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Opposing mechanisms of NADPH-cytochrome P450 oxidoreductase regulation by peroxisome proliferators

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Abstract

Peroxisome proliferators (PPs) regulate a battery of rodent P450 genes, including *CYP2B*, *CYP2C*, and *CYP4A* family members. We hypothesized that other components of the P450-metabolizing system are altered by exposure to PPs, including NADPH-cytochrome P450 oxidoreductase (P450R), an often rate-limiting component in P450-dependent reactions. In this study, we determined whether exposure to structurally diverse PPs alters the expression of P450R mRNA and protein. Increases in P450R mRNA levels were observed in male and female F-344 rat livers and in male rat kidneys after chronic exposure of the animals to PPs. Paradoxically, under the same treatment conditions in male rats, liver P450R protein levels decreased after exposure to the PPs Wy-14,643 ([4-chloro-6-(2,3-xylidino)pyrimidynylthio]acetic acid) (WY) or gemfibrozil (GEM). The down-regulation of the P450R protein was sex- and tissue-specific in that exposure to PPs led to increases in P450R protein in female rat livers [di-*n*-butyl phthalate (DBP) only] and male rat kidneys (WY, GEM, DBP). In male wild-type SV129 mice, P450R mRNA levels increased in livers after exposure to WY and diethylhexyl phthalate (DEHP) and in male kidneys after exposure to DEHP. Induction of mRNA by PPs was not observed in the liver or kidneys of mice, which lack a functional peroxisome proliferator-activated receptor α (PPARα), the central mediator of the effects of PPs in the rodent liver. In wild-type male mice, P450R protein was decreased in liver after WY and DEHP treatment and in kidneys after WY treatment. The down-regulation of the P450R protein was not observed in PPARα-null mice. These studies demonstrate the complex regulation of P450R expression by PPs at two different levels, both of which are dependent upon PPARα: up-regulation of transcript levels in liver and kidneys and down-regulation of protein levels in male rat and mouse liver by a novel posttranscriptional mechanism.

Keywords: Peroxisome proliferators; NADPH-cytochrome P450 oxidoreductase; Peroxisome proliferator-activated receptor α; Transcriptional regulation; Posttranscriptional regulation

1. Introduction

PPs are a large group of chemicals that include hypolipidemic drugs, plasticizers, and industrial solvents. These chemicals induce hepatomegaly, hepatic peroxisome proliferation, and alterations in the levels of lipid-metabolizing genes in rodents [reviewed in Ref. 1]. Responses to PP exposure are controlled by three members of the nuclear receptor superfamily called PPARs. PPARα is expressed mainly in tissues important in lipid metabolism such as intestine, heart, kidneys, and liver. PPARβ (also known as PPARδ) is expressed almost universally, but its functions have not been clearly defined. PPARγ plays a key role in adipogenesis and is an important negative regulator of the inflammatory response [2,3]. After binding PPs, these receptors heterodimerize with another member of the receptor superfamily called RXR, resulting in heterodimer interaction with DNA sequences called PPREs found in responsive genes. Studies using a mouse strain that lacks a functional form of PPARα (PPARα-null mouse)

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Abbreviations: ACO, acyl-CoA oxidase; DBP, di-*n*-butyl phthalate; DEHP, diethylhexyl phthalate; GEM, gemfibrozil; P450R, NADPH-cytochrome P450 oxidoreductase; PP, peroxisome proliferator; PBST, phosphate-buffered saline-Tween; PPAR, peroxisome proliferator-activated receptor; PPRE, PP-responsive element; RXR, retinoid X receptor; TRE, thyroid hormone response element; THX, thyroidectomized; and WY, Wy-14,643 ([4-chloro-6-(2,3-xylidino)pyrimidynylthio]acetic acid).

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demonstrated that most, if not all, of the effects induced by PPs in the liver are mediated by PPARα [4,5].

Exposure to PPs has been shown to alter the expression of members of the P450 family of enzymes including increases in the expression of members of the *CYP4A* subfamily involved in ω and ω-1 oxidation of fatty acids and prostaglandins [reviewed in Ref. 6] as well as the *CYP2B1* gene [7]. Exposure to PPs results in the down-regulation of a number of rat P450 genes, including those involved in steroid metabolism, *CYP2C11* and *CYP2C12* [8], and a mouse naphthalene hydroxylase, *Cyp2F2* [9].

Activity of P450 proteins is dependent upon the P450R (EC 1.6.2.4), a membrane-bound flavoprotein that functions as an internal electron transport chain transferring electrons from NADPH to cytochrome P450 [10]. As such, P450R indirectly participates in monooxygenation reactions catalyzed by a large number of individual P450 enzymes and plays an important role in the initial metabolism of a wide range of foreign lipophilic compounds as well as in drug biotransformation [10]. Alterations in the levels of P450R have been shown to affect the extent of P450-dependent reactions [11]. In contrast to the cytochrome P450 gene superfamily, P450R is encoded by a single well-conserved gene in mice, rats, and humans whose mRNA levels are increased independently of P450 by phenobarbital, dexamethasone, and 2-acetylamino fluorene [summarized in Ref. 10]. The P450R gene is also positively regulated by T_3 [12] through a TRE identified in the upstream promoter [13].

To gain additional insights into the mechanisms of PP regulation of the P450 enzyme system, we examined the expression of P450R in mouse and rat tissues after exposure to PPs. We found that the P450R gene transcript levels in the liver and kidneys were positively regulated by PPs. Surprisingly, we also found that the levels of the P450R protein in male rat and mouse liver decreased at the same time the P450 mRNA levels increased. Thus, opposing mechanisms of regulation of P450R are triggered after PP exposure, and both mechanisms depend upon PPARα.

2. Materials and methods

2.1. Animals

These studies were conducted under federal guidelines for the use and care of laboratory animals and were approved by the Institutional Animal Care and Use Committee of the Chemical Industry Institute of Toxicology (CIIT). Control and treated mice and rats (4–6/group) were provided with NIH-07 rodent chow (Zeigler Bros.) and deionized, filtered water *ad lib.* Lighting was on a 12-hr light–dark cycle. For the first three studies, male F-344 [CDF(F-344)/CrlBR] and Sprague–Dawley (SD-Harlan derived) rats were obtained from the Charles River Breeding Laboratories. In the first study, F-344 rats were administered by gavage a single dose of WY (ChemSyn Science Laboratories) at 50 mg/kg of body weight and killed 12 or 24 hr after treatment. In the second study, male and female F-344 rats and male Harlan Sprague–Dawley rats were fed 500 ppm WY, 8000 ppm GEM (Sigma Chemical) or 20,000 ppm DBP (Aldrich Chemical) for 13 weeks. In the third study, THX or THX male Sprague–Dawley rats given T_3 hormone (2 mg/kg in saline by injection) were given WY (25 mg/kg) or methylcellulose carrier by gavage. Intact male F344 rats also were given WY (25 mg/kg) or methylcellulose carrier by gavage. Animals were killed 48 or 72 hr after exposure. In the fourth study (conducted by the National Toxicology Program, National Institute of Environmental Health Sciences), male Harlan Sprague–Dawley rats (Harlan Sprague–Dawley) were fed the indicated doses of WY, GEM or DBP (ChemCentral) for 1, 5, or 13 weeks. In the fifth study, male SV129 wild-type (Taconic) and SV129 PPARα-null mice [4], 6–8 weeks of age, were fed WY (0.1%) or DEHP (0.6%) for 3 weeks. At the designated time after treatment, animals were deeply anesthetized with isofluorane anesthesia or pentobarbital injection and killed by exsanguination. The tissues were removed, snap-frozen in liquid nitrogen, and stored at −70° until analysis.

2.2. Northern blot analysis

Total RNA from the tissues of individual animals was isolated by a modification of the guanidinium isothiocyanate method using RNAzol B according to the instructions of the manufacturer (Tel-Test). Twenty micrograms of denatured total RNA was separated on 1.0% formaldehyde-agarose gels and transferred to Hybond-N Nylon membranes in 20× standard saline citrate buffer. The RNA was fixed by UV cross-linking using a Stratalinker UV Crosslinker (Stratagene). The membranes were prehybridized with Hybrisol I (Oncor Inc.) for 4 hr at 42° followed by hybridization overnight. The 1.5-kb *Eco*RI–*Not*I fragment encoding the rat P450R cDNA was generated from a plasmid [14] provided by Dr. Todd Porter (University of Kentucky). The β-actin cDNA was obtained from Oncor. β-Actin was selected as a loading control since the level does not change after short-term exposure to WY [15]. The probes for northern analysis were labeled with [α-^{32}P]dCTP (Amersham) using the random-primer DNA labeling kit according to instructions of the manufacturer (Stratagene). After washing, the filter was exposed to BioMax X-ray film (Eastman Kodak Co.) at −70° with an intensifying screen. The size of the mRNA species was determined by comparing the mobility relative to an RNA ladder (GIBCO/BRL).

2.3. Western blot analysis

Whole-cell protein extracts from the tissues of individual animals were made according to Wilcke and Alexson [16].

Protein concentrations were determined using a dye binding protein assay (Bio-Rad) and bovine serum albumin as the standard. Protein extracts (100 μg total protein) were denatured and size-separated by 12.5% SDS–PAGE. Proteins were transferred to nitrocellulose (Stratagene) and visualized by Ponceau Red to confirm transfer. The membrane was washed with PBST, blocked by PBST containing 5% nonfat dry milk, and incubated with anti-P450R primary antibody (Chemical International Inc.) followed by horseradish peroxidase conjugated goat-anti-rabbit IgG (Amersham) as the secondary antibody. The reaction was detected by enhanced chemiluminescence (Super-Signal®, Pierce Chemical Co.). The size of the reactive immunoproteins was estimated by gel fragment sizer software (DogStar software) by comparison to the mobility of protein standards (Rainbow Marker; Amersham).

2.4. Quantitation of RNA and protein expression

Autoradiograms for northern and western blots were densitometrically scanned using the Image-1 image analysis system (Universal Imaging Corp.) and NIH Image 1.54 software. Significant differences ($P \leq 0.05$) between groups (N = 2–3) were determined by using the Tukey–Kramer HSD test or a three-way analysis of variance (SAS). Only statistically significant increases or decreases in P450R mRNA or protein levels are discussed in the text.

3. Results

3.1. Alteration of P450R mRNA expression after exposure to PPs

The mRNA levels of P450R were determined by northern blot analysis in rats treated in the diet for 13 weeks with three PPs (WY, GEM, or DBP). The P450R mRNA was detected in the liver as a 2.6-kb mRNA in control rats (Fig. 1A). After PP treatment, the P450R mRNA levels were increased in male rats fed WY (4.4-fold) or DBP (2.2-fold) but not GEM (Fig. 1A and B). Expression of P450R mRNA increased in the livers of female rats treated with WY (7.2-fold), GEM (5.1-fold), and DBP (4.4-fold) (Fig. 1C). Increases in male kidney P450R mRNA were also observed after exposure to WY (2.5-fold), GEM (2.6-fold), and DBP (3.6-fold) (Fig. 1D). No significant changes in P450R mRNA levels were observed in testis or heart from the same rats or in the livers of rats after a 12- or 24-hr gavage dose of WY (data not shown).

3.2. Alterations in P450R protein levels after exposure to PPs

P450R protein levels were analyzed in whole-cell extracts by western blot analysis from the same tissues used above for mRNA expression. In the absence of treatment, we could detect expression of an ~75-kDa, full-length P450R protein (Fig. 2A). No other proteins were immunoreactive under our incubation conditions. In contrast to the increases observed in P450R mRNA levels, the P450R protein levels were decreased markedly in male rat livers after treatment with WY (29% of control) and GEM (18% of control), but were unaffected by DBP treatment (Fig. 2A and B).

P450R protein levels in the livers of control female rats were noticeably lower than those of male controls, as observed earlier [12]. Treatment of females with WY or GEM had little, if any, effect on liver P450R protein levels. DBP treatment, however, resulted in a 3.2-fold increase in expression (Fig. 2C).

The expression of P450R protein was examined in the kidneys of the same rats used to analyze mRNA expression. In contrast to the down-regulation of P450R protein levels in liver, there was increased expression in male kidneys after treatment with WY (3.7-fold), GEM (3.2-fold), and DBP (5.0-fold) roughly in parallel with mRNA increases (Fig. 2D). Other tissues that express P450R also were examined for changes in P450R protein expression after exposure to PPs in this same study, including testis, adrenals, heart, and brain, but no consistent changes were detected (data not shown).

To further explore differences in the ability of PPs to alter expression of P450R protein, we examined changes in liver expression at 1, 5, and 13 weeks after initiation of the feeding study using the same dietary concentrations of WY, GEM, and DBP as above. In this study, we used male Sprague–Dawley rats. No overt strain-specific differences between the Fisher and Sprague–Dawley rats were evident in response to the three PPs (data not shown). The expression of P450R did not change over the time of the experiment in the control animals. Exposure to WY altered P450R protein levels only at 13 weeks (decreased to 40% of control levels) (Fig. 3). After 1 week of exposure to GEM, expression of P450R protein increased (1.4-fold). At 13 weeks of GEM exposure, P450R protein levels decreased relative to control levels (12% of control). DBP exposure did not alter P450R protein levels significantly throughout the time course of the experiment (data not shown).

3.3. Dependence of P450R regulation by PP on PPARα

To determine if PPARα is necessary for the alteration of P450R levels after exposure to PPs, we examined mRNA and protein expression in tissues from wild-type mice and mice that lack a functional form of PPARα (PPARα-null mice). Wild-type and PPARα-null mice were fed a control diet or diets containing 0.1% WY or 0.6% DEHP for 3 weeks. Expression of the 2.5-kb P450R mRNA was increased in the livers of wild-type mice after treatment with WY or DEHP (4.6- and 5.8-fold,

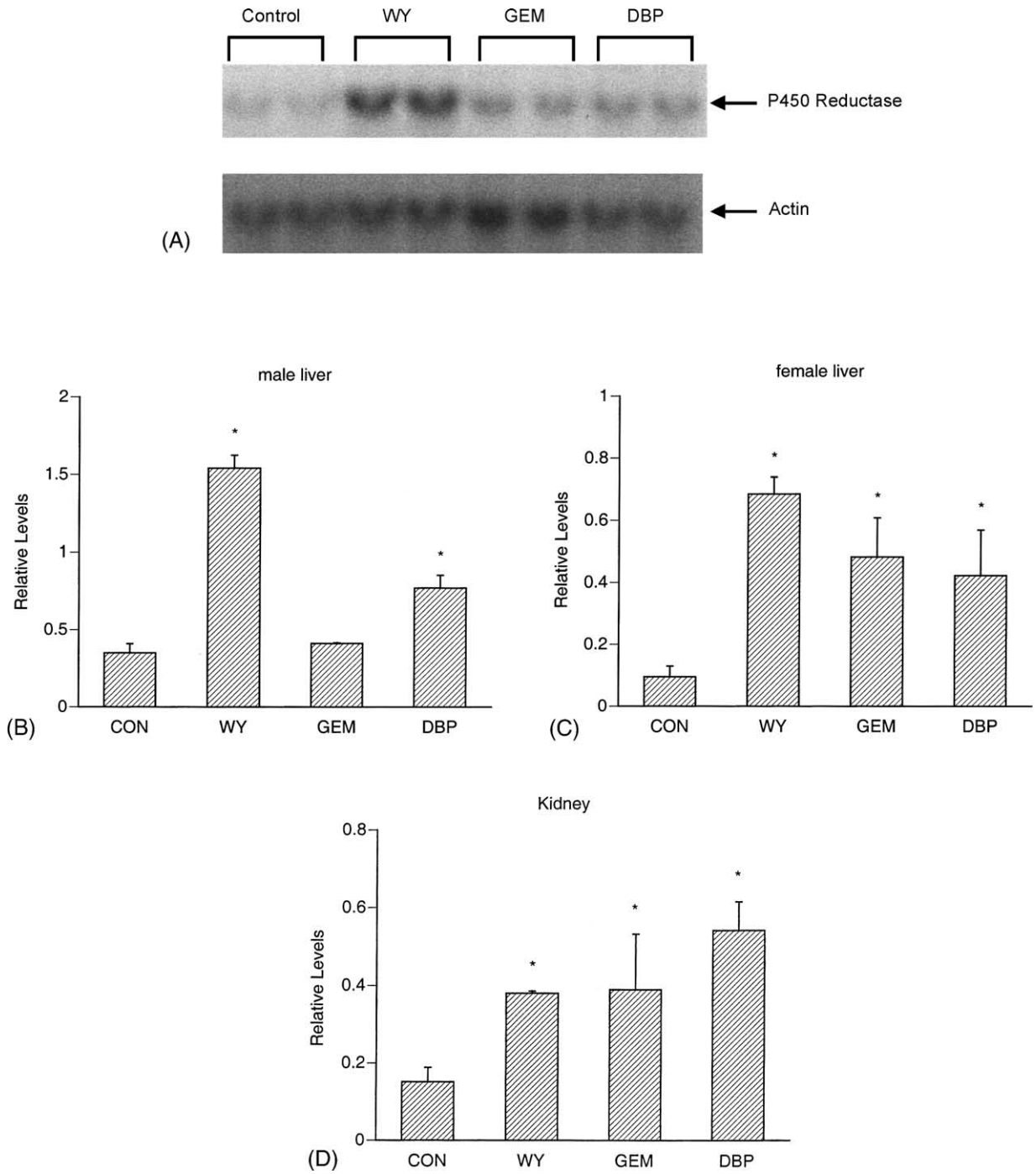

Fig. 1. Expression of P450R mRNA after exposure to PPs. (A) Northern blot analysis of P450R mRNA in livers from male control and treated rats. Total liver and kidney RNA was isolated from F-344 rats fed a control diet (CON) or diets that contained 500 ppm WY, 8000 ppm GEM, or 20,000 ppm DBP for 13 weeks. Total RNA was separated on formaldehyde-agarose gels, transferred to nylon membranes, and probed with either the full-length rat P450R cDNA or a fragment of β-actin. The autoradiograms were densitometrically scanned, and P450R expression in male liver (B), female liver (C), and male kidneys (D) was normalized to β-actin expression. The histograms show the mean induction (mean ± range) from two different samples. An asterisk (∗) indicates values significantly different from the control ($P < 0.05$).

respectively) (Fig. 4A, left). This induction was not evident in PPARα-null mice treated with either WY or DEHP (Fig. 4A, right). The expression of P450R mRNA was not altered after PP exposure in the kidneys (Fig. 4B), testis, or heart (data not shown) from wild-type or PPARα-null mice.

The expression of the P450R protein was also examined in tissues from wild-type and PPARα-null mice. In the livers of all control mice, expression of a P450R ∼75-kDa protein was detected. In wild-type mice, the P450R protein expression in livers was abolished almost completely after exposure to WY or DEHP (4 and 12% of control, respectively)

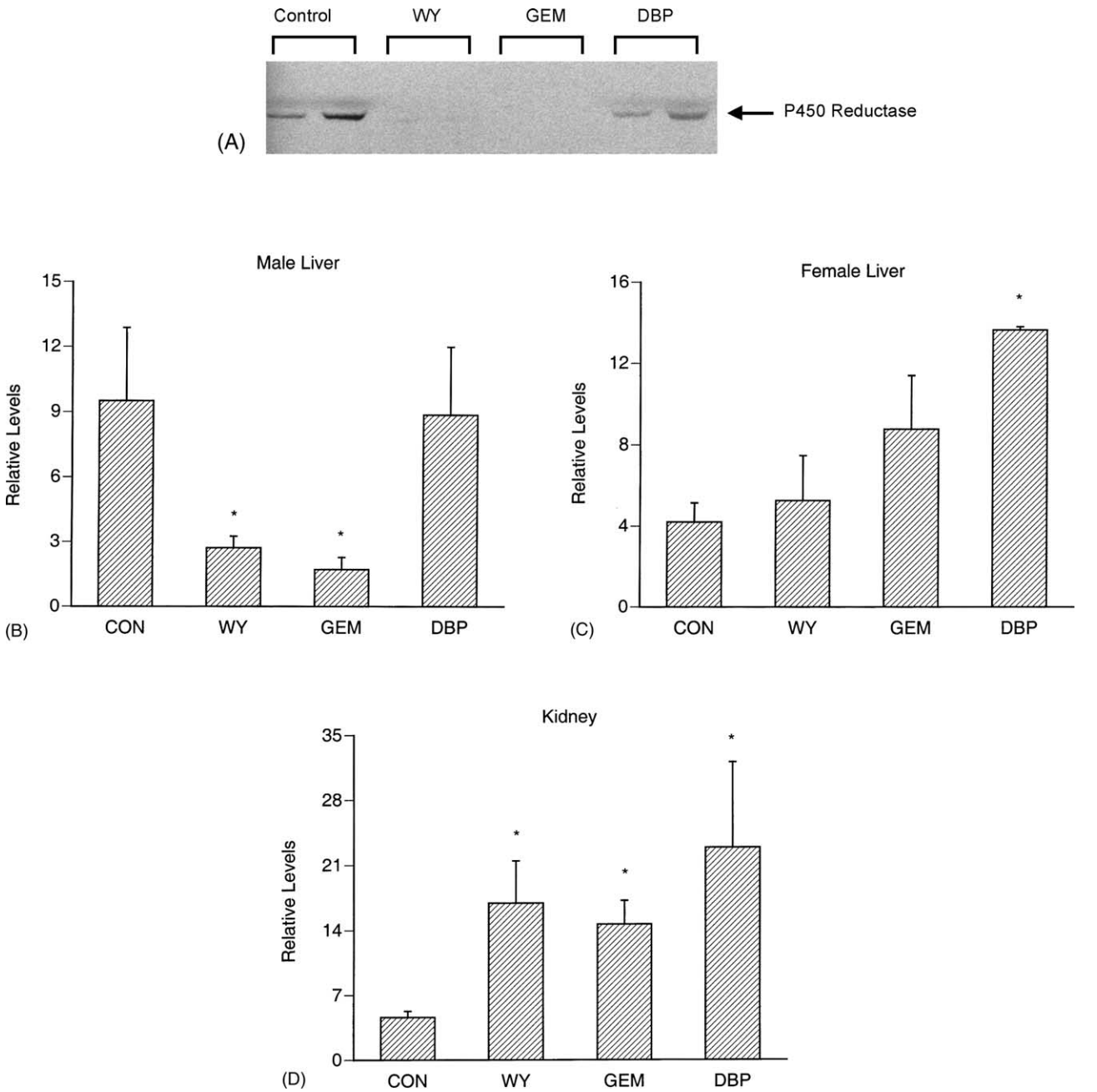

Fig. 2. Expression of P450R protein after exposure to PPs. Whole-cell liver or kidney protein extracts were made from the rats used in the experiments described in Fig. 1. Protein extracts of each sample (100 μg) were separated by 12.5% SDS–PAGE, transferred to nitrocellulose membranes, and probed with antibodies against P450R. A western blot of P450R protein expression in male livers from control and treated rats is shown in panel A. P450R protein expression was quantitated by densitometrically scanning the western blots. The histograms show the mean induction (means ± range) from two different samples from male liver (B), female liver (C), and male kidney (D). An asterisk (∗) indicates values significantly different from the control ($P < 0.05$).

(Fig. 5A, left). In contrast, WY had no effect on P450R protein levels in PPARα-null mice (Fig. 5A, right). Exposure to DEHP resulted in an increase in P450R protein (2.0-fold) that was PPARα-independent.

The dependence of P450R inducibility on PPARα expression was also examined in whole-cell extracts from kidneys. Both wild-type and PPARα-null mouse strains constitutively expressed the P450R protein in the absence of exposure to PPs (Fig. 5B, left). After exposure to WY, the expression of P450R protein was reduced dramatically (11% of control), whereas exposure to DEHP did not alter the protein levels. In PPARα-null mice, no changes in P450R kidney protein were observed after exposure to WY or DEHP (Fig. 5B, right). In testis, both wild-type and PPARα-null mice exhibited weak expression of the P450R. No changes in the expression of P450R were observed in the testis after exposure to PPs in wild-type or PPARα-null mice (data not shown).

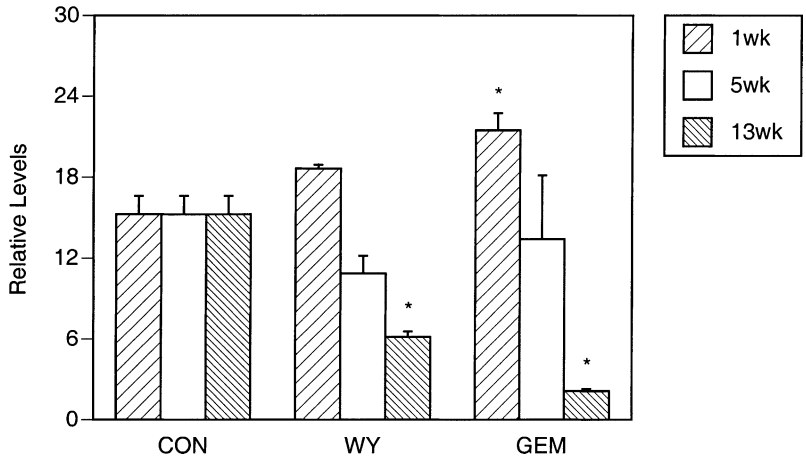

Fig. 3. Time course of P450R protein alteration by PPs. Whole-cell liver extracts from male Sprague–Dawley rats fed a control diet (CON) or a diet containing WY (500 ppm) or GEM (8000 ppm) for 1, 5, or 13 weeks were separated by 12.5% SDS–PAGE, transferred to nitrocellulose, and probed with antibodies to P450R. Expression was quantitated as described in the legend of Fig. 2. Values are means ± range, N = 2. An asterisk (∗) indicates values significantly different from the control ($P < 0.05$).

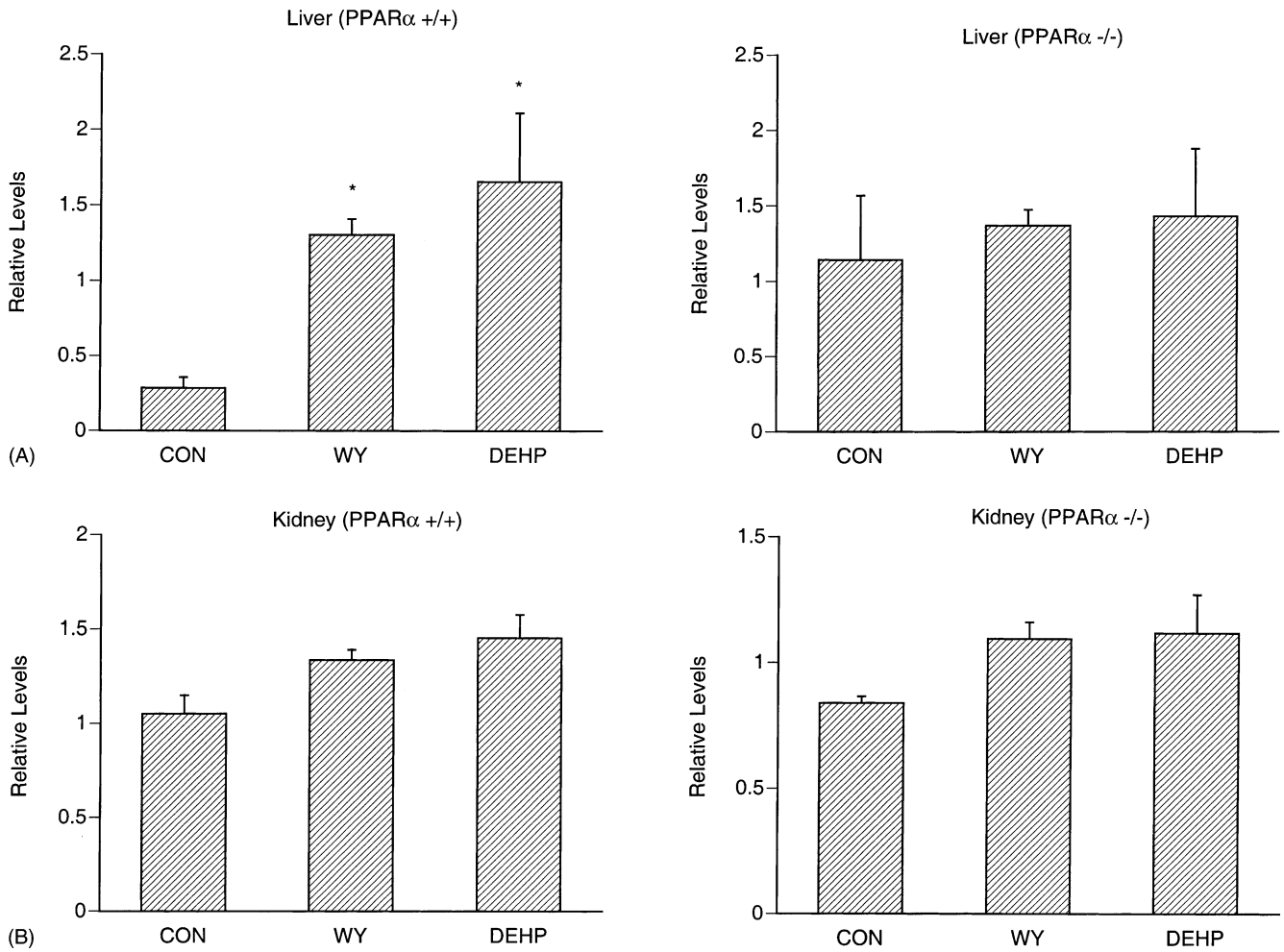

Fig. 4. Regulation of P450R mRNA levels in wild-type and PPARα-null mice after PP exposure. Northern blot analysis of P450R mRNA in liver (A) or kidneys (B) from male SV129 wild-type (left panel) and SV129 PPARα-null (right panel) mice fed a control diet (CON) or a diet containing WY (0.1%) or DEHP (0.6%) for 3 weeks. Total RNA samples were separated on formaldehyde-agarose gels, transferred to nylon membranes, and probed with either the full-length rat P450R cDNA or a fragment of β-actin. The autoradiograms were scanned densitometrically, and expression was quantitated as described in the legend of Fig. 1. Values are means ± range, N = 2. An asterisk (∗) indicates values significantly different from the control ($P < 0.05$).

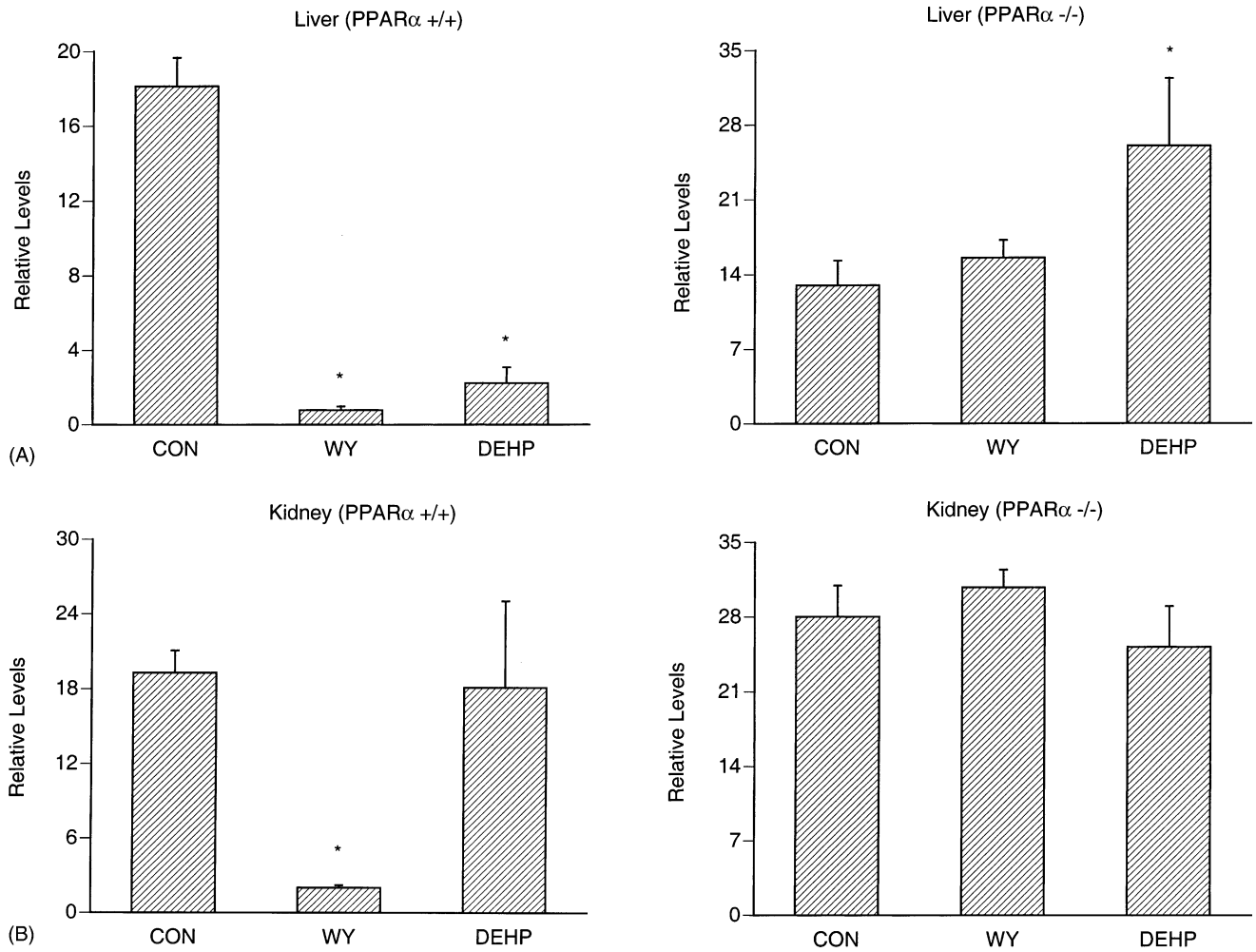

Fig. 5. Regulation of P450R protein levels in wild-type and PPARα-null mice after PP exposure. Western blot analysis of P450R protein levels in whole-cell extracts from wild-type (left) and PPARα-null (right) mouse livers (A) and kidneys (B) described in the legend of Fig. 4. Protein extracts (100 μg) were separated by 12.5% SDS–PAGE, transferred to nitrocellulose membranes, and probed with antibodies against P450R. P450R protein expression was quantitated as described in the legend of Fig. 2. Values are means ± range, N = 2. An asterisk (∗) indicates values significantly different from the control ($P < 0.05$).

3.4. Relationship between thyroid hormone and PP regulation of P450R

To determine the relationship between the known thyroid hormone dependence of P450R expression and WY-induced down-regulation of P450R protein expression, we measured P450R protein levels in the livers of intact rats (Fig. 6A), THX rats (Fig. 6B), or THX rats in which T_3 hormone was administered (Fig. 6C). All rats received either a gavage dose of WY or carrier every day for 2 or 3 days. Protein levels were quantitated and are shown in Fig. 6D. In the absence of WY treatment, thyroidectomy resulted in a dramatic decrease in the expression of P450R protein compared to intact animals, as expected. T_3 administration restored basal P450R protein to a level similar to that found in intact rats. WY treatment decreased P450R protein levels in the livers from intact, THX, and THX plus T_3 rats. ACO protein levels known to be positively regulated by PPARα after exposure to PPs were also measured in the same extracts. ACO-B protein was induced to approximately equal extents in all groups after 48 or 72 hr of exposure to WY. Our results confirm the dependence of P450R expression on T_3 and demonstrate that decreases in P450R protein after WY treatment are independent of the thyroid status of the rat.

4. Discussion

PP chemicals induce a broad spectrum of responses in the rodent liver, including peroxisome proliferation, cell proliferation, alteration of estradiol levels, increases in the metabolism of fatty acids and eicosanoids, and hepatocarcinogenesis [reviewed in Ref. 1]. Most, if not all, of the effects of PP exposure on the liver depend upon the expression of PPARα [4], the receptor for PPs, unsaturated long-chain fatty acids, and certain eicosanoids. PPARα regulates the expression of many genes involved in fatty acid metabolism, including the fatty acid β-oxidation genes and members of the cytochrome P450 4A group

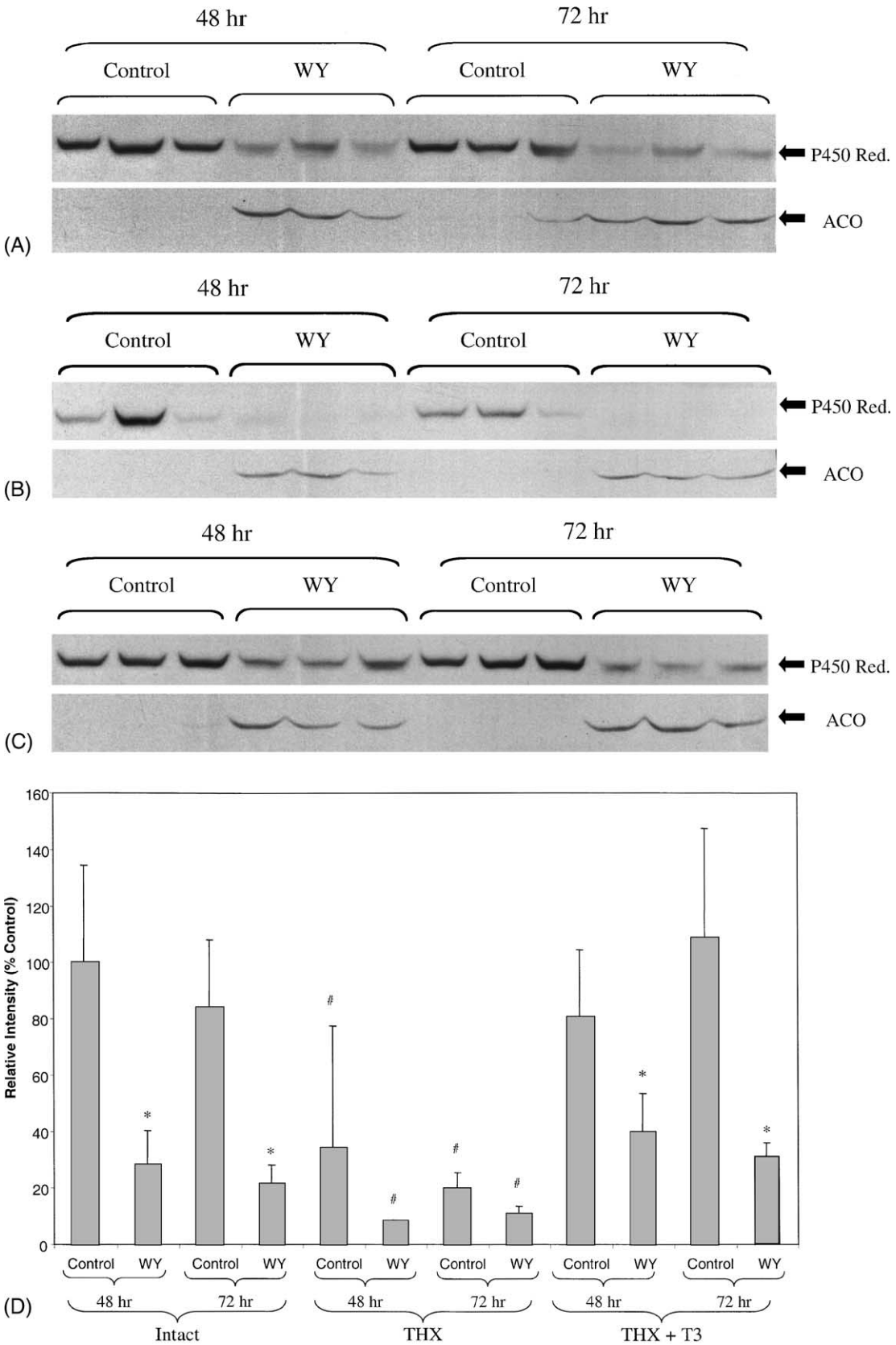

Fig. 6. Regulation of P450R protein levels after exposure of intact and THX rats to WY. Intact rats (A), THX rats (B), and THX rats given T_3 hormone (C) were given a total of two (48 hr) or three (72 hr) doses of WY (20 mg/kg body weight/day) or vehicle carrier. Protein extracts from livers were analyzed for P450R or ACO by western blot analysis (A–C) and quantitated (D) as described in the legend of Fig. 2. Values are means ± SD, N = 3. An asterisk (∗) indicates values significantly different from the control ($P = 0.05$). Values in all THX groups were significantly lower than the corresponding groups in the intact animals ($P < 0.05$), as indicated by the # sign.

of enzymes. In addition to the P450 4A family, we have shown that PPs directly regulate members of the P450 2C family that are important in the metabolism of steroids. In contrast to the inducibility of *CYP4A* genes by PPs, the male-specific *CYP2C11* and the female-specific *CYP2C12* genes are down-regulated by diverse PPs [8].

In this study, we show that PPs also regulate NADPH-P450 oxidoreductase, another component of the phase I detoxifying system by at least two general mechanisms. The first mechanism results in the up-regulation of P450R mRNA that is dependent upon PPARα. Increases in rat P450R mRNA levels were observed in the male liver after exposure to WY and DBP and in the female liver and male kidneys after exposure to WY, GEM, and DBP. Dehydroepiandrosterone (DHEA), a steroid precursor that has PP-like properties was shown previously to increase rat liver P450R mRNA after 1 week of exposure [17,18]. In our studies, P450R mRNA levels increased after exposure to WY or DEHP in the livers of wild-type mice but not those of PPARα-null mice, demonstrating the obligatory role of PPARα in mediating P450R mRNA induction. Despite shared PP-inducible regulation of P450R and β-oxidation genes by PPARα, β-oxidation gene (e.g. ACO) mRNA expression is induced rapidly after exposure to PPs and exhibits maximum expression after a 24-hr gavage dose of WY [8,19], whereas at the same time P450R mRNA levels were not altered. These results indicate that the mechanism of induction of P450R is different from that of the β-oxidation genes, despite the fact that PPARα is universally required for induction.

Using computer-assisted searches, we identified a putative DNA binding site for PPARα in the P450R promoter between −568 and −556 bp, very similar in sequence to the PPRE controlling PP induction of other regulated genes. The putative P450R PPRE, like other PPREs, consists of two direct repeats (DR) separated by 1 bp (DR-1). Interestingly, one of the direct repeats is a half site of a thyroid hormone response element (DR-4) characterized in Ref. [13]. In that study, DNase I footprinting revealed liver nuclear proteins from untreated rats covering both the DR-1 and 4 elements. The overlapping nature of the DR-1 and 4 sites leads to the prediction that PPAR–RXR and thyroid hormone receptor (TR)–RXR heterodimers compete for binding to this region. Well-characterized antagonistic crosstalk between PPs and thyroid hormone-dependent pathways has been found at the level of competition for RXR heterodimerization between PPAR and TR [summarized in Ref. 20]. We have found no evidence for antagonism between PPs and thyroid hormone, at least using the level of P450R protein expression as a measure. Demonstration of competition for binding to the overlapping TRE and putative PPRE will come from electrophoretic mobility shift and P450R promoter region analysis by transfection in appropriate cell lines.

The second mechanism controlling P450R expression by PPs operates posttranscriptionally. Evidence for this mechanism comes from inequitable alterations in mRNA and protein levels in livers from treated rats or mice. Despite increases in P450R mRNA levels after PP exposure in the male mouse and rat liver, the expression of P450R protein was decreased dramatically in these tissues. This decrease was shown to be compound-, sex-, and tissue-specific. Decreases in P450R protein levels in rat liver were observed after exposure to WY or GEM but not DBP, despite the ability of all three chemicals to induce 17β-hydroxysteroid dehydrogenase type IV and acyl-CoA oxidase [19] and down-regulate *CYP2C11* and acute-phase response genes [8] in the same tissues. In contrast to the effects in the male liver, P450R protein levels in female rat liver and male rat kidneys were either unchanged or up-regulated by treatment. Importantly, our work shows that the down-regulation of P450R protein levels by WY and DEHP was dependent upon PPARα. In contrast to our studies, P450R protein levels have been shown to be elevated in the rat liver after exposure to DHEA [17,18], although differences with our studies may be attributed to different times of exposure (5 or 7 days) and use of a different PP. Paradoxically, we observed decreases in P450R protein levels in rat livers after 48 or 72 hr but not after 1 or 5 weeks of exposure to WY. As decreases in P450R protein expression after short-term exposure were observed only in animals administered WY by gavage, it is possible that the stress incurred by the animal during the gavage procedure contributes to the sensitivity of the down-regulation. In addition, the uncoupling of P450R mRNA and protein expression was observed earlier. Treatment of euthyroid male rats with T_3 resulted in increases in P450R mRNA but no changes in P450 activity or protein [12]. PP exposure in a limited number of rat and mouse tissues may lead to alteration of a PPARα-dependent posttranscriptional mechanism that overrides increases in P450R mRNA, resulting in decreases in P450R protein synthesis, increases in P450R protein degradation, or both. We are presently profiling the expression of liver genes after PP exposure, using microarrays, to give us further clues as to the mechanism of action of PPs including the down-regulation of P450 protein levels. Interestingly, the expression of a number of genes encoding proteases is up-regulated in wild-type but not PPARα-null mice (Corton, manuscript in preparation), indicating that P450R protein levels may be regulated indirectly by PPARα through the regulation of protease levels.

Other examples of nonparallel coexpression of PP-dependent mRNA and protein are rare, partly due to the paucity of studies that examine expression at multiple levels after exposure. A comparison of mRNA and protein levels of PP-inducible 17β-hydroxysteroid dehydrogenase type IV in liver demonstrated that exposure to WY or GEM leads to approximately equal mRNA induction, whereas exposure to GEM greatly increased protein levels relative to WY treatment [19]. Our studies reinforce the growing concern that biochemical or physiological alterations predicted from

measured mRNA levels are not necessarily valid [21]. Given the increased emphasis on the use of high-density cDNA arrays to determine global expression patterns, confirmation of altered expression at the protein or functional level will likely reveal other genes with inequitable levels of mRNA and protein expression.

Metabolism is a major factor in determining whether a chemical agent will produce toxic responses in an exposed organism. Physiological conditions and chemical exposures that alter expression of drug-metabolizing enzymes such as members of the P450 family or P450R will alter the risk of toxicity to humans exposed to chemical agents and the sensitivity of animals in short- and long-term tests used to predict that risk. In our studies, we have identified dosing conditions that almost completely abolish expression of P450R protein and thus provide a potential intact animal model to understand the role of P450R in the bioactivation of numerous chemicals. Due to P450R protein levels becoming rate-limiting, we would predict that at high doses of some PPs there would be a decrease in the CYP4A enzyme levels from those induced at lower doses. Evidence for this phenomenon could not be found in the literature. It is possible that even low levels of P450R are sufficient for maintaining activities of the P450s. However, because P450R is involved directly in the bioactivation of a number of carcinogens and cytotoxicants [22–27], we predict that chronic treatment of rats with high doses of WY or GEM (resulting in down-regulation of P450R) would at least partially protect from DNA damage or cytotoxicity by agents that require P450R-dependent metabolic activation. With this in mind, pretreatment of rodents with the PP clofibrate protects against hepatocyte toxicity after exposure to a number of liver toxicants by an unknown PPARα-dependent mechanism [summarized in Refs. 20 and 28].

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