DP, Li N, Johnson DR and Klaassen CD (2002) Organ distribution of multidrug resistance proteins 1, 2, and 3 (Mrp1, 2, and 3) mRNA and hepatic induction of Mrp3 by constitutive androstane receptor activators in rats. *J. Pharmacol Exp. Ther.* **300:**97-104.

Cherrington NJ, Slitt AL, Maher JM, Zhang X, Zhang J, Huang W, Wan YY, Moore DD and Klaassen CD (2003) Induction of multidrug resistance protein 3 (MRP3) in vivo is independent of constitutive androstane receptor. *Drug Disp Metab* **31:**1315-1319.

Choi HS, Chung M, Tzameli I, Simha D, Lee YK, Seol W and Moore DD (1997) Differential transactivation by two isoforms of the orphan nuclear hormone receptor CAR. *J Biol Chem* **272:**23565-23571.

Guo GL, Lambert G, Negishi M, Ward JM, Brewer HB, Jr., Kliewer SA, Gonzalez FJ and Sinal CJ (2003) Complementary roles of farnesoid X receptor, pregnane X receptor, and constitutive androstane receptor in protection against bile acid toxicity. *Journal of Biological Chemistry* **278:**45062-45071.

Harper PA, Riddick DS and Okey AB (2006) Regulating the regulator: factors that control levels and activity of the aryl hydrocarbon receptor. *Biochem Pharmacol* **72:**267-279.

Hayashi A, Suzuki H, Itoh K, Yamamoto M and Sugiyama Y (2003) Transcription factor Nrf2 is required for the constitutive and inducible expression of multidrug resistance-associated protein 1 in mouse embryo fibroblasts. *Biochemical & Biophysical Research Communications* **310:**824-829.

Honkakoski P, Kojo A and Lang MA (1992) Regulation of the mouse liver cytochrome P450 2B subfamily by sex hormones and phenobarbital. *Biochem J* **285 (Pt 3):**979-983.

Honkakoski P and Negishi M (1997) Characterization of a phenobarbital-responsive enhancer module in mouse P450 Cyp2b10 gene. *J Biol Chem* **272:**14943-14949.

Huang W, Zhang J, Chua SS, Qatanani M, Han Y, Granata R and Moore DD (2003) Induction of bilirubin clearance by the constitutive androstane receptor (CAR). *Proc Natl Acad Sci U S A* **100:**4156-4161.

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

Kawamoto T, Kakizaki S, Yoshinari K and Negishi M (2000) Estrogen activation of the nuclear orphan receptor CAR (constitutive active receptor) in induction of the mouse Cyp2b10 gene. *Mol Endocrinol* **14:**1897-1905.

Kawamoto T, Sueyoshi T, Zelko I, Moore R, Washburn K and Negishi M (1999) Phenobarbital-responsive nuclear translocation of the receptor CAR in induction of the CYP2B gene. *Mol Cell Biol* **19:**6318-6322.

Kedderis LB, Diliberto JJ, Linko P, Goldstein JA and Birnbaum LS (1991) Disposition of 2,3,7,8-tetrabromodibenzo-p-dioxin and 2,3,7,8-tetrachlorodibenzo-p-dioxin in the rat: biliary excretion and induction of cytochromes CYP1A1 and CYP1A2. *Toxicol Appl Pharmacol* **111:**163-172.

Klaassen CD and Slitt AL (2005) Regulation of hepatic transporters by xenobiotic receptors. *Curr Drug Metab* **6:**309-328.

Kliewer SA, Forman BM, Blumberg B, Ong ES, Borgmeyer U, Mangelsdorf DJ, Umesono K and Evans RM (1994) Differential expression and activation of a family of murine peroxisome proliferator-activated receptors. *Proc Natl Acad Sci U S A* **91:**7355-7359.

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

Ledda-Columbano GM, Pibiri M, Concas D, Molotzu F, Simbula G, Cossu C and Columbano A (2003) Sex difference in the proliferative response of mouse hepatocytes to treatment with the CAR ligand, TCPOBOP. *Carcinogenesis* **24:**1059-1065.

Lee SS, Pineau T, Drago J, Lee EJ, Owens JW, Kroetz DL, Fernandez-Salguero PM, Westphal H and Gonzalez FJ (1995) Targeted disruption of the alpha isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators. *Mol Cell Biol* **15:**3012-3022.

Li W, Donat S, Dohr O, Unfried K and Abel J (1994) Ah receptor in different tissues of C57BL/6J and DBA/2J mice: use of competitive polymerase chain reaction to measure Ah-receptor mRNA expression. *Arch Biochem Biophys* **315:**279-284.

Ma Q, Kinneer K, Bi Y, Chan JY and Kan YW (2004) Induction of murine NAD(P)H:quinone oxidoreductase by 2,3,7,8-tetrachlorodibenzo-p-dioxin requires the CNC (cap 'n' collar) basic leucine zipper transcription factor Nrf2 (nuclear factor erythroid 2-related factor 2): cross-interaction between AhR (aryl hydrocarbon receptor) and Nrf2 signal transduction. *Biochem J* **377:**205-213.

Maher JM, Cheng X, Slitt AL, Dieter MZ and Klaassen CD (2005a) Induction of the multidrug resistance-associated protein family of transporters by chemical activators of receptor-mediated pathways in mouse liver. *Drug Metab Dispos* **33:**956-962.

Maher JM, Slitt AL, Cherrington NJ, Cheng X and Klaassen CD (2005b) Tissue distribution and hepatic and renal ontogeny of the multidrug resistance-associated protein (Mrp) family in mice. *Drug Metab Dispos* **33:**947-955.

Marchand A, Barouki R and Garlatti M (2004) Regulation of NAD(P)H:quinone oxidoreductase 1 gene expression by CYP1A1 activity. *Mol Pharmacol* **65:**1029-1037.

McMahon M, Itoh K, Yamamoto M, Chanas SA, Henderson CJ, McLellan LI, Wolf CR, Cavin C and Hayes JD (2001) The Cap'n'Collar basic leucine zipper transcription factor Nrf2 (NF-E2 p45-related factor 2) controls both constitutive and inducible expression of intestinal detoxification and glutathione biosynthetic enzymes. *Cancer Research* **61:**3299-3307.

Moi P, Chan K, Asunis I, Cao A and Kan YW (1994) Isolation of NF-E2-related factor 2 (Nrf2), a NF-E2-like basic leucine zipper transcriptional activator that binds to the tandem NF-E2/AP1 repeat of the beta-globin locus control region. *Proc Natl Acad Sci U S A* **91:**9926-9930.

Nebert DW, Roe AL, Dieter MZ, Solis WA, Yang Y and Dalton TP (2000) Role of the aromatic hydrocarbon receptor and [Ah] gene battery in the oxidative stress response, cell cycle control, and apoptosis. *Biochem Pharmacol* **59:**65-85.

Reyes H, Reisz-Porszasz S and Hankinson O (1992) Identification of the Ah receptor nuclear translocator protein (Arnt) as a component of the DNA binding form of the Ah receptor. *Science* **256:**1193-1195.

Slitt AL, Cherrington NJ, Maher JM and Klaassen CD (2003) Induction of multidrug resistance protein 3 in rat liver is associated with altered vectorial excretion of acetaminophen metabolites. *Drug Disp Metab* **31:**1176-1186.

Staudinger JL, Goodwin B, Jones SA, Hawkins-Brown D, MacKenzie KI, LaTour A, Liu Y, Klaassen CD, Brown KK, Reinhard J, Willson TM, Koller BH and Kliewer SA (2001) 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 Metabolism & Disposition* **31:**523-527.

Venugopal R and Jaiswal AK (1996) Nrf1 and Nrf2 positively and c-Fos and Fra1 negatively regulate the human antioxidant response element-mediated expression of NAD(P)H:quinone oxidoreductase1 gene. *Proc Natl Acad Sci U S A* **93:**14960-14965.

Vollrath V, Wielandt AM, Iruretagoyena M and Chianale J (2006) Role of Nrf2 in the regulation of the Mrp2 (ABCC2) gene. *Biochem J* **395:**599-609.

Wang H and Negishi M (2003) Transcriptional regulation of cytochrome p450 2B genes by nuclear receptors. *Curr Drug Metab* **4:**515-525.

Wei P, Zhang J, Egan-Hafley M, Liang S and Moore DD (2000) The nuclear receptor CAR mediates specific xenobiotic induction of drug metabolism.[see comment]. *Nature* **407:**920-923.

Xie W and Evans RM (2001) Orphan nuclear receptors: the exotics of xenobiotics. *J Biol Chem* **276:**37739-37742.

Footnote

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Legends for Figures

Fig. 1. Tissue distribution of AhR, CAR, PXR, PPARα, and Nrf2 mRNA expression in C57BL/6 mice. Tissues were harvested from 8-week-old male and female C57BL/6 mice, as described in Materials and Methods. Total RNA isolates from mouse tissues (n=5) were analyzed for gene expression using the bDNA assay. Data represent mean ± S.E.M. Asterisks (*) indicate a statistically significant difference between male and female mice ($p<0.05$).

Fig. 2. Induction of AhR and Cyp1a1 mRNA expression in male C57BL/6 mouse liver by microsomal enzyme inducer compounds. Animals were treated with inducer agents as described in Table 1. Abbreviations for inducers can also be found in Table 1. Gene expression from livers of five mice was determined using the bDNA assay. Data represent mean ± S.E.M. Asterisks (*) indicate a statistically significant difference between treated and control mice ($p<0.05$).

Fig. 3. Induction of CAR and Cyp2b10 mRNA expression in male C57BL/6 mouse liver by microsomal enzyme inducer compounds. Animals were treated with inducer agents as described in Table 1. Abbreviations for inducers can also be found in Table 1. Gene expression from livers of five mice was determined using the bDNA assay. Data represent mean ± S.E.M. Asterisks (*) indicate a statistically significant difference between treated and control mice ($p<0.05$).

Fig. 4. Induction of PXR and Cyp3a11 mRNA expression in male C57BL/6 mouse liver by microsomal enzyme inducer compounds. Animals were treated with inducer agents as described in Table 1. Abbreviations for inducers can also be found in Table 1. Gene expression from livers of five mice was determined using the bDNA assay. Data represent mean ± S.E.M. Asterisks (*) indicate a statistically significant difference between treated and control mice ($p<0.05$).

Fig. 5. Induction of PPARα and Cyp4a14 mRNA expression in male C57BL/6 mouse liver by microsomal enzyme inducer compounds. Animals were treated with inducer agents as described in Table 1. Abbreviations for inducers can also be found in Table 1. Gene expression from livers of five mice was determined using the bDNA assay. Data represent mean ± S.E.M. Asterisks (*) indicate a statistically significant difference between treated and control mice ($p<0.05$).

Fig. 6. Induction of Nrf2 and Nqo1 mRNA expression in male C57BL/6 mouse liver by microsomal enzyme inducer compounds. Animals were treated with inducer agents as described in Table 1. Abbreviations for inducers can also be found in Table 1. Gene expression from livers of five mice was determined using the bDNA assay. Data represent mean ± S.E.M. Asterisks (*) indicate a statistically significant difference between treated and control mice ($p<0.05$).

Fig. 7. Gender differences in expression of CAR, Cyp2b10, Mrp2, Mrp3, and Mrp4 in C57BL/6 mouse liver and gender-divergent responses to the CAR ligand TCPOBOP. Male and female mice were treated with corn oil or TCPOBOP for four days, as described in Table 1. Gene expression from livers of five mice was determined using the bDNA assay. Data represent mean ± S.E.M. Statistical significance was evaluated using Student's t-test and significant differences ($p<0.05$) are indicated as follows: 1) between male and female is indicated by a double dagger (‡), 2) between corn oil and TCPOBOP treatment is indicated by an asterisk (*), 3) ratio of corn oil to TCPOBOP treated in males versus females is indicated by a cross (†).

Fig 8. Induction of CAR, and Cyp2b10 mRNA in male C57BL/6 mouse liver by TCCD, TCPOBOP, or combined treatment of TCDD and TCPOBOP. Animals were treated with: 1) 5 ml/kg/day corn oil for four days, 2) 37 μg/kg/day TCDD for four days, 3) 5 ml/kg/day corn oil for four days followed by 3 mg/kg TCPOBOP for 12 hours, or 4) 37 μg/kg/day TCDD for four days followed by a 12 hour 3 mg/kg TCPOBOP treatment (See details in Materials and Methods). Gene expression (n=5) was analyzed using the bDNA assay (See Materials and Methods). Data represent mean ± S.E.M. Statistically significant differences ($p<0.05$) are indicated as follows: *significantly different from corn oil treatment, † significantly different from TCDD treatment, and ‡ significantly different from TCPOBOP treatment.

Fig. 9. Induction of Mrp2, Mrp3, and Mrp4 transporter mRNA in C57BL/6 mouse liver by TCCD, TCPOBOP, or combined treatment of TCDD and TCPOBOP. Animals were treated with: 1) 5 ml/kg/day corn oil for four days, 2) 37 μg/kg/day TCDD for four days, 3) 5 ml/kg/day corn oil for four days followed by 3 mg/kg TCPOBOP for 12 hours, or 4) 37 μg/kg/day TCDD for four days followed by a 12 hour 3 mg/kg TCPOBOP treatment (See details in Materials and Methods). Gene expression (n=5) was analyzed using the bDNA assay (See Materials and Methods). Data represent mean ± S.E.M. Statistically significant differences ($p<0.05$) are indicated as follows: *significantly different from corn oil treatment, † significantly different from TCDD treatment, and ‡ significantly different from TCPOBOP treatment.

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Tables

Table 1. Dosing regimen, target receptors, and marker genes of xenobiotic activator compounds used for inducer studies.

Compound	CAS Number	Dosing Regimen	Activated Receptor	Marker Gene
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)	1746-01-6	37 μg/kg, CO, ip	AhR	Cyp1a1
Polychlorinated Biphenyl 126 (PCB-126)	57465-28-8	300 μg/kg, CO, ip		
β-naphthoflavone (BNF)	6051-87-2	200 mg/kg CO, ip		
Phenobarbital (PB)	50-06-6	100 mg/kg, SAL, ip	CAR	Cyp2b10
1,4-bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP)	76150-91-9	3 mg/kg, CO, ip		
Diallylsulfide (DAS)	592-88-1	200 mg/kg, CO, ip		
Pregnenolone-16α-carbonitrile (PCN)	1434-54-4	200 mg/kg, CO, ip	PXR	Cyp3a11
Spironolactone (SPR)	52-01-7	200 mg/kg, CO, ip		
Dexamethasone (DEX)	50-02-2	75 mg/kg, CO, ip		
Clofibrate (CLOF)	637-07-0	500 mg/kg CO, ip	PPARα	Cyp4a14
Ciprofibrate (CPFB)	52214-84-3	40 mg/kg, CO, ip		
Di (2-ethylhexyl) phthalate (DEHP)	117-81-7	1000 mg/kg, CO, ip		
Oltipraz (OPZ)	64224-21-1	150 mg/kg, CO, po	Nrf2	Nqo1
Ethoxyquin (EXQ)	91-53-2	250 mg/kg, CO, po		
Butylated Hydroxyanisole (BHA)	25013-16-5	350 mg/kg, CO, ip		

Note: CO denotes corn oil vehicle and SAL denotes saline vehicle. Compounds were dosed intraperitoneally (ip) or by oral gavage (po). Cyp is the abbreviation for cytochrome P450, and Nqo1 is the abbreviation for NAD(P)H quinone oxidoreductase.

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

Target[a]	Function[b]	Sequence	Target	Function	Sequence
		AhR (NM_013464)			**CAR (NM_009803)**
2766-2784	CE	cttccgccaggtgatggagTTTTTctcttggaaagaaagt	42-64	CE	ttccttgagatctcaggaactgcTTTTctcttggaaagaaagt
2841-2865	CE	ggtatcctgttcctgaatgaatttcTTTTTctcttggaaagaaagt	84-102	CE	gatgcctcctctccccaggTTTTctcttggaaagaaagt
3004-3024	CE	tgtgcactctgaggtgcctgaTTTTTctcttggaaagaaagt	190-209	CE	cagttcctcggcccatattcTTTTctcttggaaagaaagt
3072-3094	CE	ccaaatacctccctgtttttcctTTTTTctcttggaaagaaagt	271-291	CE	tgttcgtctgaagaagcccttTTTTTctcttggaaagaaagt
2705-2726	LE	gtcggacgaataggtttcattgTTTTTaggcataggacccgtgtct	22-41	LE	caggaattagggactgcggtTTTTaggcataggacccgtgtct
2727-2745	LE	cgtggccaatgctgctcaaTTTTTaggcataggacccgtgtct	65-83	LE	cctcctcgctgaccctgctTTTTTaggcataggacccgtgtct
2821-2840	LE	atcctggcatgggagctacaTTTTTaggcataggacccgtgtct	124-143	LE	tcctgtttcccttaggcagcTTTTTaggcataggacccgtgtct
2866-2888	LE	tgatgtccaaccctcacagttctTTTTTaggcataggacccgtgtct	169-189	LE	ttcttcactggccatggtttcTTTTTaggcataggacccgtgtct
2943-2963	LE	gggagcatctcaggtacgggtTTTTTaggcataggacccgtgtct	210-229	LE	cccggtctccacacaccacaTTTTTaggcataggacccgtgtct
2964-2984	LE	tccgagctctcccatcgtataTTTTTaggcataggacccgtgtct	251-270	LE	gcagccctcacaagtcagggTTTTTaggcataggacccgtgtct
2985-3003	LE	actcctcccatgtgctccgTTTTTaggcataggacccgtgtct	292-312	LE	gggaccaatggttttgctgacTTTTTaggcataggacccgtgtct
3025-3049	LE	cccgagaatttttcacagtaaacacTTTTTaggcataggacccgtgtct	313-333	LE	ccttccagcaaacggacagatTTTTTaggcataggacccgtgtct
2746-2765	BL	atggccagtggtctgagcag	335-353	LE	tgggccttgctgacctcacTTTTTaggcataggacccgtgtct
2785-2800	BL	tcgggaagaggccggg	103-123	BL	aggccacaagattgggtgtag
2801-2820	BL	ggaatccaccgggtgtgata	144-168	BL	tagtgttagcatagctgtcatggtc
2889-2914	BL	cgaaatctgttttggagaaagtgtac	230-250	BL	cgtggaaatgatagcctgtgg
2915-2942	BL	tttaaactccttctctaaacacaagaat			
3050-3071	BL	gctgaagttactgagcagggaa			
		PXR (AF031814)			**PPARα (NM_011144)**
495-510	CE	ggcccgcctctgctccTTTTTctcttggaaagaaagt	1245-1265	CE	gaagaatcggacctctgcctcTTTTTctcttggaaagaaagt
571-589	CE	gcgcctgctgttcttccgtTTTTTctcttggaaagaaagt	1453-1473	CE	actcgcgtgtgataaagccatTTTTTctcttggaaagaaagt
762-784	CE	gctgcagagagatcttcattggaTTTTTctcttggaaagaaagt	1474-1495	CE	ggcttcctcaggttcttaaggaTTTTTctcttggaaagaaagt
843-864	CE	tggcagaagagggatgatctctTTTTTctcttggaaagaaagt	1611-1632	CE	agcctatgtttagaaggccaggTTTTTctcttggaaagaaagt
435-452	LE	tccaggcacttgcgcaaaTTTTTaggcataggacccgtgtct	1226-1244	LE	tttgtcttcgacgccgttgTTTTTaggcataggacccgtgtct
477-494	LE	acagcggcatcggacatgTTTTTaggcataggacccgtgtct	1286-1303	LE	tccgtgacggtctccacgTTTTTaggcataggacccgtgtct
590-610	LE	cgtccatcagctcctggatcaTTTTTaggcataggacccgtgtct	1368-1392	LE	cttcatacacaccgtactttagcaaTTTTTaggcataggacccgtgtc
611-633	LE	tgtgtcaaaggtttgcatctgagTTTTTaggcataggacccgtgtct	1414-1434	LE	tcccgtctttgttcatcaaggTTTTTaggcataggacccgtgtct
718-737	LE	catgtggcagggtcttccaaTTTTTaggcataggacccgtgtct	1435-1452	LE	tgccgtacgcgatcagcaTTTTTaggcataggacccgtgtct
738-761	LE	accctgtctttcatgatttgactcTTTTTaggcataggacccgtgtct	1496-1518	LE	tgggttccatgatgtcacagaacTTTTTaggcataggacccgtgtct
785-801	LE	gccgtcttctccgcgcaTTTTTaggcataggacccgtgtct	1544-1566	LE	cactgtcatccagttctaaggcaTTTTTaggcataggacccgtgtct
865-883	LE	ttgacacatcggccaggtgTTTTTaggcataggacccgtgtct	1633-1654	LE	ccctcctgcaacttctcaatgtTTTTTaggcataggacccgtgtct
453-476	BL	atcatctctttcttcatgccactc	1655-1676	LE	gagcttaagcacgtgcacaatcTTTTTaggcataggacccgtgtct
511-533	BL	tccctcttcttcctcttgatcaa	1677-1696	LE	ggatggttgctctgcaggtgTTTTTaggcataggacccgtgtct
534-553	BL	gcggtggagcctcaatcttt	1266-1285	BL	gacatgcactggcagcagtg
554-570	BL	cagcccctgccctccag	1304-1324	BL	gccttggcaaattctgtgagc
634-657	BL	gaaatccttgaagtgggagaaagt	1325-1345	BL	aagtttgcaaagcctgggata
658-675	BL	gaacactgcaggcagccg	1346-1367	BL	ggtgacttggtcgttcaagtcc
676-696	BL	tggaagctcacagccactgtg	1393-1413	BL	aggacagcatcgtgaagatgg
697-717	BL	cagtgaggcctgcagaaactc	1519-1543	BL	ttgaacttcatagcgaagtcaaact
802-823	BL	ggggttggtagttccagatgct	1567-1587	BL	cagccacaaacagggaaatgt
824-842	BL	ttcccgtcgctcttggaag	1588-1610	BL	ccgatctccacagcaaattatag

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Target[a]	Function[b]	Sequence
		Nrf2 (NM_010902)
50-67	CE	tgctcgctggagtggcagTTTTTctcttggaaagaaagt
110-127	CE	gcagcagaggtgaggcggTTTTTctcttggaaagaaagt
322-345	CE	ttccttttcgagtttttttctgtttTTTTTctcttggaaagaaagt
32-49	LE	gccagccttagtccgcccTTTTTaggcataggacccgtgtct
68-88	LE	gccgggaactaggagatagccTTTTTaggcataggacccgtgtct
89-109	LE	cggcaatggctagtccgagtaTTTTTaggcataggacccgtgtct
128-145	LE	ccgacggcgaggctacttTTTTTaggcataggacccgtgtct
146-164	LE	gacgctgtggtagggctccTTTTTaggcataggacccgtgtct
165-182	LE	tccatcatgctgagggcgTTTTTaggcataggacccgtgtct
183-200	LE	ggcggtggcaactccaagTTTTTaggcataggacccgtgtct
201-222	LE	gtcctgctgggactgtagtcctTTTTTaggcataggacccgtgtct
223-246	LE	ccaaaggatgtcaatcaaatccatTTTTTaggcataggacccgtgtct
298-321	LE	ttccaactcatagtccttctgtcgTTTTTaggcataggacccgtgtct
247-271	BL	ttactccaagatctatgtcttgcct
272-297	BL	ctgactaaagtcaaacacttctcgac

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

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FIGURE 1

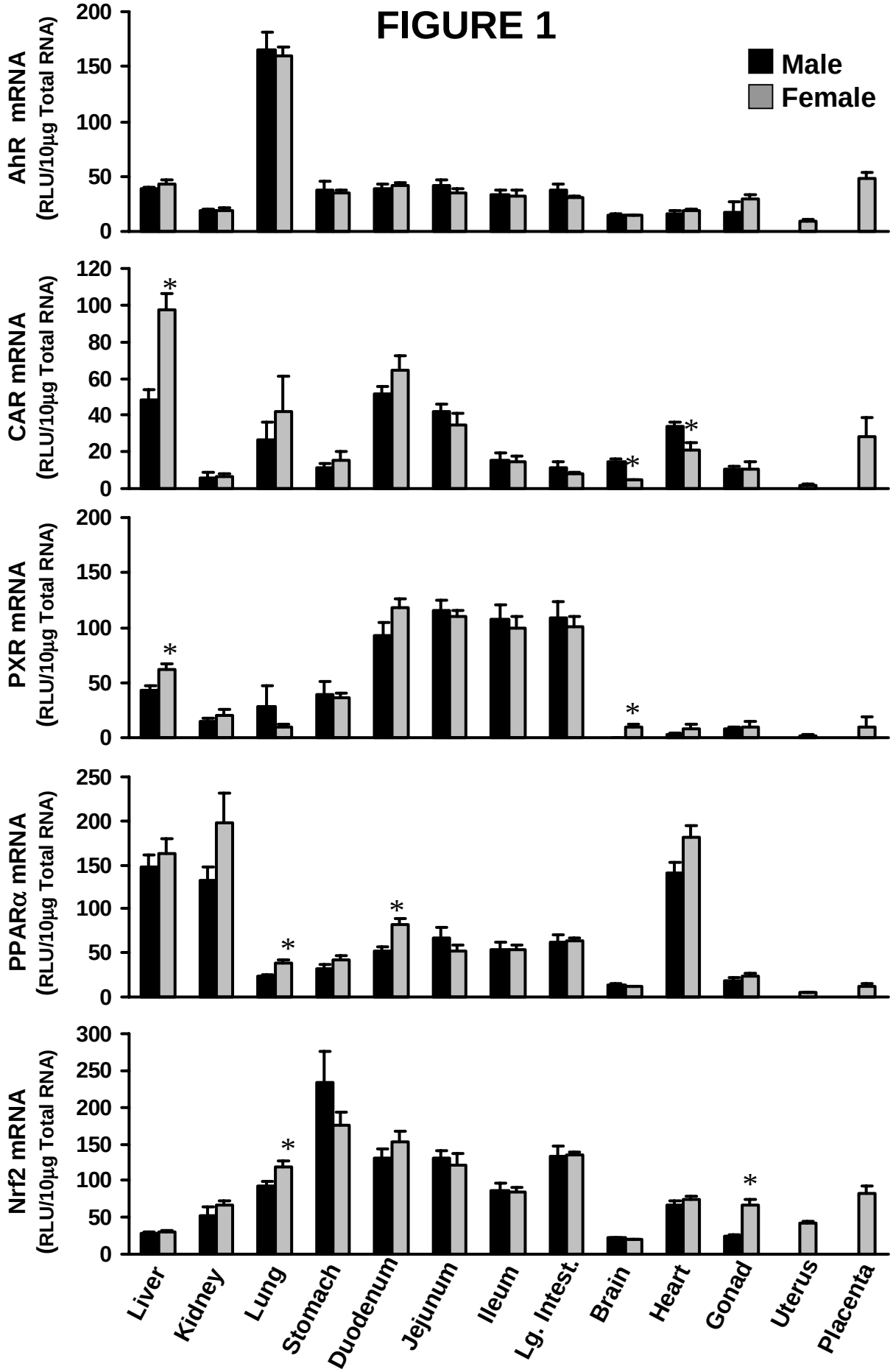

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FIGURE 2

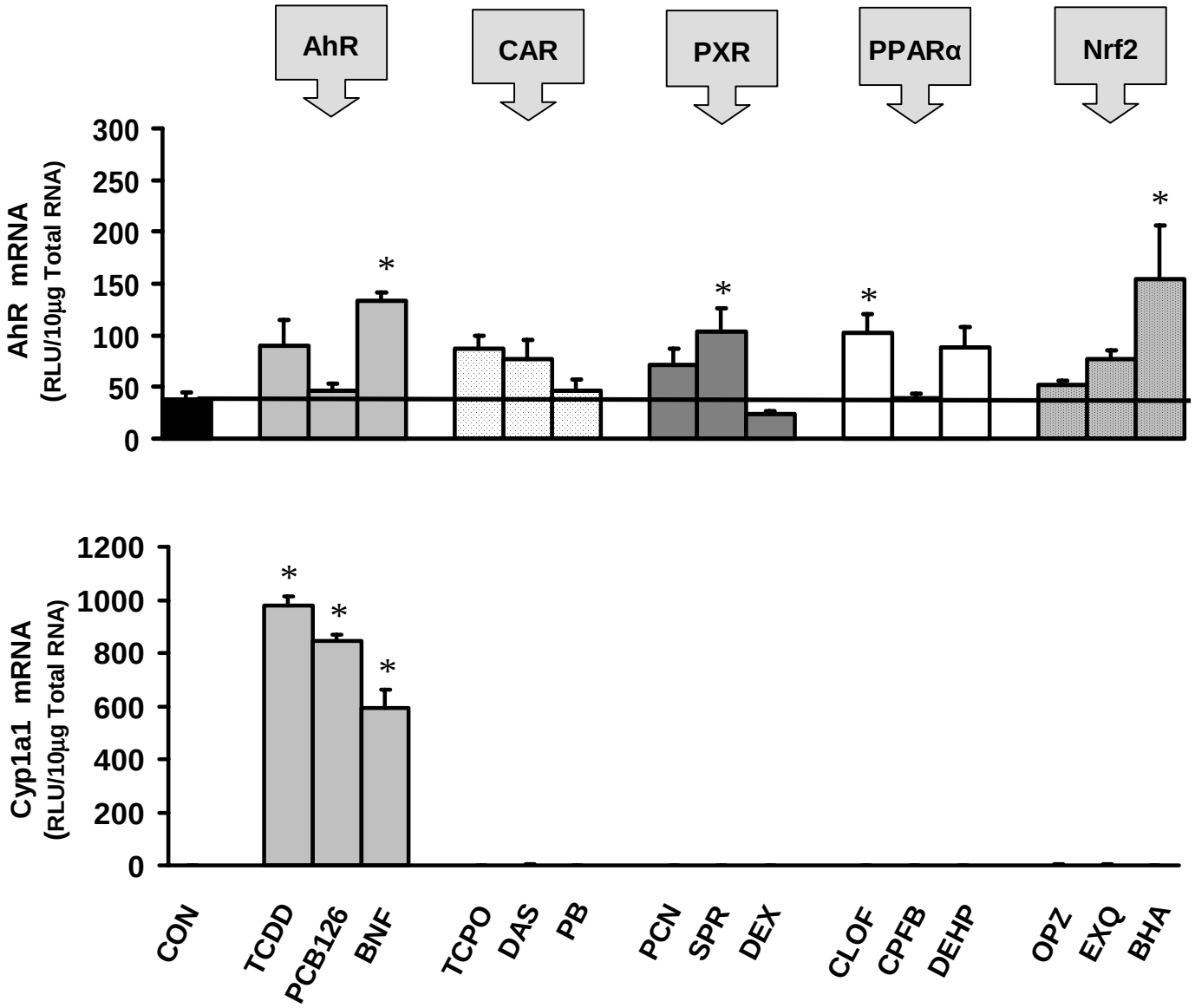

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FIGURE 3

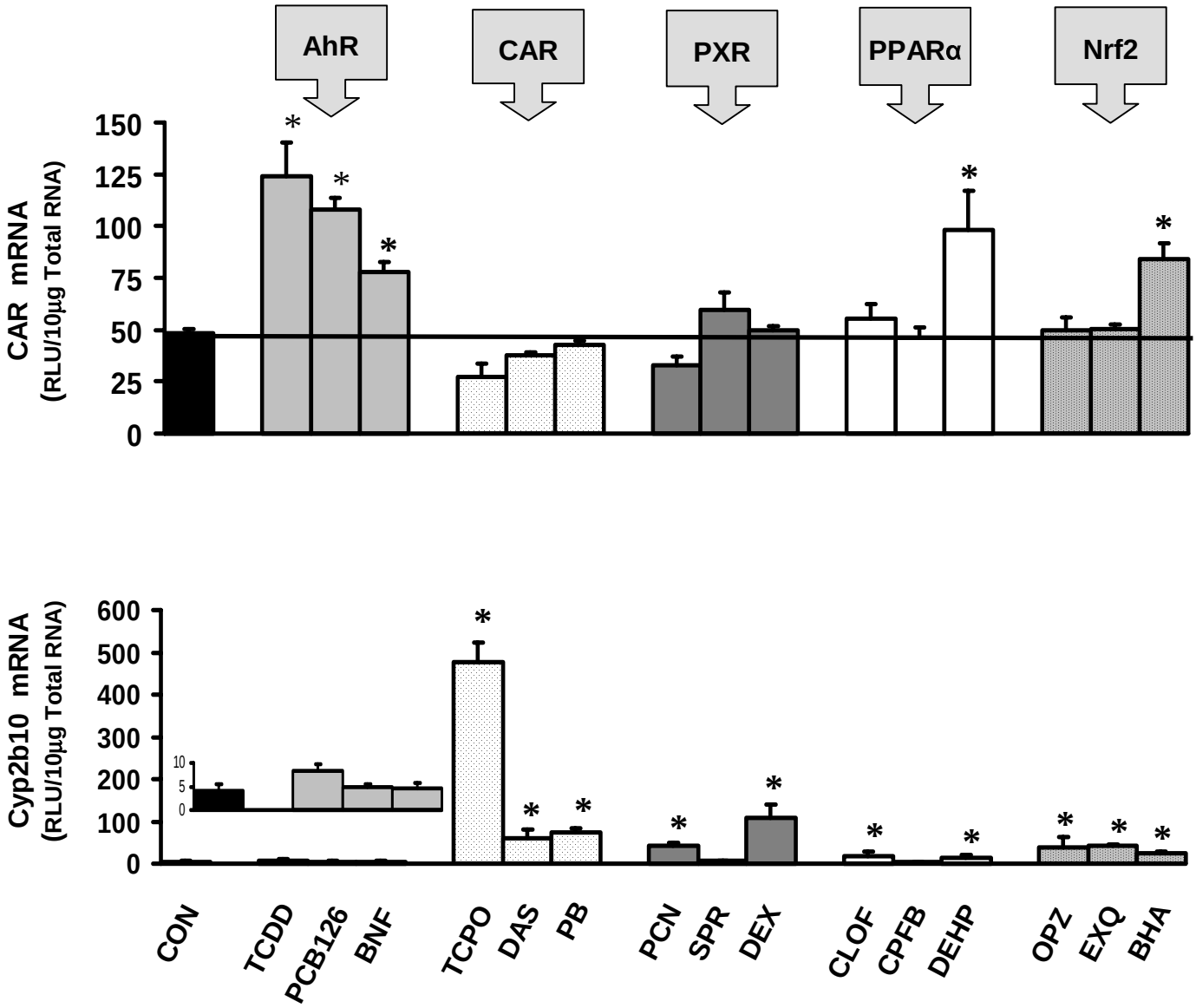

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FIGURE 4

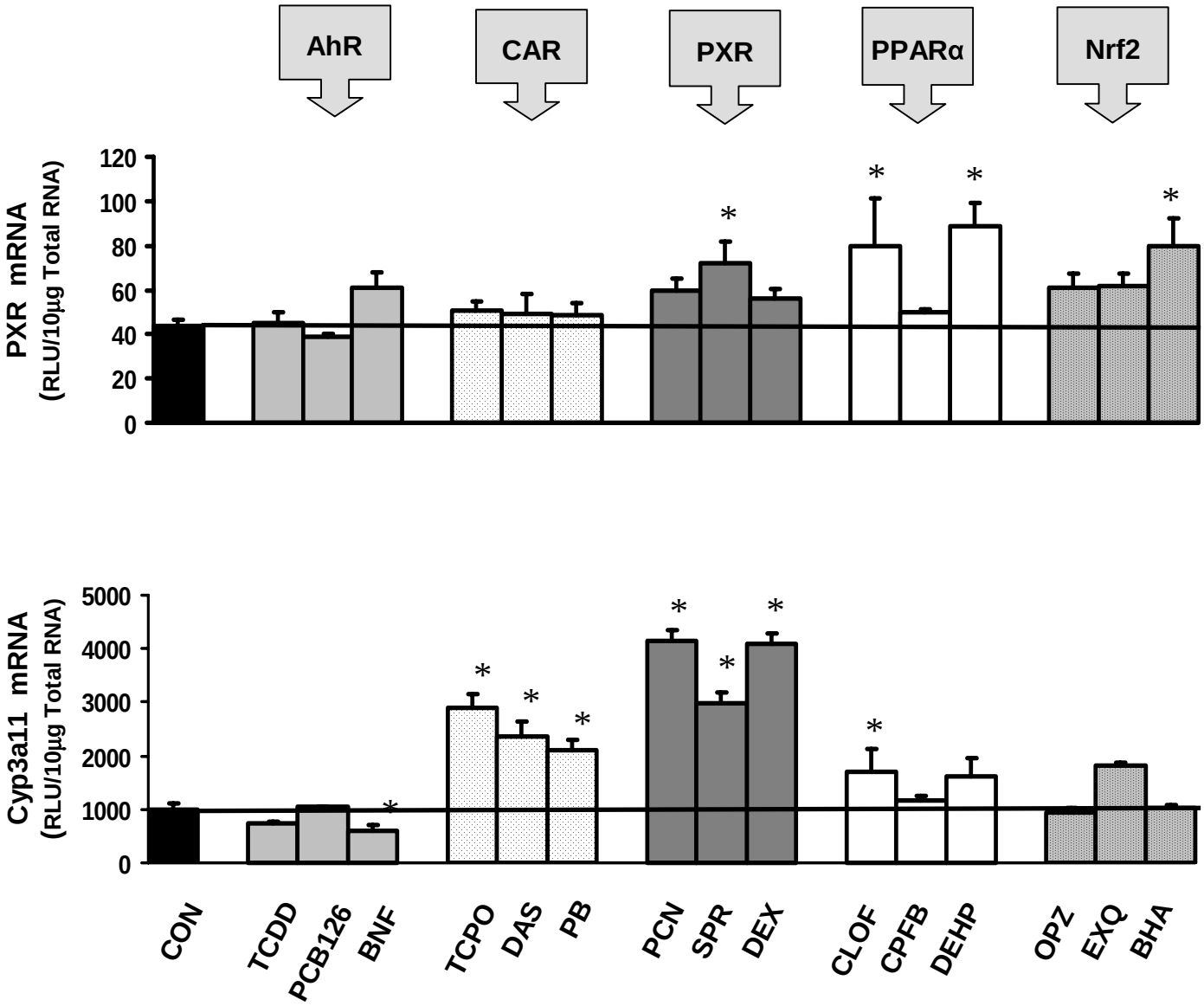

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FIGURE 5

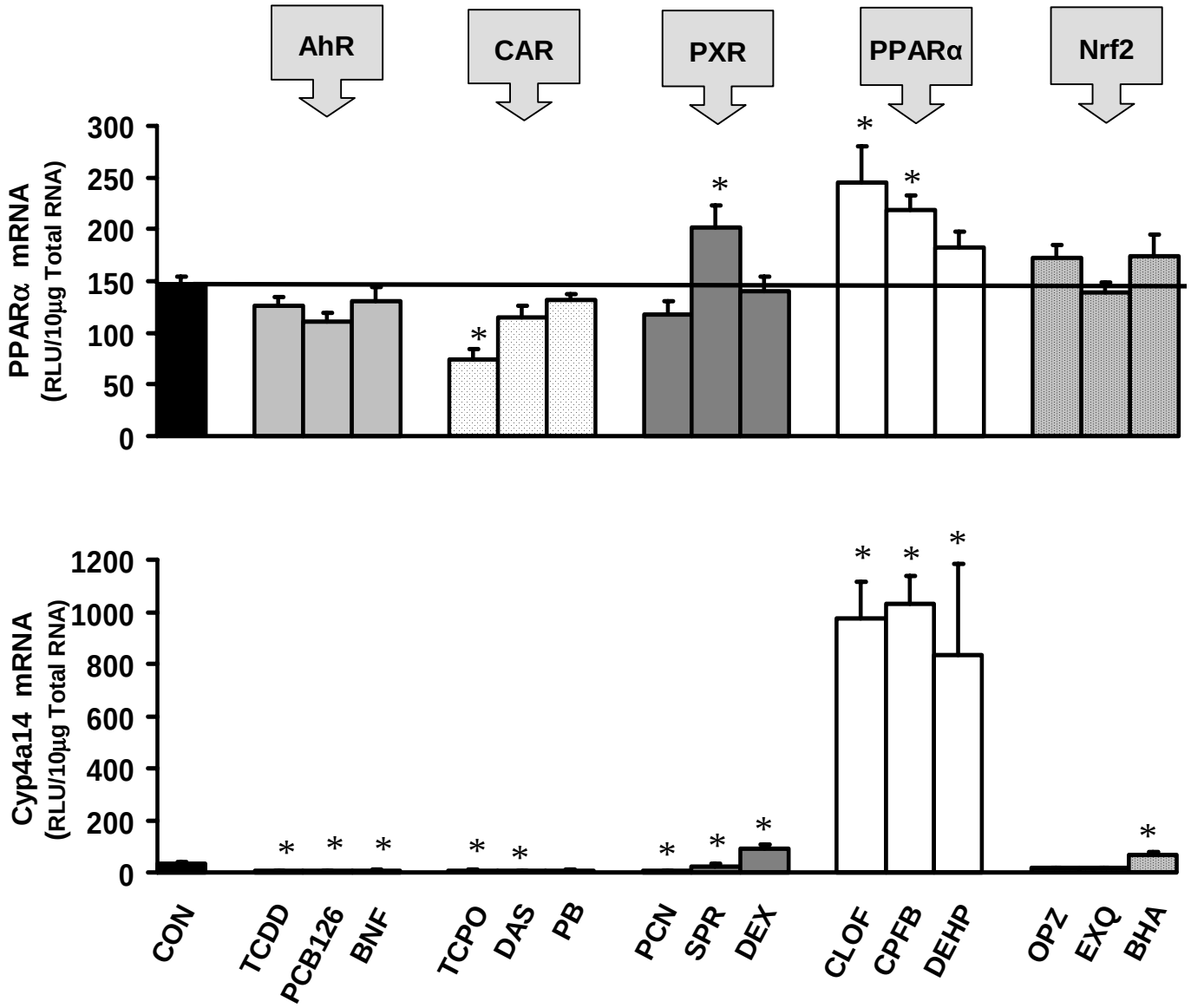

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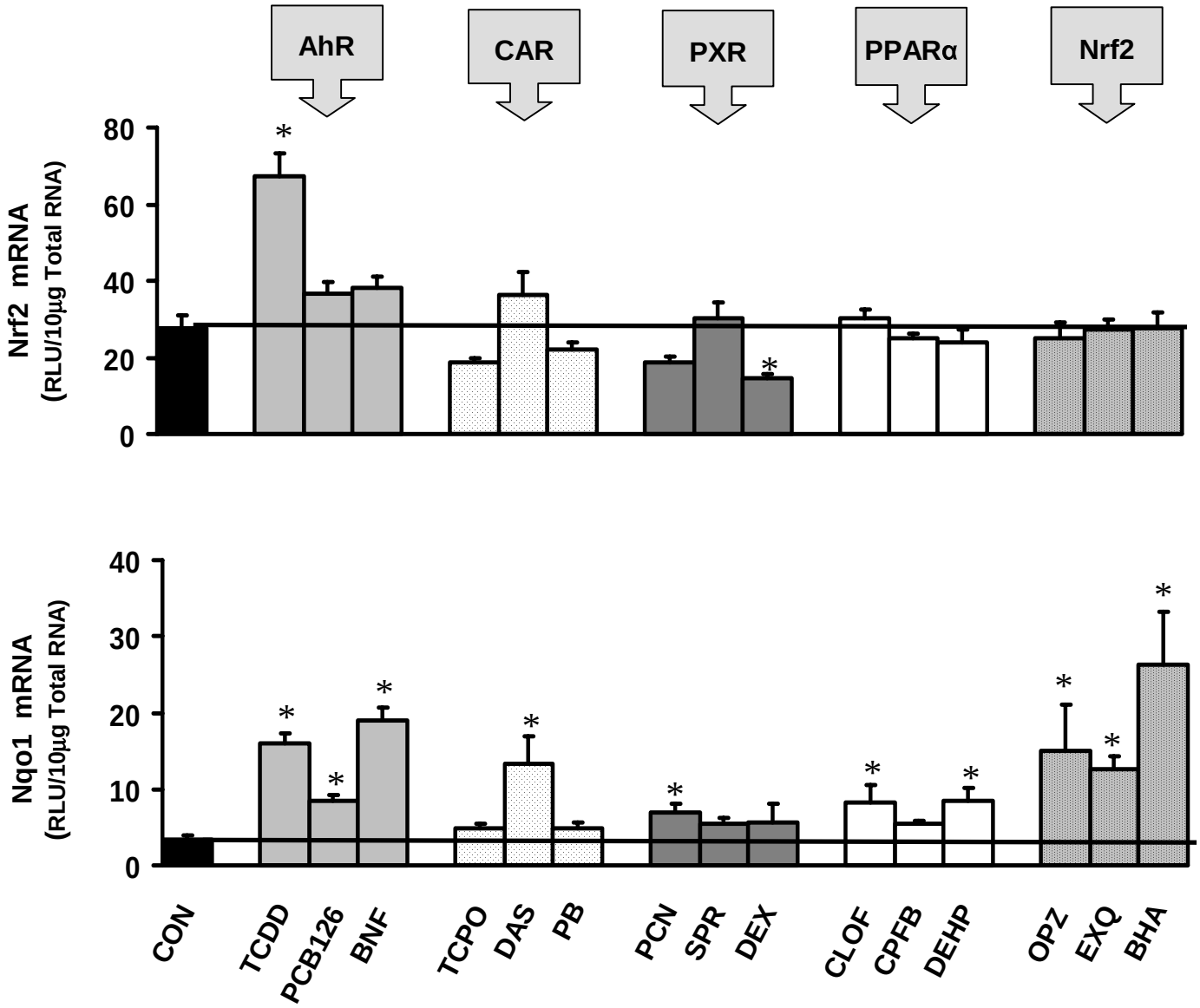
FIGURE 6



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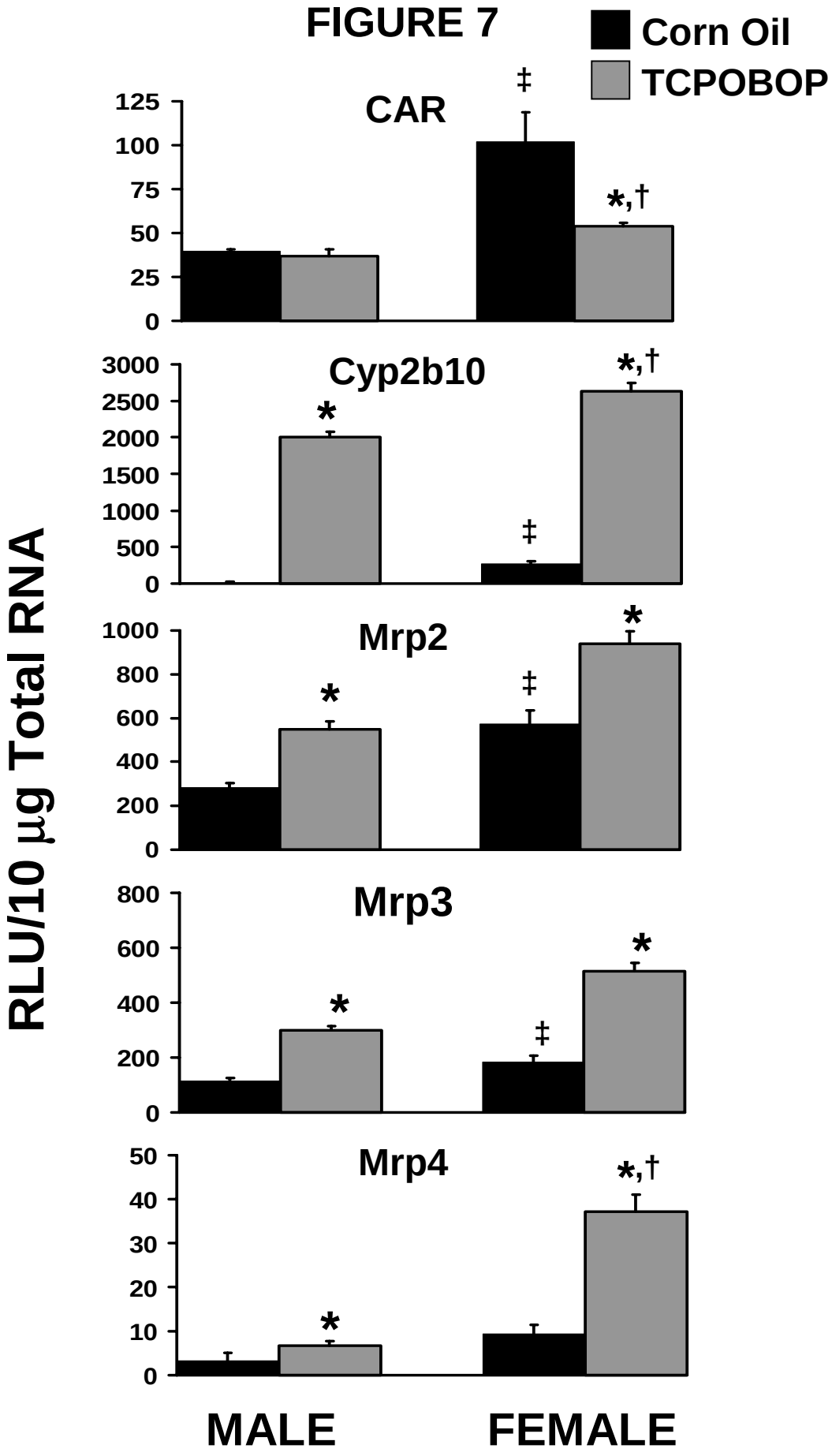

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FIGURE 8

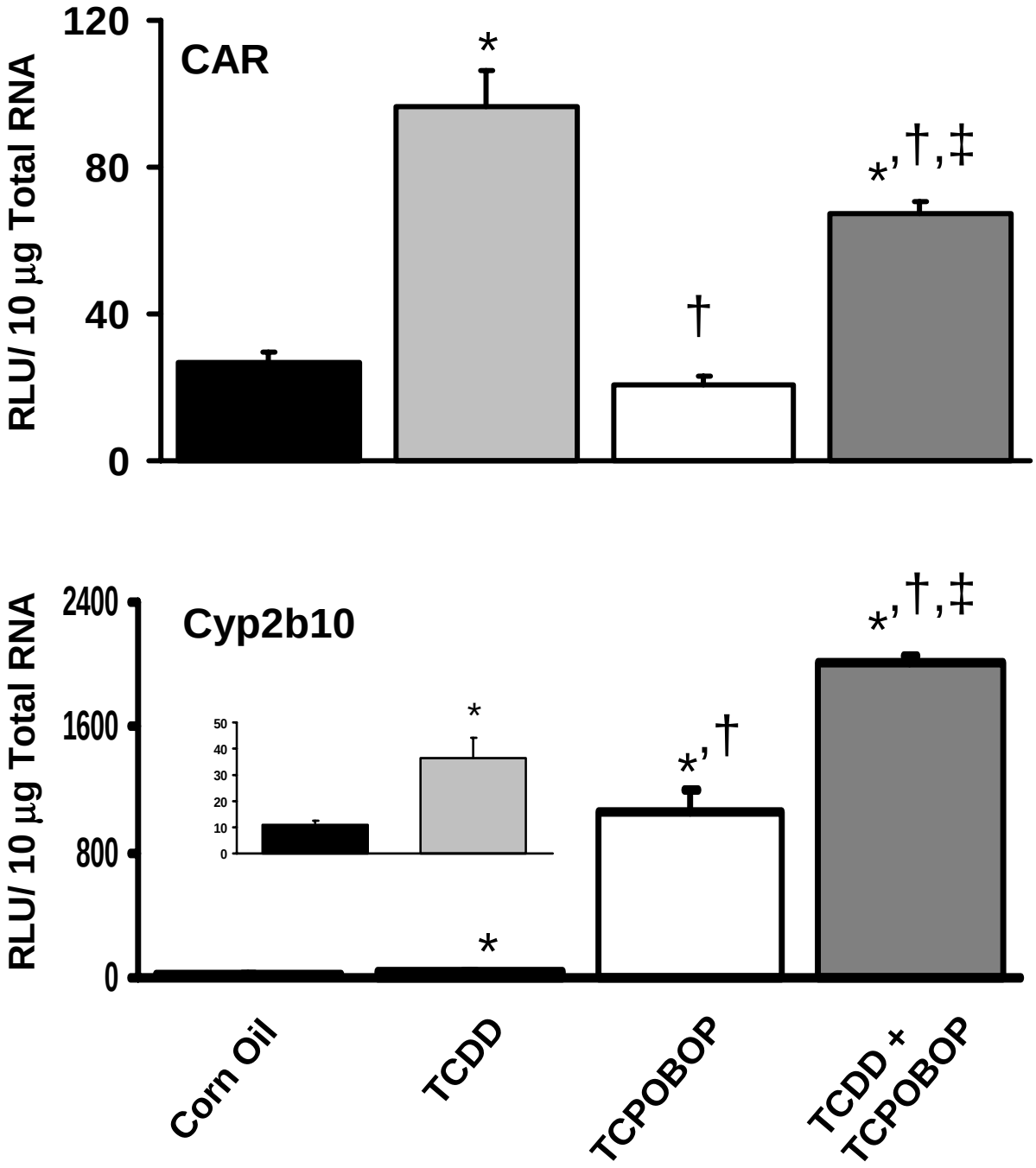

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FIGURE 9