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Induction of Rat UDP-Glucuronosyltransferases in Liver and Duodenum by
Microsomal Enzyme Inducers That Activate Various Transcriptional Pathways

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glucuronosyltransferases; CYP, cytochrome P450; AhR aryl hydrocarbon

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PPAR α , peroxisome proliferator-activated receptor alpha; Nrf2, nuclear factor

erythroid 2-related factor 2; microsomal enzyme inducer (MEI); TCDD, 2,3,7,8-

tetrachlorodibenzo-*p*-dioxin; PCB126, polychlorinated biphenyl congener 126;

PCB99, polychlorinated biphenyl congener 99; PCN, pregnenolone-16 α -

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carbonitrile; DEHP, di-(2-ethylhexyl)phthalate; PFDA, perfluorodecanoic acid;
PP, peroxisome proliferators; XRE, xenobiotic response element.

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ABSTRACT

Microsomal enzyme inducers (MEIs) up-regulate phase-I biotransformation enzymes, most notably cytochrome P450s (CYPs). Transcriptional up-regulation by MEIs occurs through at least three nuclear receptor mechanisms: constitutive androstane receptor (CAR; CYP2B inducers), pregnane-X-receptor (PXR; CYP3A inducers), and peroxisome proliferator-activated receptor α (PPAR α ; CYP4A inducers). Other mechanisms include transcription factors aryl hydrocarbon receptor (AhR; CYP1A inducers), and NF-E2-related factor 2 (Nrf2; Nadph quinone oxidoreductase inducers). UDP-glucuronosyltransferases (UGTs) are phase-II biotransformation enzymes that are predominantly expressed in liver and intestine. MEIs increase UGT activity; however, transcriptional regulation of individual UGT isoforms is not completely understood. The purpose of this study was to examine inducibility of individual UGT isoforms and potential mechanisms of transcriptional regulation in rat liver and duodenum. UGT mRNA levels were assessed in liver and duodenum of rats treated with MEIs that activate various transcriptional pathways. All four CAR activators induced UGT2B1 in liver, but not duodenum. UGT1A1, 1A5, 1A6, and 2B12 were induced by at least two CAR activators in liver only. Two PXR ligands induced UGT1A2, but only in duodenum. Two PPAR α ligands induced UGT1A1 and 1A3 in liver only. AhR ligands induced UGT1A6 and 1A7 in liver, but not duodenum. Nrf2 activators increased UGT2B3 and 2B12 in both liver and duodenum, and UGT1A6, 1A7, and 2B1 in liver only. In summary, only UGT1A2 and 1A8 were not inducible in

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liver by MEIs. MEIs differentially regulate hepatic expression of individual UGT isoforms, though no one transcriptional pathway dominated. In duodenum, MEIs had minimal effects on UGT expression.

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Individuals are continually exposed to potentially toxic endogenous and xenobiotic compounds. Thus, individuals require a means of eliminating such compounds from the body. UDP-glucuronosyltransferases (UGTs) comprise a superfamily of phase-II biotransformation enzymes that generally function to detoxify by catalyzing the glucuronidation of structurally diverse compounds, making them more water-soluble, and more easily excreted. UGTs are mainly expressed in liver and gastrointestinal tract and are involved in the metabolic homeostasis of endogenous compounds including bilirubin, steroid hormones, and thyroid hormone, as well as the metabolism of a wide variety of xenobiotics including opioids, flavonoids, acetaminophen, non-steroidal anti-inflammatory drugs, and carcinogens (Dutton, 1980; Clarke and Burchell, 1987; Mackenzie and Rodbourn, 1990; Burchell *et al.*, 1991; Emi *et al.*, 1995; Grams *et al.*, 2000; Shelby *et al.*, 2003).

To date, there are numerous reports of increased UGT activity in response to xenobiotic exposure. Many of these xenobiotics are known as microsomal enzyme inducers (MEIs), which are classic inducers of phase-I biotransformation enzymes, most notably cytochrome P450s (CYPs). Induction of CYPs by MEIs is thought to be due mostly to transcriptional activation (Nebert and Gonzalez, 1987). UGTs may be coordinately regulated with CYPs and other phase-I enzymes through the same transcriptional pathways.

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Recently two members of the nuclear receptor family of ligand-activated transcription factors, Constitutive Androstane Receptor/Constitutive Active Receptor (CAR) and Pregnane-X Receptor (PXR), have been termed xenobiotic sensors or “xenosensors” for their ability to up-regulate detoxifying enzymes in response to chemical exposure (Swales and Negishi, 2004; Kliewer, 2005). Although CAR and PXR have somewhat redundant function and frequently share target genes, they differ in the compounds that activate them, and mechanism of activation (Xie *et al.*, 2000; Wei *et al.*, 2002). CAR is unique in that it can be activated both directly by ligand binding and indirectly (Yoshinari *et al.*, 2003). Consequently, activation of CAR, and resultant induction of its target gene CYP2B (Honkakoski *et al.*, 1998), occurs by structurally diverse compounds such as the anti-epileptic drug phenobarbital, and non-planar polychlorinated biphenyls (PCB99). However, activation of PXR requires direct binding of a ligand, and resultant induction of its target gene CYP3A, occurs by pregnanes, glucocorticoids, and numerous xenobiotic compounds (Kliewer *et al.*, 1998).

Peroxisome proliferator-activated receptor alpha (PPAR α) is another member of the nuclear receptor family that has been implicated in the regulation of biotransformation enzymes. PPAR α induces expression of CYP4A in response to hypolipidemic drugs, such as clofibrate, and increased fatty acids in liver that occur with fasting or uncontrolled diabetes (Issemann and Green, 1990; Singh, 1997).

Other mechanisms of transcriptional activation and induction of biotransformation enzymes include transcription factors such as Aryl hydrocarbon receptor (AhR)

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and nuclear factor erythroid 2 (NF-E2)-related factor 2 (Nrf2). AhR is well known for its ability to induce the phase-I biotransformation enzyme CYP1A1 (Jaiswal *et al.*, 1985; Fernandez-Salguero *et al.*, 1996). Highly lipophilic compounds such as the herbicide contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), and planar polychlorinated biphenyls (PCBs) bind to AhR, and trigger its activation and subsequent binding to xenobiotic response elements (XREs). Contrary to AhR, which can induce both phase-I and -II enzymes, Nrf2 is mainly involved in the regulation of phase-II enzymes. Alterations in the oxidative environment within the cell can cause the activation/release of Nrf2 from sequestration within the cytosol. Nrf2 subsequently translocates to the nucleus and forms a complex with small Maf proteins that binds antioxidant/electrophile response elements and turns on the antioxidant response gene battery (Itoh *et al.*, 1999).

Transcriptional up-regulation of biotransformation enzymes by MEIs occurs through nuclear receptor-mediated pathways including CAR, PXR, and PPAR α , as well as transcription factors such as AhR, and Nrf2. However for some MEIs, such as the CYP2E inducers isoniazid and streptozotocin, no transcriptional pathway has been identified.

Induction of phase-II biotransformation enzymes, such as UGTs, may be coordinately regulated with phase-I biotransformation enzymes through the same transcriptional pathway. Inducibility of individual UGT isoforms by MEIs and potential mechanisms of induction are not completely understood. Induction of UGT activity reportedly occurs in both liver and duodenum, tissues in which

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UGTs are predominantly expressed. Therefore, the purpose of this study was to determine the inducibility of individual UGT isoforms in liver and duodenum by MEIs that act through various transcriptional pathways.

Materials and Methods

Chemicals. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) was a generous gift from Dr. Karl Rozman (Kansas City, KS). 3,3',4,4',5-Pentachlorobiphenyl (PCB126) and 2,2',4,4',5-pentachlorobiphenyl (PCB99) were purchased from Accustandard (New Haven, CT). Pregnenolone-16 α -carbonitrile (PCN) was synthesized from 16-dehydropregnenolone precursor (Steraloids, Newport, RI) according to Sonderfan and Parkinson (1988). β -Naphthoflavone, phenobarbital, diallyl sulfide, *trans*-stilbene oxide, spironolactone, dexamethasone, clofibrate, di-(2-ethylhexyl)phthalate (DEHP), perfluorodecanoic acid (PFDA), ethoxyquin, oltipraz, streptozotocin, isoniazid, and acetylsalicylic acid were purchased from Sigma Chemical Co. (St. Louis, MO).

Animals and Tissues. Adult male rats were used in this study to avoid age-related expression of nuclear receptors and transcription factors that may occur in immature rats, as well as cyclic variation in hormone status that occurs in females. Male Sprague-Dawley (SD) rats (n=5 per treatment, 225-250g) were purchased from Charles River Laboratories, Inc. (Wilmington, MA). Animals were housed according to the American Animal Association of Laboratory Animal

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Care guidelines. The rats were fed Laboratories Rodent Chow W (Harlan Laboratories, Madison, WI) and water *ad libitum*. Animals were decapitated and livers were removed and snap-frozen in liquid nitrogen. The small intestine was removed and sectioned into thirds. The duodenum was then dissected longitudinally, rinsed in saline, and intestinal epithelium was scraped and snap-frozen in liquid nitrogen. Tissues were stored at -80°C.

Treatments. Male SD rats were treated with the following chemicals for 4 days unless otherwise noted: TCDD (3.9 µg/kg in corn oil, ip, 1 day), β-naphthoflavone (100 mg/kg in corn oil, ip), PCB126 (40µg/kg in corn oil, po, 7 days), phenobarbital (80 mg/kg in saline, ip), diallyl sulfide (500 mg/kg in corn oil, ip), *trans*-stilbene oxide (200 mg/kg in corn oil, ip, 5 days), PCN (50 mg/kg in corn oil, ip), spironolactone (75 mg/kg in corn oil, ip), dexamethasone (50 mg/kg in corn oil, ip), clofibrate (200 mg/kg in saline, ip), di-(2-ethylhexyl)phthalate (200 mg/kg in corn oil, gav), perfluorodecanoic acid (40 mg/kg in corn oil, ip, 1 day), ethoxyquin (150 mg/kg in corn oil, po), oltipraz (150 mg/kg in corn oil, po), streptozotocin (100 mg/kg in 100mM sodium citrate, ip, 1 day), isoniazid (200 mg/kg in saline, ip), and acetylsalicylic acid (500 mg/kg in corn oil, po). Control rats (n=4 per treatment) were treated by ip injection with either saline or corn oil, or by po with corn oil. Injection volumes for all treatments were 5 ml/kg. Induction of cytochrome P450 1A1/2, 2B1/2, 3A1/23, 4A2/3, 2E1, and NADPH-quinone oxidoreductase mRNA served as positive controls for chemical

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treatments. This data, as well as the chemical structure of individual inducers, is reported by Johnson and Klaassen, (2002).

RNA extraction. Total tissue RNA was extracted using RNeasy Reagent (Qiagen, Crawfordsville, IN) according to the manufacturer's protocol, and resuspended in water treated with diethyl pyrocarbonate. RNA samples were analyzed by formaldehyde-agarose gel electrophoresis and integrity was confirmed by visualization of intact 18S and 28S rRNA.

Branched DNA (Signal Amplification Assay). UGT mRNA was measured using branched signal amplification analysis (Quantigene branched DNA Signal Amplification Kit, Bayer Diagnostics, East Walpole, MA) with modifications (Hartley and Klaassen, 2000). Rat UGT sequences of interest were obtained from Genbank. Multiple oligonucleotide probe sets were designed using Probe DesignerTM software v1.0 to be specific to a single mRNA transcript. The probes were designed with a T_m of approximately 63°C to enable hybridization conditions to be held constant (53°C) during each hybridization step and for each probe set. Each oligonucleotide probe was submitted to the National Center for Biotechnological Information for nucleotide comparison by the basic logarithmic alignment search tool to ensure minimal cross reactivity with other known rat sequences and expressed sequence tags. Oligonucleotide probes with a high degree of similarity ($\geq 80\%$) to other rat gene transcripts were eliminated from the probe set. Probe sets for UGT1A1, 2, 5, 6, 7, 2A1, and 2B1 (Vansell and

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Klaassen, 2002) and UGT1A3, 8, 2B2, 3, 6, 8, and 12 were described previously (Shelby *et al.*, 2003).

Total RNA (1 µg/µl; 10 µl/well; n= 4-5) was allowed to hybridize to the probe sets overnight at 53°C. Subsequent hybridization steps were carried out and alkaline phosphatase substrate was added. Luminescence was measured with a Quantiplex™ 320 branched DNA Luminometer interfaced with Quantiplex™ Data Management Software Version 5.02. Luminescence was reported as relative light units per 10 µg of total RNA.

Statistics. Analysis of variance followed by Duncan's multiple range post hoc analysis was used to determine statistical differences between control groups and between control and treated animals. Statistical significance was accepted at $p < 0.05$ and is denoted by an asterisk (*).

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Results

Induction of UGT1A mRNA in Liver. The effects of MEI treatment on UGT1A mRNA expression in liver are shown in Figure 1. UGT1A1 mRNA was up-regulated by numerous MEIs including the CAR activators PCB99 (~2-fold), diallyl sulfide (~4-fold), and *trans*-stilbene oxide (~3-fold); two PXR ligands PCN (~5-fold) and spironolactone (~2-fold); the PPAR α ligands clofibrate (~2 fold), DEHP (~3.5-fold), and PFDA (~3-fold); and the CYP2E1 inducer streptozotocin (~3-fold) (Fig. 1a). No effects on UGT1A1 mRNA expression were observed following treatment with AhR ligands or Nrf2 activators. UGT1A2 is predominantly expressed in the gastrointestinal tract and is barely detectable in liver; nonetheless inducibility by classical MEIs was examined because constitutive expression of inducible enzymes is often low. However, UGT1A2 mRNA expression in liver was not affected by any MEI treatment (Fig. 1a). Similar to UGT1A2, UGT1A3 is barely detectable in liver and predominantly expressed in intestine. Conversely, UGT1A3 mRNA was up-regulated by many MEIs including all CYP2E1 inducers, two of three AhR and PPAR α ligands, and the Nrf2 activator oltipraz (Fig 1a). Despite this up-regulation, resultant UGT1A3 mRNA levels in liver were still very low. UGT1A5 mRNA was up-regulated by various MEIs including the two CAR activators diallyl sulfide and *trans*-stilbene oxide, PCN, DEHP, and ethoxyquin (Fig. 1a). This seemingly sporadic up-regulation of UGT1A5 by individual compounds within a class hinders elucidation of specific nuclear receptors involved in the regulation of UGT1A5. UGT1A6

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mRNA was up-regulated by all AhR ligands and Nrf2 activators, and by three of four CAR activators (Fig. 1b). Similar to UGT1A6, UGT1A7 mRNA was up-regulated by all AhR ligands and Nrf2 activators, and the CAR activator *trans*-stilbene oxide (Fig. 1b). Like other UGT1A isoforms, UGT1A8 mRNA is barely detectable in liver. UGT1A8 does not appear to be inducible by classic nuclear receptor mechanisms (Fig. 1b).

Induction of UGT1A mRNA in Duodenum. The effects of MEI treatment on UGT1A mRNA expression in duodenum are shown in Figure 2. Unlike UGT1A1 mRNA expression in liver, UGT1A1 mRNA was not up-regulated by any MEI treatment in duodenum. Although PFDA and streptozotocin, which induced UGT1A1 mRNA expression in liver, tended to decrease UGT1A1 mRNA in duodenum (Fig. 2a). UGT1A2 mRNA was up-regulated by the two PXR ligands spironolactone and dexamethasone, the Nrf2 activator oltipraz, and the CYP2E1 inducer isoniazid (Fig. 2a). In liver, numerous MEIs up-regulated UGT1A3 mRNA levels; however in duodenum only oltipraz was able to induce UGT1A3 mRNA (Fig. 2a). Similar to the situation with UGT1A3, multiple MEIs induced UGT1A5 mRNA in liver but none induced UGT1A5 mRNA in duodenum (Fig. 2a). This was also the case for UGT1A6 and 1A7 (Fig. 2b). UGT1A8 mRNA was not induced by any MEI treatment (Fig. 2b).

Induction of UGT2B mRNA in Liver. The effects of MEI treatment on UGT2B mRNA expression in liver are shown in Figure 3. UGT2B1 mRNA levels were

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up-regulated by all CAR activators and Nrf2 activators, as well as PCN (Fig. 3a). UGT2B2 mRNA levels were not significantly increased by any MEI treatment (Fig. 3A). UGT2B3 mRNA levels were up-regulated by both Nrf2 activators and the CAR activator *trans*-stilbene oxide (Fig. 3a). UGT2B6 mRNA levels were not significantly up-regulated by any MEI treatment. Although both Nrf2 activators and two CAR activators (PCB99 and *trans*-stilbene oxide) tended to increase UGT2B6 mRNA levels (Fig. 3b). UGT2B8 mRNA levels were up-regulated by the CAR activator *trans*-stilbene oxide and the Nrf2 activator oltipraz (Fig. 3b). UGT2B12 mRNA levels were up-regulated by the AhR ligand PCB126 and by the two CAR activators PCB99 and *trans*-stilbene oxide (Fig. 3b).

Induction of UGT2B mRNA in Duodenum. The effects of MEI treatment on UGT2B mRNA expression in duodenum are shown in Figure 4. UGT2B1 mRNA levels are barely detectable in duodenum and were not increased by any MEI treatment. However, all four CAR activators tended to increase UGT2B1 mRNA levels (Fig. 4a). Like UGT2B1, UGT2B2 mRNA is barely detectable in duodenum and was not increased by any MEI treatment (Fig. 4a). UGT2B3 mRNA was up-regulated by the Nrf2 activator oltipraz and tended to be increased by the Nrf2 activator ethoxyquin (Fig. 4a). UGT2B6 mRNA levels were not induced by any MEI treatment, but tended to be increased by the Nrf2 activator oltipraz (Fig. 4b). UGT2B8 mRNA was not up-regulated by any MEI. However, both Nrf2 activators, the CAR activator diallyl sulfide, and the PXR

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ligand PCN tended to increase UGT2B8 mRNA levels in duodenum (Fig. 4b). UGT2B12 mRNA levels were up-regulated by both Nrf2 activators (Fig. 4b).

Discussion

The ability of drugs and other chemicals to increase the expression of detoxifying biotransformation enzymes, such as UGTs, and subsequently alter the disposition of both endogenous and xenobiotic compounds, has been of interest for several decades. For instance, in the sixties, administration of the antiepileptic drug phenobarbital to a hyperbilirubinemic infant was shown to increase bilirubin UGT activity resulting in amelioration of hyperbilirubinemia (Yaffe *et al.*, 1966). More recently in mice, induction of UGTs by the chemopreventive agent oltipraz increased glucuronidation of the bladder carcinogen *N*-nitrosobutyl (4-hydroxybutyl) amine resulting in decreased incidence of bladder carcinoma (Iida *et al.*, 2004). There are numerous studies, in addition to the aforementioned studies, which have demonstrated an increase in UGT expression or activity in response to xenobiotic exposure. In the present study, different groups of compounds known to induce phase-I biotransformation enzymes through various transcriptional pathways involving nuclear receptors and other transcription factors were utilized to indicate potential mechanisms of transcriptional regulation of individual UGT isoforms in rat liver and duodenum and coordinate regulation of UGTs with phase-I biotransformation enzymes.

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With the exception of UGT1A1, most UGT1A isoforms are expressed at low levels in naïve rat liver. Only UGT1A1, 1A5, and 1A6 have appreciable expression in rat liver (Grams *et al.*, 2000; Shelby *et al.*, 2003). Despite low levels of expression, many of the UGT1A isoforms are inducible in liver. For example, some studies have not detected UGT1A7 in naïve rat liver, but have readily detected it after treatment with oltipraz (Emi *et al.*, 1995; Grove *et al.*, 1997). Unlike in liver, most UGT1A isoforms have appreciable, if not predominant, expression in intestine (Grams *et al.*, 2000; Shelby *et al.*, 2003). In contrast, most rat UGT2B isoforms have predominant expression in liver and minimal expression in other tissues (Shelby *et al.*, 2003). Only UGT2B8 has higher expression in intestine than in liver (Shelby *et al.*, 2003).

Although CAR and PXR are reportedly expressed in liver and intestine, induction of UGTs by CAR activators and PXR ligands was observed primarily in liver. In duodenum, CAR activators did not induce any UGTs, and only UGT1A2 was induced by PXR ligands. UGT1A2, which is predominantly expressed in intestine and barely detectable in liver (Shelby *et al.*, 2003), was the only UGT to be induced in duodenum but not liver.

Overall, CAR activators had more effects on UGT mRNA expression in liver than did PXR ligands. UGTs induced in liver by CAR activators include UGT1A1, 1A5, 1A6, 2B1, and 2B12. UGT2B1, which is predominantly expressed in liver, was the only UGT to be induced by all four CAR activators, strongly indicating it

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is a potential CAR target gene. This is not surprising as UGT2B1 was originally identified by its ability to be induced by phenobarbital, a CAR activator (Mackenzie, 1986). UGT1A1, 1A5, 1A6, and 2B12, were all induced in liver by at least two of four CAR activators. Interestingly, none of these UGTs were induced by the prototypical CAR activator phenobarbital. However, in the case of UGT1A1, other groups have also reported a lack of induction of rat UGT1A1 mRNA following phenobarbital treatment (Emi *et al.*, 1995). Furthermore, other investigators have reported that rat bilirubin glucuronidation activity is not readily inducible by phenobarbital, which is in contrast to bilirubin glucuronidation activity in humans (Bock *et al.*, 1973). UGT2B12 has been reported previously to be inducible in liver by phenobarbital though induction was not observed in the present study (Green *et al.*, 1995).

UGT1A1, 1A5, and 2B1, were induced in liver by the PXR ligand PCN, and only UGT1A1 was also induced by spironolactone. In another study, northern and western blot analysis revealed induction of UGT1A1, 1A5, and 2B1 following spironolactone treatment (Catania *et al.*, 2003). Thus, rat UGT1A1, 1A5, and 2B1 may be both CAR and PXR target genes in liver, however further evidence is needed. Hepatic expression of UGT1A6 and 2B12 is also potentially regulated by CAR, although PXR does not appear to regulate these genes.

Unlike CAR and PXR, PPAR α does not seem to play a major role in the body's defense mechanism against xenobiotics. PPAR α is activated by fatty acids and

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compounds referred to as peroxisome proliferators (PP) for their ability to produce a pleiotropic response including hepatomegaly, peroxisome proliferation, and induction of specific enzymes involved in both peroxisomal β -oxidation and microsomal ω -oxidation (Cyp4A) of fatty acids (Reddy *et al.*, 1982; Van Rafelghem *et al.*, 1987; Chinje *et al.*, 1994; Johnson *et al.*, 1996). The pleiotropic response, normally observed after administration of PPs such as clofibrate, is not observed in PPAR α -null mice (Lee *et al.*, 1995). Moreover, liver and kidney, which express high amounts of PPAR α , are the tissues that are most responsive to PPs (Kliwer *et al.*, 1994). As PPAR α is not expressed appreciably in intestine, induction of UGTs in intestine by PPAR α agonists was not observed or expected.

Despite its seemingly predominant role in regulating genes involved in fatty acid metabolism and transport, PPAR α can also mediate induction of biotransformation enzymes. Recently UGT1A9, a human UGT that metabolizes fibrates, was shown to be induced by fibrates via activation of PPAR α (Barbier *et al.*, 2003). Fibrates have also been demonstrated to increase bilirubin glucuronidation activity (Lilienblum *et al.*, 1982). In a study by Emi *et al.* (1995), clofibrate induced UGT1A1 mRNA and had lesser effects on UGT1A5 mRNA. This is in agreement with the present study, in which, all three PPAR α activators induced UGT1A1 in liver, whereas only the PPAR α activator DEHP induced UGT1A5. Our data suggest that in rat, UGT1A1 is a PPAR α target gene and that exposure to PPs can potentially increase its mRNA expression in liver.

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In addition to the nuclear receptors CAR, PXR, and PPAR α , the transcription factors AhR and Nrf2 are involved in induction of detoxifying enzymes in response to chemical exposure. The AhR pathway of transcriptional activation has been known for numerous years and is well established, whereas the role of Nrf2 in induction of detoxifying enzymes is a newer concept. Increasing evidence suggests AhR and Nrf2 are somehow linked. Analysis of the mouse Nrf2 promoter revealed the presence of XRE-like elements. Additionally Nrf2 was induced by the AhR ligand TCDD, and AhR was shown to bind the Nrf2 promoter by the chromatin immunoprecipitation assay (Miao *et al.*, 2005). Thus, administration of AhR ligands should result in increased Nrf2, however the functional consequence of this remains unknown. Previous studies have reported the presence of XRE's in regulatory regions of UGT1A6 and UGT1A7 and inducibility of these UGTs by the AhR ligand 3-methylcholanthrene (Emi *et al.*, 1995). UGT1A6 and UGT1A7 are also inducible by the Nrf2 activator, oltipraz (Grove *et al.*, 1997; Kessler and Ritter, 1997). A recent study demonstrated that the Nrf2 activator oltipraz is capable of activating AhR, triggering binding of the AhR/ARNT heterodimer to XREs, thereby mediating transcription of UGT1A6 through the AhR pathway (Auyeung *et al.*, 2003). More recently, UGT1A6 and 1A7 induction by oltipraz was abrogated in Nrf2-null mice, suggesting a major role for Nrf2 in induction of these genes by oltipraz (Iida *et al.*, 2004). In our study, all AhR ligands induced hepatic expression of UGT1A6 and UGT1A7. Two AhR ligands induced expression of UGT1A3 in liver. However

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even the increased expression of UGT1A3 is extremely low, and thus, likely of nominal consequence. AhR ligands did not affect expression of UGTs in duodenum. This is in agreement with a previous study that examined harmol and acetaminophen glucuronidation following TCDD treatment (Goon and Klaassen, 1992), but in disagreement with Kobayashi et al., (1998) who saw induction of UGT1A7 in duodenum following administration of β -naphthoflavone, as shown by 1-naphthol glucuronidation. A potential reason for the discrepancy between this study and (Kobayashi *et al.*, 1998) is the route of administration of β -bapthoflavone, which was given orally in their study.

Similar to AhR ligands, both Nrf2 activators induced hepatic expression of UGT1A6 and 1A7. However, both Nrf2 activators also induced hepatic expression of UGT2B1 and 2B3. In contrast to AhR ligands, Nrf2 activators also induced expression of UGTs in duodenum. UGT2B12 mRNA expression was induced by Nrf2 activators, ethoxyquin and oltipraz, whereas UGT1A2, 1A3, and 2B3 were induced, but only by oltipraz.

In conclusion, the regulation of inducible expression of UGTs is complex and needs more in depth study as to the specific mechanisms of regulation. This study was to indicate potential mechanisms of regulation of UGT isoforms in rat liver and duodenum. Each individual UGT has its own inducible profile. Some UGTs were not induced by any treatment whereas others were induced by many treatments. Several UGT1A isoforms were readily induced in liver but not

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duodenum. Thus, tissue specific factors seem to be involved in inducible expression. Overall, CAR/PXR and Nrf2 seem to be major pathways of transcriptional activation of UGTs in response to xenobiotics though further analysis is needed. Understanding the transcriptional mechanisms that govern expression of individual UGT enzymes could be important in drug design, pharmacological manipulation of UGT expression, as well as avoiding drug-drug interactions.

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FOOTNOTES

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Figure Legends

Figure 1. Effects of Microsomal Enzyme Inducer Treatment on UGT1A mRNA expression in liver. UGT1A1, UGT1A2, UGT1A3, and UGT1A5 (1a.) and UGT1A6, UGT1A7, and UGT1A8 (1b.) mRNA expression in liver following treatment with (n=4-5 per treatment) TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; BNF, β -Naphthoflavone; PCB126, polychlorinated biphenyl congener 126; PB, Phenobarbital; DAS, diallyl sulfide; PCB99, polychlorinated biphenyl congener 99; TSO, *trans*-stilbene oxide; PCN, pregnenolone-16 α -carbonitrile; SPIRO, spironolactone; DEX, dexamethasone; CLOF, clofibrate; DEHP, di-(2-ethylhexyl)phthalate; PFDA, perfluorodecanoic acid; ETH, ethoxyquin; OLT, oltipraz; STREP, streptozotocin; ISO, isoniazid; ASA, acetylsalicylic acid, saline, or cornoil. Control groups were not statistically different and were combined. Total RNA was isolated from liver and mRNA levels were evaluated using branched DNA signal amplification analysis. Data are reported as relative light units (RLU) /10 μ g Total RNA. Statistical significance was accepted at $p < 0.05$ and is denoted by an asterisk (*).

Figure 2. Effects of Microsomal Enzyme Inducer Treatment on UGT1A mRNA expression in duodenum. UGT1A1, UGT1A2, UGT1A3, and UGT1A5 (2a.) and UGT1A6, UGT1A7, and UGT1A8 (2b.) mRNA expression in duodenum following treatment with (n=4-5 per treatment) TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; BNF, β -Naphthoflavone; PCB126, polychlorinated biphenyl congener 126; PB, Phenobarbital; DAS, diallyl sulfide; PCB99, polychlorinated biphenyl congener

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99; TSO, *trans*-stilbene oxide; PCN, pregnenolone-16 α -carbonitrile; SPIRO, spironolactone; DEX, dexamethasone; CLOF clofibrate; DEHP di-(2-ethylhexyl)phthalate; PFDA, perfluorodecanoic acid; ETH, ethoxyquin; OLT, oltipraz; STREP, streptozotocin; ISO, isoniazid; ASA acetylsalicylic acid, saline, or cornoil. Control groups were not statistically different and were combined. Total RNA was isolated from liver and mRNA levels were evaluated using branched DNA signal amplification analysis. Data are reported as relative light units (RLU) /10 μ g Total RNA. Statistical significance was accepted at $p < 0.05$ and is denoted by an asterisk (*).

Figure 3. Effects of Microsomal Enzyme Inducer Treatment on UGT2b mRNA expression in liver. UGT2B1, UGT2B2, and UGT2B3 (3a.) and UGT2B6, UGT2B8, and UGT2B12 (3b.) mRNA expression in liver following treatment with (n=4-5 per treatment) TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; BNF, β -Naphthoflavone; PCB126, polychlorinated biphenyl congener 126; PB, Phenobarbital; DAS, diallyl sulfide; PCB99, polychlorinated biphenyl congener 99; TSO, *trans*-stilbene oxide; PCN, pregnenolone-16 α -carbonitrile; SPIRO, spironolactone; DEX, dexamethasone; CLOF clofibrate; DEHP di-(2-ethylhexyl)phthalate; PFDA, perfluorodecanoic acid; ETH, ethoxyquin; OLT, oltipraz; STREP, streptozotocin; ISO, isoniazid; ASA acetylsalicylic acid, saline, or cornoil. Control groups were not statistically different and were combined. Total RNA was isolated from liver and mRNA levels were evaluated using branched DNA signal amplification analysis. Data are reported as relative light

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units (RLU) /10 μ g Total RNA. Statistical significance was accepted at $p < 0.05$ and is denoted by an asterisk (*).

Figure 4. Effects of Microsomal Enzyme Inducer Treatment on UGT2B mRNA expression in duodenum. UGT2B1, UGT2B2, and UGT2B3 (4a.) and UGT2B6, UGT2B8, and UGT2B12 (4b.) mRNA expression in duodenum following treatment with (n=4-5 per treatment) TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; BNF, β -Naphthoflavone; PCB126, polychlorinated biphenyl congener 126; PB, Phenobarbital; DAS, diallyl sulfide; PCB99, polychlorinated biphenyl congener 99; TSO, *trans*-stilbene oxide; PCN, pregnenolone-16 α -carbonitrile; SPIRO, spironolactone; DEX, dexamethasone; CLOF, clofibrate; DEHP, di-(2-ethylhexyl)phthalate; PFDA, perfluorodecanoic acid; ETH, ethoxyquin; OLT, oltipraz; STREP, streptozotocin; ISO, isoniazid; ASA, acetylsalicylic acid, saline, or cornoil. Control groups were not statistically different and were combined. Total RNA was isolated from liver and mRNA levels were evaluated using branched DNA signal amplification analysis. Data are reported as relative light units (RLU) /10 μ g Total RNA. Statistical significance was accepted at $p < 0.05$ and is denoted by an asterisk (*).

Fig 1a

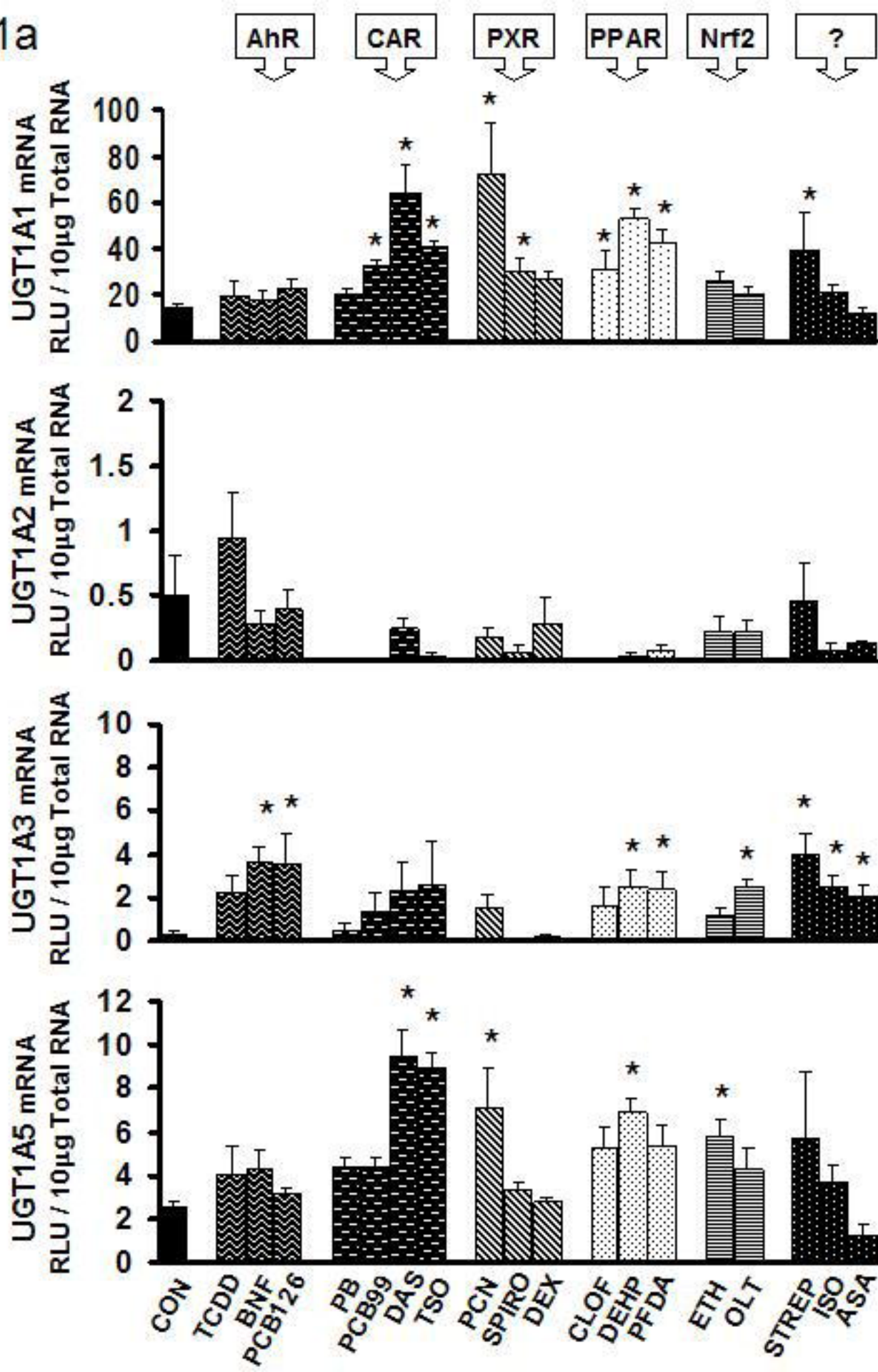


Fig 1b

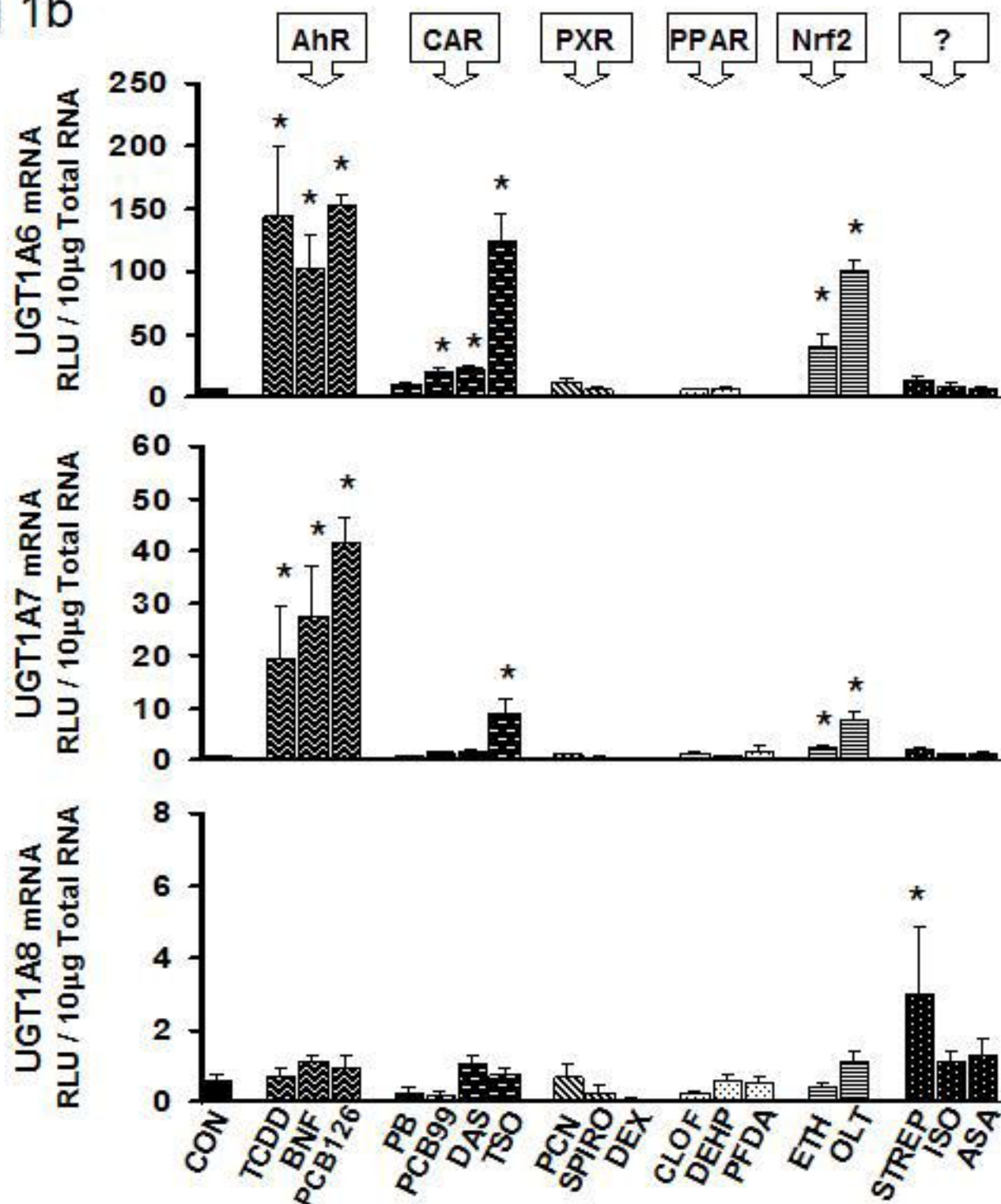


Fig 2a

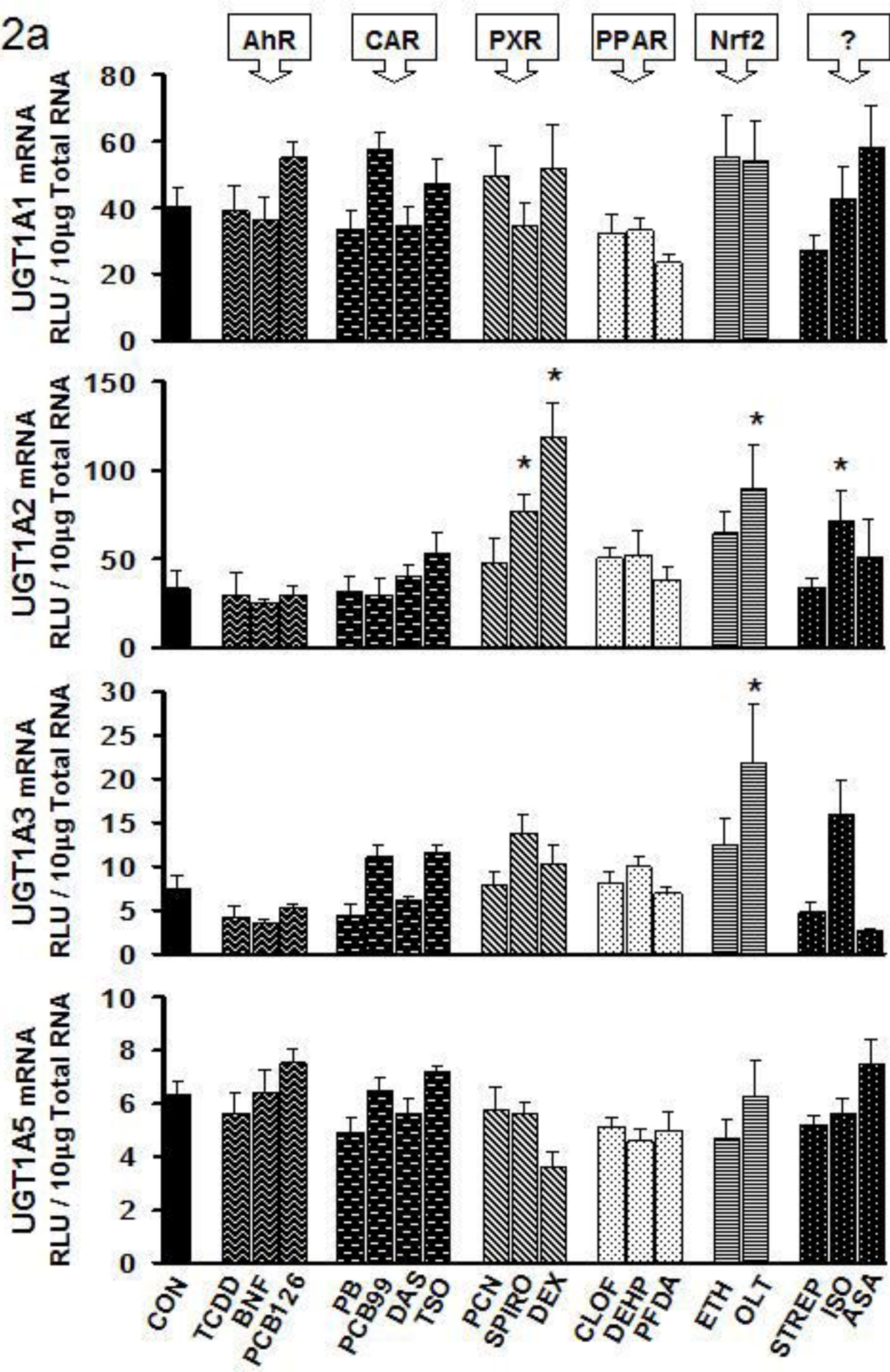


Fig 2b

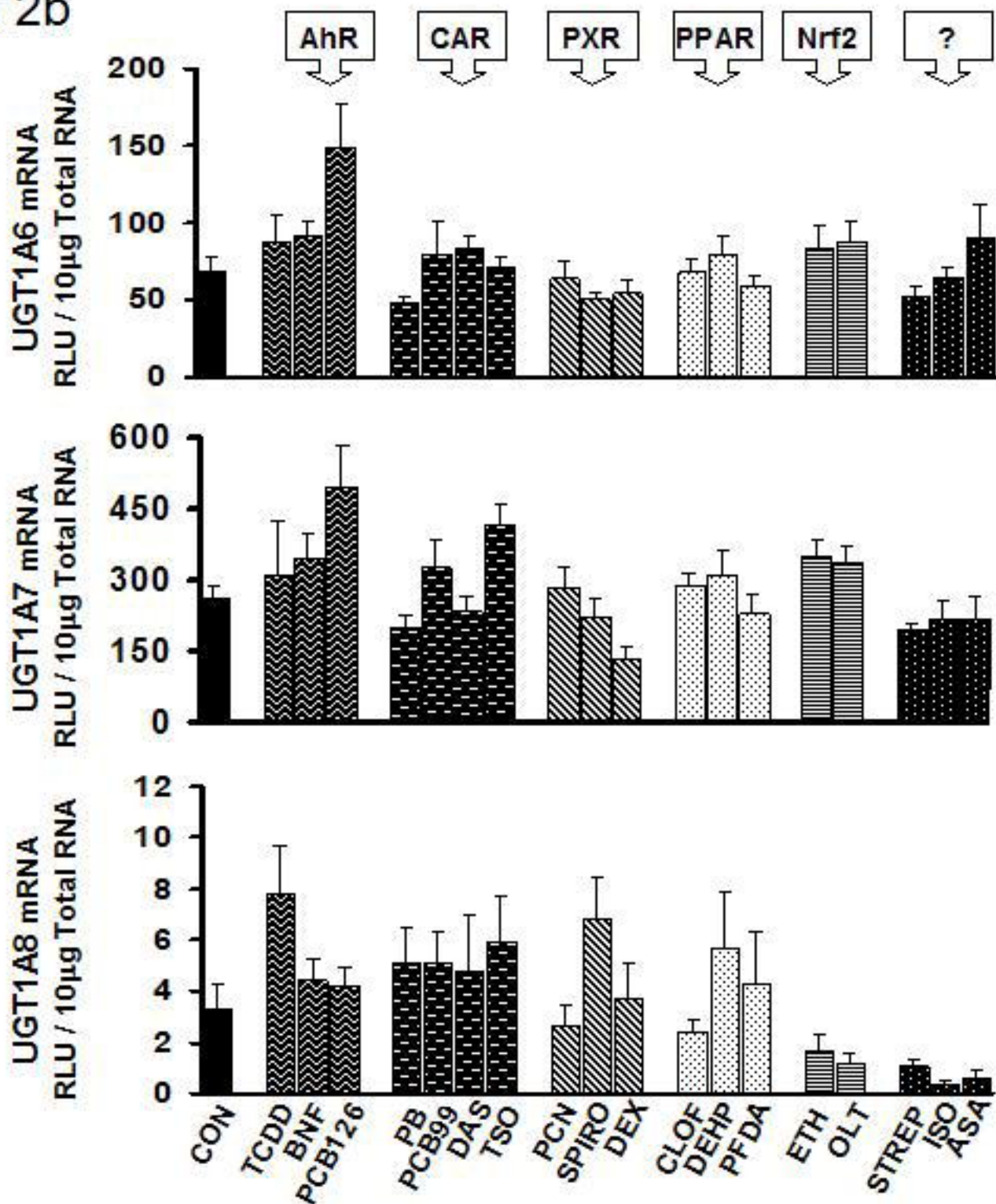


Fig 3a

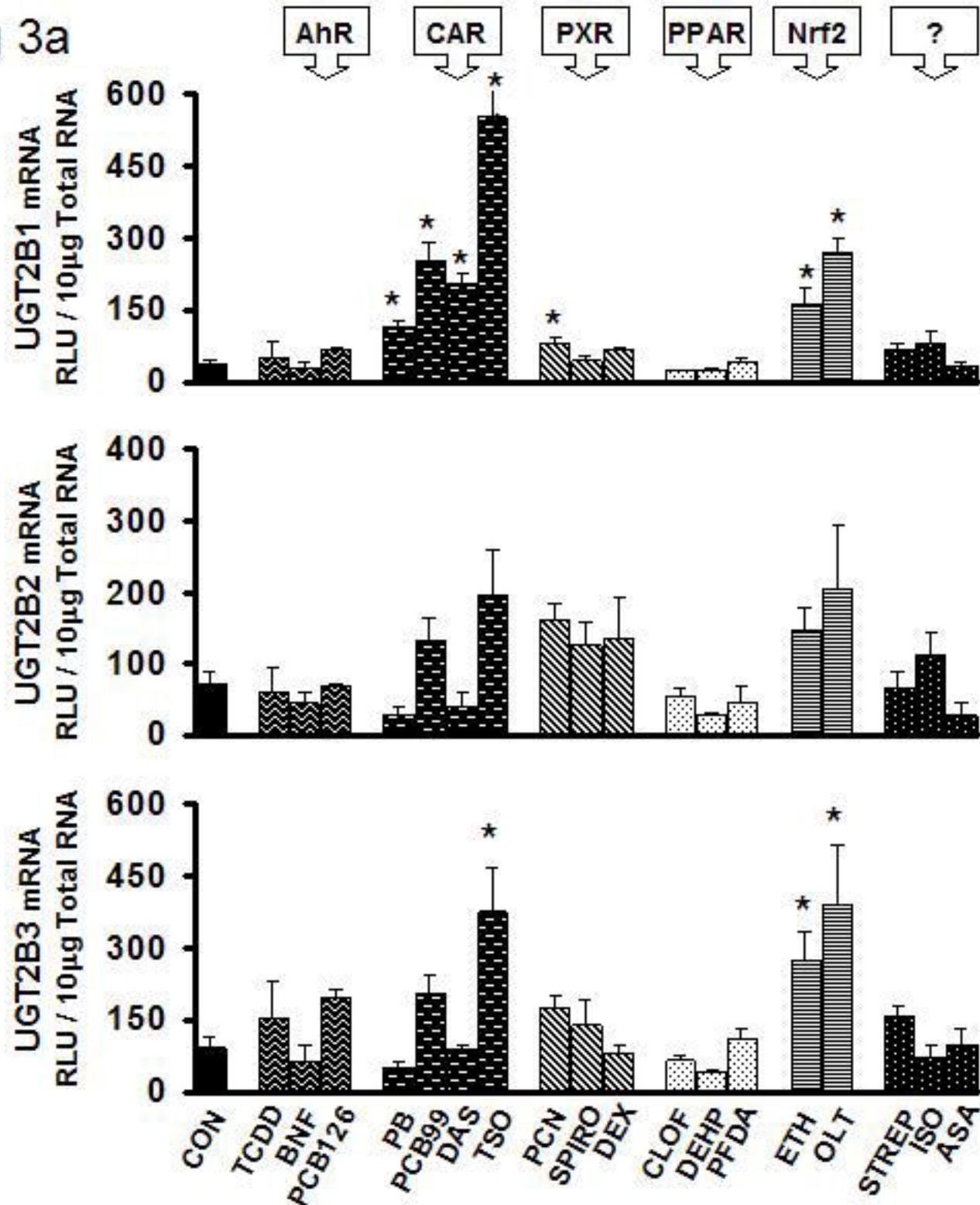


Fig 3b

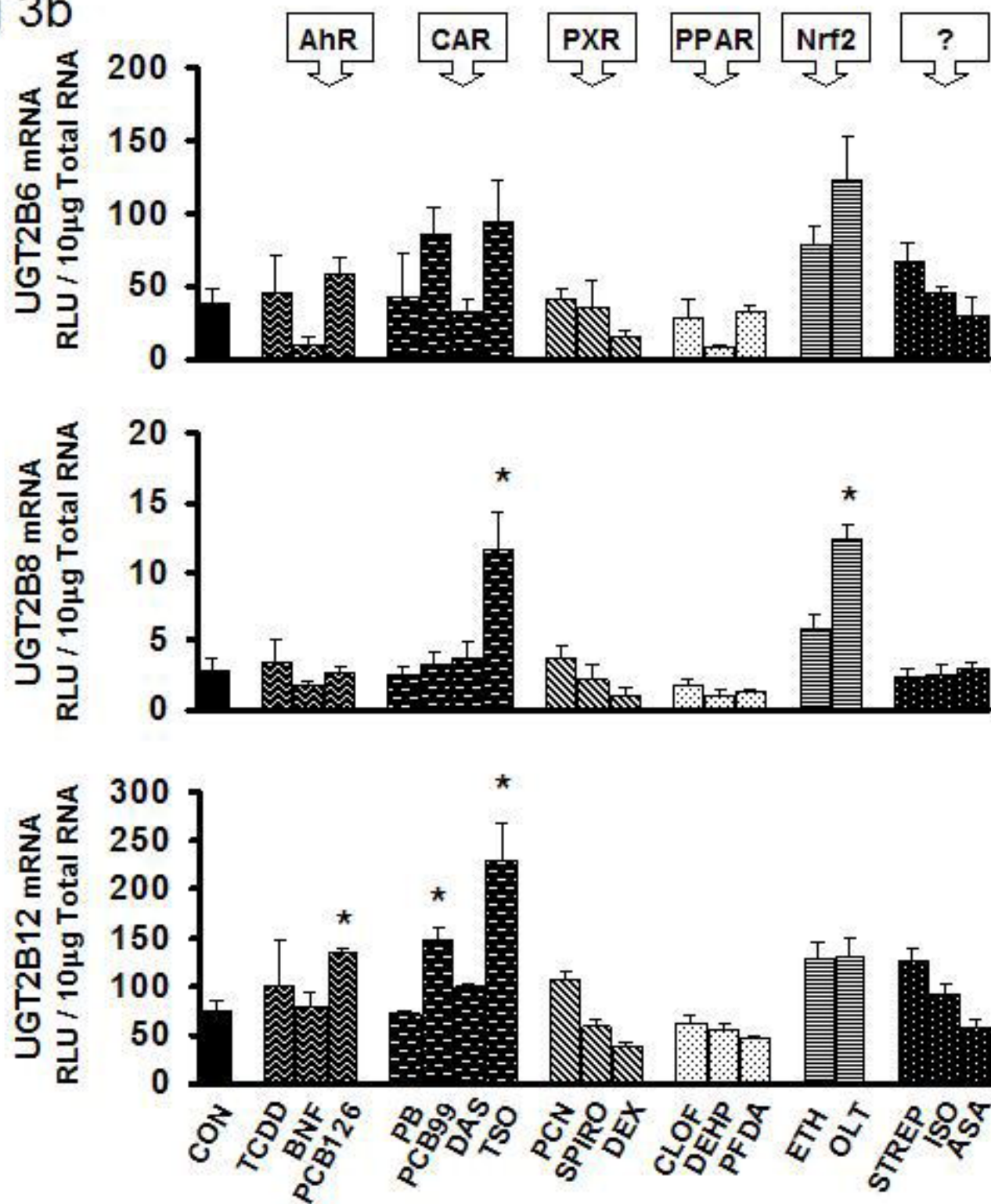


Fig 4a

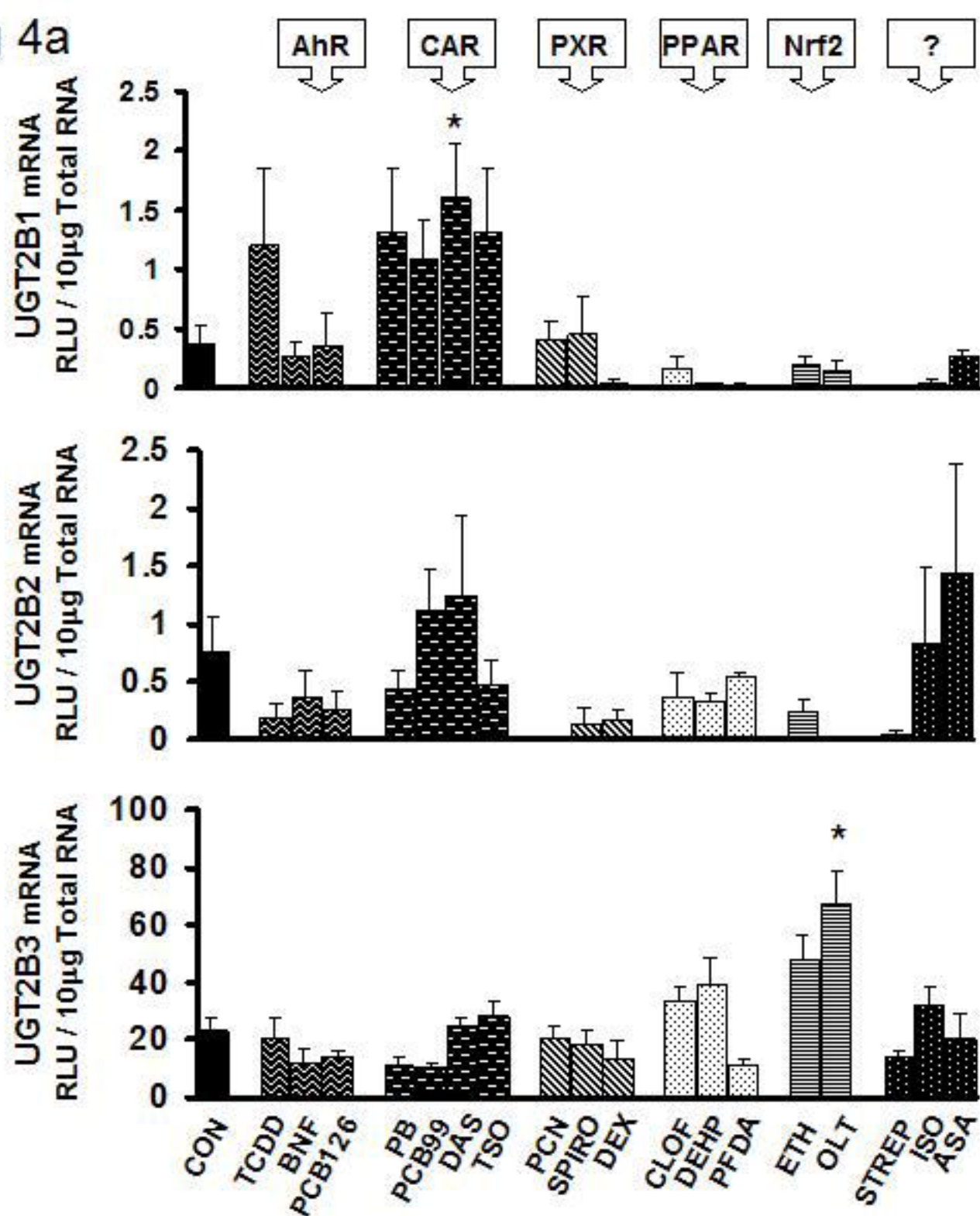


Fig 4b

