Peroxisome proliferator-activated receptor α (PPARα) and agonist inhibit cholesterol 7α-hydroxylase gene (*CYP7A1*) transcription

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Abstract Fibrates are widely used hypolipidemic drugs that regulate the expression of many genes involved in lipid metabolism by activating the peroxisome proliferator-activated receptor α (PPARα). The objective of this study was to investigate the mechanism of action of peroxisome proliferators and PPARα on the transcription of cholesterol 7α-hydroxylase, the rate-limiting enzyme in the conversion of cholesterol to bile acids in the liver. When cotransfected with the expression vectors for PPARα and RXRα, Wy14,643 reduced human and rat cholesterol 7α-hydroxylase gene (*CYP7A1*)/luciferase reporter activities by 88% and 43%, respectively, in HepG2 cells, but not in CV-1 or CHO cells. We have mapped the peroxisome proliferator response element (PPRE) to a conserved sequence containing the canonical AGGTCA direct repeats separated by one nucleotide (DR1). This DR1 sequence was mapped previously as a binding site for the hepatocyte nuclear factor 4 (HNF-4) which stimulates *CYP7A1* transcription. Electrophoretic mobility shift assay (EMSA) showed no direct binding of *in vitro* synthesized PPARα/RXRα heterodimer to the DR1 sequence. PPARα and Wy14,643 did not affect HNF-4 binding to the DR1. However, Wy14,643 and PPARα/RXRα significantly reduced HNF-4 expression in HepG2 cells. These results suggest that PPARα and agonist repress cholesterol 7α-hydroxylase activity by reducing the availability of HNF-4 for binding to the DR-1 sequence and therefore attenuates the transactivation of *CYP7A1* by HNF-4.—Marrapodi, M., and J. Y. L. Chiang. **Peroxisome proliferator-activated receptor α (PPARα) and agonist inhibit cholesterol 7α-hydroxylase gene (*CYP7A1*) transcription.** *J. Lipid Res.* **2000.** 41: **514–520.**

Supplementary key words bile acid synthesis • gallstones • peroxisome proliferators • hypolipidemic drugs • cytochrome P450 • HNF-4

Fibrates are extensively used drugs in the treatment of hyperlipidemia, a risk factor for the development of coronary heart diseases (1). This class of compounds exerts its effect on plasma lipids by altering the expression of genes involved in lipid metabolism through activation of PPAR (2). Upon activation by ligands, such as fatty acids, eicosanoids, and hypolipidemic drugs, PPAR forms a heterodimer with RXRα, which binds to the peroxisome proliferator response element (PPRE) and modulates gene transcription (3). The PPRE consists of a direct repeat of the canonical AGGTCA sequence separated by one base pair (bp) (2). Three PPAR isoforms, α, δ, and γ have been identified (4). PPARα is the predominant form in the liver and plays a pivotal role in regulation of lipid metabolism. Disruption of PPARα in mice caused a defect in lipid and lipoprotein metabolisms (5–7).

Of clinical importance is the fact that treatment with fibric acid derivatives leads to cholesterol saturation of the bile resulting in an increased risk of gallstone formation (8). Fibrates reduce bile acid synthesis, presumably by inhibition of cholesterol 7α-hydroxylase, the rate-limiting enzyme in the catabolic pathway of cholesterol to bile acids in the liver (9). Cholesterol 7α-hydroxylase (CYP7A1) is regulated mainly at the transcriptional level by factors including negative feedback by bile acids, insulin, and phorbol esters (10, 11). Bezafibrate has been shown to reduce the activity of cholesterol 7α-hydroxylase in rodents (12, 13), as well as in gallstone (14) and hyperlipoproteinemia patients (15). Interestingly, development of supersaturated bile is prevented when fibrate therapy is combined with the bile acid sequestrant, cholestyramine (15). This suggests that increased expression of CYP7A1 activity by cholestyramine treatment may increase bile acids or decrease cholesterol in bile.

The *CYP7A1* expression is restricted to the liver and its liver-specific promoter contains functional binding sites for the liver-enriched transcription factors, HNF-3, HNF-4, and COUP-TFII (16, 17). The orphan nuclear receptor

Abbreviations: CYP7A1, cholesterol 7α-hydroxylase; *CYP7A1*, cholesterol 7α-hydroxylase gene; PPARα, peroxisome proliferator activated receptor α; PPRE, peroxisome proliferator response element; HNF-4, hepatocyte nuclear factor 4; DR; direct repeat; bp, base pair; nt, nucleotide; Luc, luciferase; AOX, acyl-CoA oxidase; EMSA, electrophoretic mobility shift assay, BARE, bile acid response element; PCR, polymerase chain reaction.

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HNF-4 binds as a homodimer to the DR-1 without ligand binding (18). Previous study has shown that HNF-4 regulates *CYP7A1* transcription through a completely conserved DR-1 at nt −146 to −130 of the rat *CYP7A1* promoter, which was also mapped as a bile acid response element (BARE) (16, 19). We have proposed a receptor–mediated mechanism for down-regulation of *CYP7A1* transcription by bile acids (9). Recently, orphan nuclear receptor FXR has been identified as a bile acid-activated receptor, which mediates the repression of *CYP7A1* transcription by bile acids (20–23). However, the mechanism by which FXR down-regulates *CYP7A1* transcription is not known. Interaction of transcription factors bound to the BARE may explain the negative regulation of *CYP7A1* transcription by physiological regulators such as bile acids, fibrates, insulin, and phorbol esters. The goal of this study was to identify the *cis*-acting elements conferring the inhibitory effect of PPARα and agonist on *CYP7A1* transcription. We show that peroxisome proliferators regulate *CYP7A1* at the transcriptional level by reducing the availability of HNF-4 for binding to the DR-1 sequence and therefore interfering with transactivation of *CYP7A1* by HNF4.

EXPERIMENTAL PROCEDURES

Plasmids

Construction of human *CYP7A1*/Luc reporter genes has been described previously (11). The rat *CYP7A1*/Luc mutant plasmids and the HNF-4 expression vector pCMV-HNF-4, were generated as described previously (16). The rat acyl-CoA oxidase gene (*AOX*) from nucleotides −1198 to −463 contains a well-characterized PPRE and was obtained by polymerase chain reaction (PCR) amplification of rat genomic DNA (24). PCR primer pair, 5′-ATAG GTACCATCCCCAGTAGAACCTTGTTCAGG-3′ and 5′-ATGAG ATCTTTCAGGGTCTCGGGCGGAGTGAAG-3′, was used to generate a 736 bp fragment. The reporter gene plasmid *AOX*-1198/-463 was constructed by fusing the PCR fragment upstream to the SV40 promoter in pGL3-Promoter (Promega, Madison, WI). The expression plasmid for human PPARα, pcDNA3-hPPARα, was constructed by subcloning the human PPARα cDNA (Dr. F. J. Gonzalez, NCI, Bethesda, MD) into pcDNA3 vector (Invitrogen, Carlsbad, CA). The expression plasmid for the retinoid X receptor, pCMX-RXRα, was obtained from Dr. R. Evans (Salk Institute, La Jolla, CA).

Transfection assays

HepG2, CHO, and CV-1 cells, grown in 12-well tissue culture plates to 100% and 70% and 70% confluence, respectively, were transfected with the chimeric gene constructs by the calcium phosphate co-precipitation method as described previously (25). In cotransfection experiments, expression plasmids for PPARα and RXRα (0.5 μg each) or an equal amount of the empty vector were co-transfected with 2.5 μg of the test plasmid. After transfection, cells were treated with 100 μm Wy14, 643 or an equal volume of ethanol as the vehicle. Cells were harvested 42 h after treatment and luciferase activity was determined with the luciferase assay kit (Promega) using a Lumat LB9501 luminometer (Berthold System, Inc., Pittsburgh, PA). Luciferase activities were normalized for transfection efficiency by dividing relative light units by β-galactosidase activity expressed from cotransfected pCMVβ plasmid. Transfection assays were performed in triplicate and the results were analyzed for statistical significance by Student's *t*-test using Sigma plot software (Jandel Scientific, San Rafael, CA). Each experiment was repeated at least twice.

Electrophoretic Mobility Shift Assays (EMSA)

PPARα, RXRα, and HNF-4 were synthesized in vitro by programming transcription/translation (TNT) system (Promega) with the expression vectors pcDNA3-hPPARα, pCMX-RXRα, and pCMV-HNF4, respectively. Synthetic oligonucleotides carrying the 5′ overhang GATC were annealed by heating at 100°C in 2× SSC buffer (0.3 m NaCl, 0.03 m sodium citrate, pH 7.0) and slowly cooling to room temperature. The double-stranded oligonucleotides were labeled by filling-in with [α ^{32}P]dCTP using the Klenow fragment of DNA polymerase I. In vitro translated proteins were pre-incubated for 15 min on ice in a buffer containing 12 mm HEPES, pH 7.9, 50 mm KCl, 1 mm EDTA, 1 mm dithiothreitol, 15% glycerol, and 1 μg poly (dI-dC). After incubation for 20 min at room temperature with 75,000 cpm of labeled probe, the DNA/protein complexes were separated on a 4% native polyacrylamide gel in 0.5 × TBE running buffer, at 200 V for 2 h at room temperature. One μl of an antibody directed against PPARα (Santa Cruz Biotechnology, Santa Cruz, CA) was added to the reaction mixture. Results were quantified with IP Lab Gel software (Signal Analytics, Corp., Vienna, VA) in conjunction with a Molecular Dynamics PhosphoImager 445Si (Sunnyvale, CA).

Quantitative Western immunoblot analysis

Confluent HepG2 cells were transfected with the expression vectors for PPARα/RXRα or the empty vector pcDNA3 as a control. These cells were then treated with 100 μm Wy14,643 or the vehicle ethanol. Nuclear extracts were isolated from HepG2 cell preparations according to Dent and Latchman (26). Ten μg of protein was resolved by 10% SDS-PAGE and electroblotted onto nitrocellulose filters. The membranes were blocked in non-fat dry milk at 4°C overnight and then incubated with anti-HNF4 antisera (Dr. F. Sladek, University of California, Riverside, CA) for 6 h at 4°C. After washing with PBS and 0.05% Tween-20, the blot was incubated with the horseradish peroxidase-conjugated secondary antibody for 2 h at room temperature, washed again, and HNF-4 was detected with enhanced chemiluminacent ECL kit (Amersham Life Science, Arlington Heights, IL). Filters were then washed and blocked again. Antibody against Sp1 was used to detect Sp1 protein as an internal standard. X-ray films were analyzed with the Kodak digital science 1D 2.0.2 software and the intensity of HNF-4 band was normalized by dividing the Sp1 band intensity. Data from three independent nuclear extract preparations were analyzed. Statistical significance between two treatments of three independent experiments was analyzed using ANOVA procedure (SAS software).

RESULTS

Mapping of PPARα response element in CYP7A1 promoter activity

It has been established that fibrate treatment in rodents (12, 13) and humans (14, 15) results in a decrease of cholesterol 7α-hydroxylase activity by 65%. To study the effect of PPAR and agonist Wy14,643 on CYP7A1 transcription, transient transfection assays in HepG2 cells were performed. Wy14,643 (100 μm) alone and over-expression of PPARα/RXRα alone reduced human *CYP7A1*/luciferase reporter (ph-3025luc) activity by about 40% and 60%, respec-

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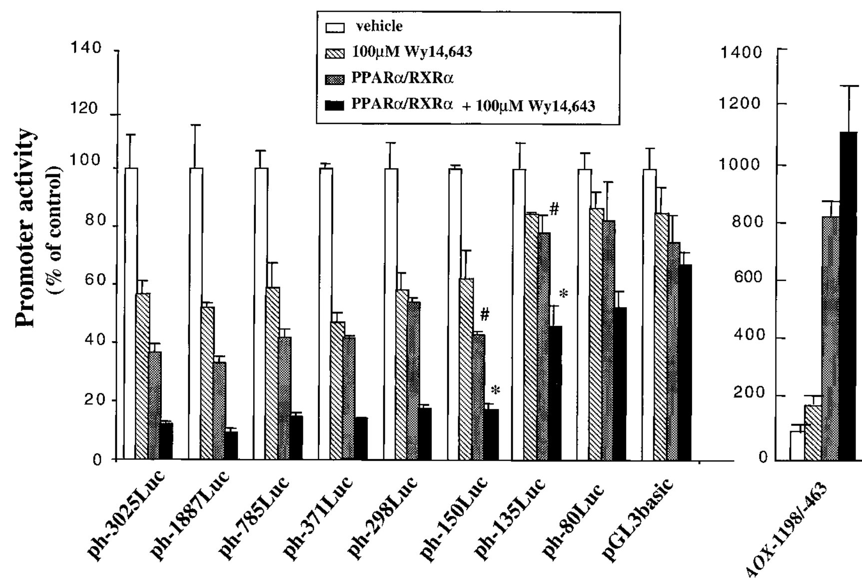

Fig. 1. Effects of Wy14,643 and PPARα/RXRα on reporter activities of human *CYP7A1*/luciferase chimeric constructs in transient transfection assays in HepG2 cells. Confluent HepG2 cells were transfected with the human *CYP7A1*/Luc reporter genes and the expression vectors for PPARα and RXRα as described under Experimental Procedures. The *AOX*-1198/-463 plasmid was used as a positive control. Results are expressed as the percent of reporter activity in HepG2 cells cotransfected with reporter plasmid and the empty vector pcDNA3 and incubated with the vehicle ethanol. Data presented are representative of three independent experiments each performed in triplicate. Symbols (*, #) indicate statistically significant difference in reporter activity between two constructs. Basal reporter activities (RLU/β-gal) for the chimeric constructs were 2.2, 7.9, 3.0, 3.7, 6.0, 1.1, 0.49, 0.34, and 0.21 $\times 10^6$ for ph-3025Luc, ph-1887Luc, ph-785Luc, ph-372Luc, ph-298Luc, ph-150Luc, ph-135Luc, ph-80Luc, and pGL3basic, respectively. Error bar indicates standard error of the mean (SEM) from three determinations.

tively (**Fig. 1**). The combination of both Wy14,643 and PPARα/RXRα reduced the promoter activity by 90%. These results suggested that endogenous PPARα might be activated by Wy14,643 and PPARα/RXRα suppressed *CYP7A1* transcription. As expected, the reporter activity of the positive control plasmid *AOX*-1198/463 was stimulated 2-fold by Wy14,643. PPARα and Wy14,643 strongly stimulated this reporter activity by about 11-fold (Fig. 1). The 5′ deletion mutants of human *CYP7A1*/Luc were transfected in HepG2 cells to map the PPRE. As shown in Fig. 1, deletion of nucleotides between −150 and −136 significantly reduced the inhibitory effect of Wy14,643 and PPARα. This result suggest that a negative PPRE is localized between −150 and −136. This region includes the DR1, TGGACT-tAGTTCA (**Fig. 2**). Figure 2 shows the alignment of rat and corresponding human nucleotide sequences in this region. Three AGGTCA-like sequences form overlapping DR1 and DR5 motifs, which have been mapped as an HNF-4 and RXRα/RARα binding site in the rat *CYP7A1* promoter, respectively (17). As the DR-1 motif is perfectly conserved in the rat and human *CYP7A1* promoter (Fig. 2), we also tested the effect of Wy14,643 on the wild-type rat *CYP7A1*/luciferase chimeric plasmid, p-376luc, and mutant plasmids with mutations in AGGTCA-like sequences (Figs. 2 and **Fig. 3**). Overexpression of PPARα/RXRα in the presence of Wy14,643 suppressed the activity of the wild-type rat p-376luc construct by 50%. Mutations in the 5′ half-site of the DR1 (m-146/-141) reduced the basal reporter activity (1.7 $\times 10^6$ RLU/β-gal vs. 4.8 $\times 10^6$ of the wild-type reporter). The T at position 1 of the 5′ half-site TGGACT has been shown to be more specific for HNF-4 than for PPAR binding (27). This mutant reporter did not respond to Wy14,643. On the other hand, the plasmid m-139/-132, which has mutations in the 3′ half-site of the DR1 or 5′ half-

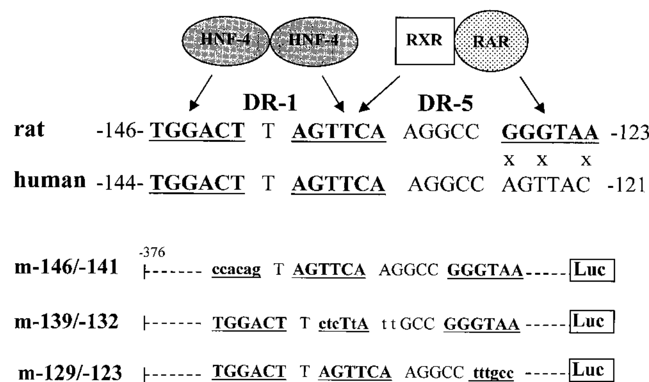

Fig. 2. Alignment of the rat and human *CYP7A1* promoter in the region under study. The canonical AGGTCA sequences are represented by bold characters and half-sites of direct repeats are underlined. X indicates a non-conserved nucleotide between the rat and human *CYP7A1* sequence. Arrows point to nuclear receptors binding sites. Relevant sequences in the rat *CYP7A1*/luciferase mutants used in transfection assays in Fig. 3 are also shown (mutations indicated in lower case).

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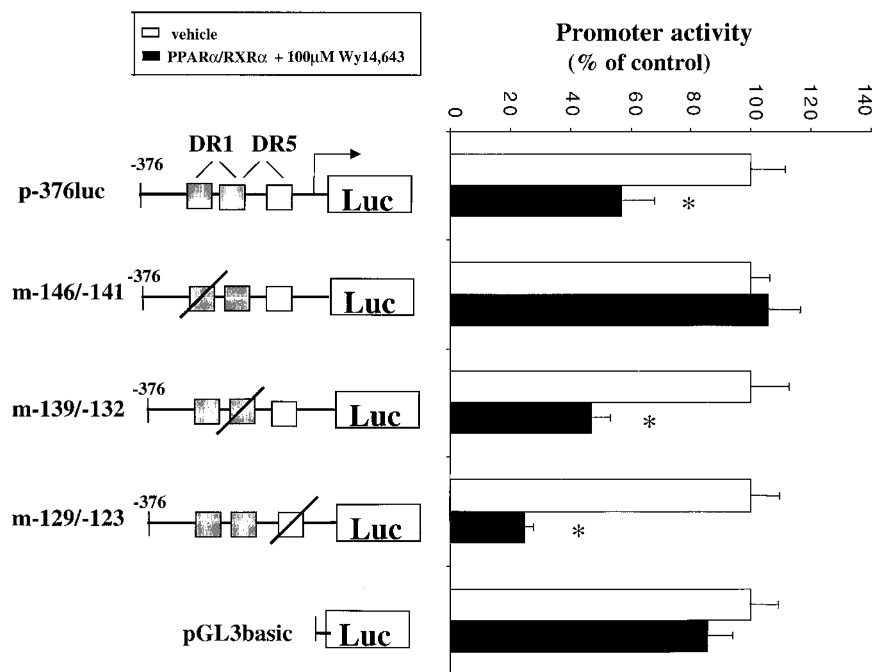

Fig. 3. Effect of Wy14,643 and PPARα/RXRα on reporter activities of the wild-type and mutant rat *CYP7A1*/luciferase chimeric gene constructs. The wild-type rat *CYP7A1*/luciferase chimeric reporter plasmid p-376Luc and its mutant constructs were transfected in confluent HepG2 cells. Results (filled bars) are expressed as percent of the reporter activity in HepG2 cells cotransfected with PPARα/RXRα plasmids versus that with the empty vector pcDNA3 and treated with vehicle ethanol. Data are representative of three independent experiments each performed in triplicate. Basal promoter activities (RLU/β-gal) for the chimeric constructs were 4.8, 1.7, 0.43, 8.8, and 0.53 × 10^6 for p-376luc, m-146/-141luc, m-139/-132luc, m-129/-123luc, and pGL3basic, respectively. On the left, AGGTCA half-sites are represented by a box. The shaded boxes represent DR1 motif. A box with a line crossed indicates a half-site with mutations as indicated in Fig. 2. The (*) indicates reporter activity significantly different from the control (100%). Error bar indicates SEM.

site of the DR5, had the lowest basal activity (0.43 × 10^6 RLU/β-gal) among wild-type and three mutant reporters, but was responsive to Wy14,643. Previously, we showed that mutations in the 3′ half-site of the DR-1 allowed some, although weakened, interaction with HNF-4 (28). This may explain the observed inhibitory effect of Wy14,643 on m-139/-132. The RXRα/RARα heterodimer only binds to the rat *CYP7A1* promoter and competes with HNF-4 for binding to a partially overlapped consensus sequence (16). As previously reported, plasmid m-129/-123, which has mutations in the 3′ half-site of the DR5, showed higher basal activity when compared with the wild-type plasmid (8.8 × 10^6 RLU/β-gal vs. 4.8 × 10^6). This is expected as the mutation disrupted RXRα/RARα binding site but allowed HNF-4 to bind to the intact DR-1. Interestingly, abolishing the RXRα/RARα binding site in plasmid m-129/-123 enhanced the down-regulation by Wy14,643. This mimics the stronger inhibitory effect of Wy14,643 on the human *CYP7A1* promoter, which lacks the RXRα/RARα binding site. These data provided strong evidence for mapping the PPRE to the DR1 sequence of the *CYP7A1* promoter.

PPARα/RXRα does not bind to the human and rat DR-1

Electrophoretic mobility shift assay (EMSA) was used to determine whether the effect of PPARα on *CYP7A1* was due to direct binding of PPARα/RXRα to the DR1 region, which is the preferred binding site for PPAR. As a positive control, the *AOX*-PPRE probe bound in vitro synthesized the PPARα/RXRα heterodimer but not PPARα or RXRα alone (**Fig. 4A**). An antibody against PPARα was able to super-shift the PPARα/RXRα/DNA complex (Fig. 4A). PPARα/RXRα did not bind to the human h*CYP7A1* −157/−127 or the rat r*CYP7A1* −149/−128 probe (Figs. 4A and B). Addition of Wy14,643 did not have any effect on EMSA. The h*CYP7A1* −157/−127 probe bound the in vitro synthesized HNF-4 (Fig. 4A). Incubation of the h*CYP7A1* −157/−127 probe with the in vitro synthesized PPARα/RXRα did not affect HNF-4 bound to the probe, both in the presence and in absence of the ligand Wy14,643 (Fig. 4A). These results clearly demonstrated that PPARα/RXRα did not bind to the functional PPRE in the *CYP7A1* promoter and did not compete with HNF-4 for the same binding site.

HNF-4 protein expression level is reduced by Wy14,643 treatment in HepG2 cells

To further investigate the mechanism by which PPARα represses *CYP7A1* transcription, we studied the effect of Wy14,643 and PPARα on the expression of HNF-4 in HepG2 cells. Western immunoblot assay was used to measure HNF-4 expression levels in HepG2 cells. **Figure 5** shows that treatment with Wy14,643 and overexpression

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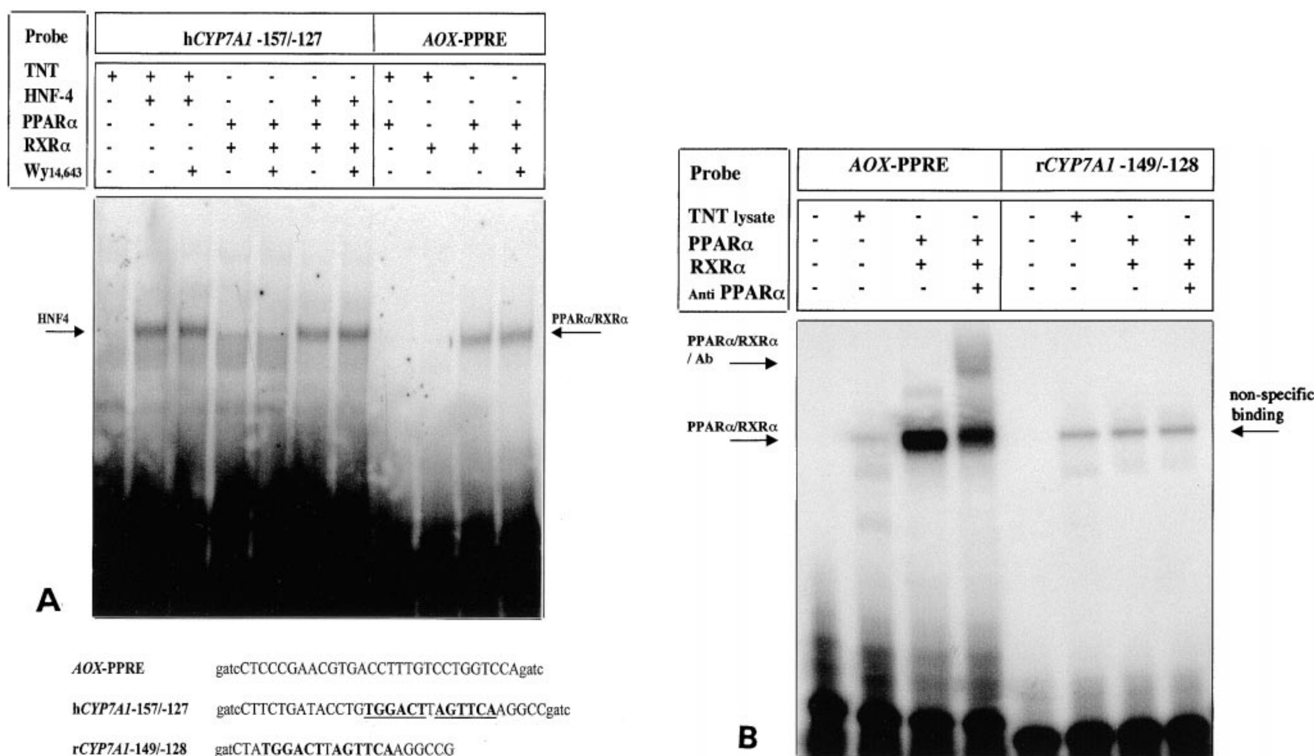

Fig. 4. EMSA of PPARα/RXRα binding to the human and rat *CYP7A1* probes. A: EMSA using ^{32}P-labeled oligonucleotide probe of the human *CYP7A1* sequences from −157 to −127 (h*CYP7A1*-157/-127) and in vitro synthesized PPARα, RXRα, and HNF-4. Where indicated Wy14,643 (100 μm) was added to the binding reaction. The *AOX*-PPRE probe containing a known PPRE was the positive control for binding of PPARα/RXRα. DNA/protein complexes are indicated by an arrow. B: EMSA using ^{32}P-labeled oligonucleotide probe of the rat *CYP7A1* sequences from −149 to −128 (r*CYP7A1*-149/-128), and PPARα and RXRα. An antibody against PPARα was used to super-shift the *AOX*-PPRE/protein complex. Nucleotide sequences of oligonucleotide probes used are shown on the bottom. Overhanging sequences are in lower case. DR-1 sequence is represented by bold characters and half-sites are underlined.

of PPARα/RXRα reduced HNF-4 levels in nuclear extracts isolated from HepG2 cells. Wy14,643 also reduced HNF-4 levels in control cells transfected with the pcDNA3 empty vector. **Table 1** summarizes the quantitative analysis of three independent experiments. Wy14,643 or PPARα/RXRα overexpression reduced HNF-4 levels to 59% and 69% of the control, respectively. Addition of Wy14,643 significantly repressed the HNF-4 levels to 42% ($P < 0.0085$) in HepG2 cells overexpressed with PPARα/RXRα. PPARα overexpression also reduced HNF-4 levels in HepG2 cells treated with Wy14,643 ($P < 0.0528$). These results clearly demonstrated that modulation of HNF-4 protein expres-

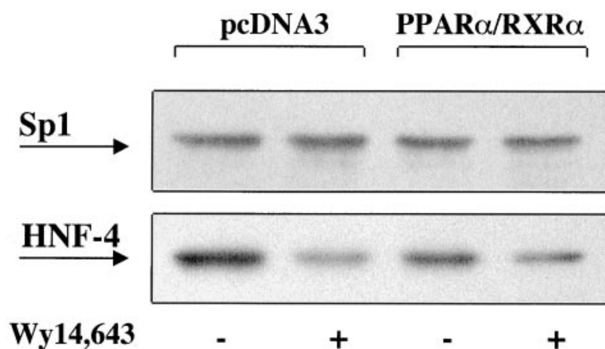

Fig. 5. Western immunoblot analysis of the effect of PPARα on HNF-4 protein levels in HepG2 cells. A representative autoradiograph of Western immunoblot performed with nuclear extracts from confluent HepG2 cells transfected with the expression vectors for PPARα/RXRα or the empty vector pcDNA3 and incubated with Wy14,643 (100 μm) or the vehicle ethanol is shown. Arrows indicate the HNF-4 and the internal standard Sp1 detected with antibodies. Quantitative analysis of the band intensity is shown in Table 1.

TABLE 1. Quantitative analysis of HNF-4 protein levels in HepG2 cells

HepG2 Nuclear Extracts	HNF-4/Sp1
	% of control
pcDNA3 + Wy14,643	59 ± 13[a]
PPARα/RXRα	69 ± 21[b]
PPARα/RXRα + Wy14,643	42 ± 6[a,b]
Control (ethanol,pcDNA3)	100

Western blots detected with ECL system were analyzed with the Kodak 1D 2.0.2. software for quantification the band intensity. The quantity of HNF-4 in HepG2 nuclear extracts was determined by dividing the band intensity of HNF4 by that of Sp1, which served as an internal control for normalization. Data were analyzed from three nuclear extract preparations and expressed as percent of the control represented by HepG2 cells transfected with the empty vector pcDNA3. Statistical significance between two treatments was analyzed from three independent experiments using ANOVA procedure.

The P values are: < 0.0528 for Wy14,643 vs. PPAR + Wy14,643 ([a]), and < 0.0085 for PPAR vs. PPAR + Wy14,643 ([b]).

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sion might be a mechanism for the suppression of *CYP7A1* transcription by PPARα and Wy14,643.

Suppression of CYP7A1 promoter activity by Wy14,643 is a liver-specific effect

In order to determine whether the down-regulation of *CYP7A1* by fibrates is a phenomenon restricted to the liver, we tested the effect of Wy14,643 on several non-hepatic cell lines. As shown in **Fig. 6A**, Wy14,643 (100 μm) stimulated human p-372/Luc reporter activity by 20% in CHO and did not have any effect in CV-1 cells. Cotransfection with increasing amounts of PPARα/RXRα heterodimer (0.1 to 0.5 μg) and Wy14,643 (100 μm) stimulated reporter activity by 20 to 40% in CHO cells (Fig. 6B). Therefore, liver-specific factors important for regulation of *CYP7A1* transcription must be present to mediate the negative effect of Wy14,643 and PPARα/RXRα.

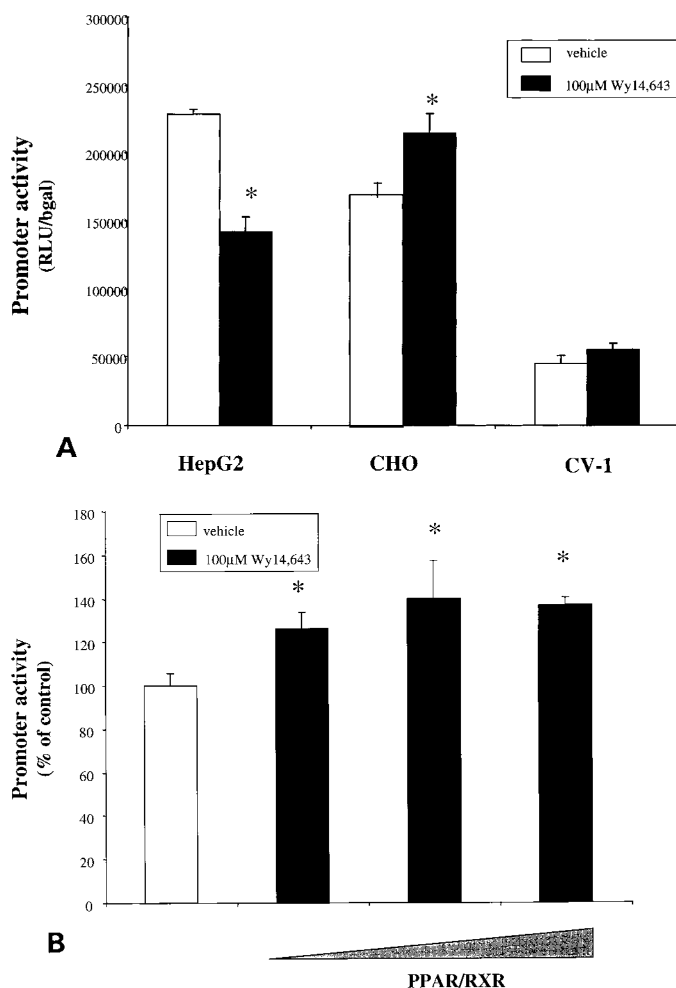

Fig. 6. Effect of Wy14,643 and PPARα/RXRα on *CYP7A1* promoter activity in transient transfection assays using HepG2 and non-hepatic cells. A: HepG2, CHO, and CV-1 cells were transfected with the human reporter construct ph-372Luc and incubated for 42 h with Wy14,643 or the vehicle ethanol. B: CHO cells were cotransfected with ph-372Luc and increasing amounts of the expression vectors for PPARα and RXRα (0.1 μg, 0.25 μg, and 0.5 μg each vector, respectively) and treated with Wy14.643 or the vehicle ethanol. The (*) indicates statistically significant difference from the control (100%).

DISCUSSION

In this study, we showed that the potent peroxisome proliferator Wy14,643 suppressed *CYP7A1* expression in HepG2 cells. The inhibition of *CYP7A1* transcription by Wy14,643 explains the reduced cholesterol 7α-hydroxylase activity in patients treated with fibrates, resulting in diminished excretion of bile acids and consequently increased incidence of cholesterol gallstones formation. Peroxisome proliferators have been shown to activate many genes involved in lipid metabolism through transcriptional activation mediated by binding of PPARα to PPREs in the gene promoter. This mechanism involves heterodimerization of PPARα with RXR and ligand-dependent recruitment of co-activators (29). However, the mechanism for suppression of genes by peroxisome proliferators is less understood. Several mechanisms have been proposed which includes competition with HNF-4 for binding site, suppression of HNF-4 expression (30, 31), and up-regulation of the repressor Rev-erbα (32). Our mutagenesis analysis of the human and rat *CYP7A1* promoter in transfection assays showed that the PPRE co-localizes with a functional HNF-4 binding site (DR1). Mutations in this conserved sequence reduced the basal promoter activity and abolished the inhibitory effect of not only PPAR but also bile acids and phorbol esters (25). This indicates that HNF-4 is required in maintaining basal level expression of the *CYP7A1* as well as in mediating the response to physiological regulators. The DR-1 elements are quite promiscuous in allowing the binding of RXR homodimer, PPAR/RXR and RAR/RXR heterodimers, HNF-4, and COUP-TFII (27). However, PPARα/RXRα did not bind to the DR-1 sequence of the rat and human *CYP7A1* promoter and did not interfere with the binding of HNF-4. A reduction of HNF-4 protein levels in HepG2 cell nuclear extracts upon treatment with Wy14,643 suggests that one possible mechanism for down-regulation of *CYP7A1* transcription by fibrates is through reduced availability of HNF-4 for binding to the DR1, hence reduced *CYP7A1* expression. This mechanism has been suggested for the repression of HNF-4-activated liver genes, apolipoprotein C-III, and transferrin by PPAR (30, 31). Although functional PPRE has not been identified in the promoter of HNF-4, it is known that HNF-4 regulates its own synthesis (33). It is possible that the ligand-activated PPARα may compete with HNF-4 for the same binding site on the HNF-4 promoter and that may result in the reduced synthesis of HNF4 protein in liver cells. Thus, reduction of HNF-4 expression and interference with HNF-4 function by PPAR explains the pleiotropic effect exerted on liver gene expression by hypolipidemic peroxisome proliferators.

Our results show that the suppression of *CYP7A1* promoter activity by Wy14,643 and PPARα is restricted to HepG2 cells. Overexpression of PPARα/RXRα caused a small but significant increase of *CYP7A1* reporter activity

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in CHO cells. Therefore, liver-enriched transcription factors must be involved in the suppression of *CYP7A1* transcription by peroxisome proliferators. This again supports our conclusion that HNF4 is involved in PPARα-mediated repression of *CYP7A1* transcription.

Screening of fibrates for their minimal effects on cholesterol 7α-hydroxylase gene expression may be considered for reducing the risk of developing cholesterol gallstone disease in hyperlipidemic patients under fibrates therapy. A better understanding of the basic mechanisms by which fibrates affect the expression of genes involved in lipid metabolism will allow the development of more rational lipid-lowering strategies with enhanced specificity and reduced side effects.

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