Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 11593–11597, December 1995
Biochemistry

Thyroid hormone (T_3) inhibits ciprofibrate-induced transcription of genes encoding β-oxidation enzymes: Cross talk between peroxisome proliferator and T_3 signaling pathways

RUIYIN CHU*, LAIRD D. MADISON†, YULIAN LIN*, PETER KOPP†, M. SAMBASIVA RAO*, J. LARRY JAMESON†, AND JANARDAN K. REDDY*‡

*Department of Pathology and †Department of Medicine, Northwestern University Medical School, 303 East Chicago Avenue, Chicago, IL 60611

Communicated by Emanuel Margoliash, The University of Illinois, Chicago, IL, August 9, 1995

ABSTRACT **Peroxisome proliferators cause rapid and coordinated transcriptional activation of genes encoding peroxisomal β-oxidation system enzymes by activating peroxisome proliferator-activated receptor (PPAR) isoform(s). Since the thyroid hormone (T_3; 3,3′,5-triiodothyronine) receptor (TR), another member of the nuclear hormone receptor superfamily, regulates a subset of fatty acid metabolism genes shared with PPAR, we examined the possibility of interplay between peroxisome proliferator and T_3 signaling pathways. T_3 inhibited ciprofibrate-induced luciferase activity as well as the endogenous peroxisomal β-oxidation enzymes in transgenic mice carrying a 3.2-kb 5′-flanking region of the rat peroxisomal enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase gene fused to the coding region of luciferase. Transfection assays in hepatoma H4-II-E-C3 and CV-1 cells indicated that this inhibition is mediated by TR in a ligand-dependent fashion. Gel shift assays revealed that modulation of PPAR action by TR occurs through titration of limiting amounts of retinoid X receptor (RXR) required for PPAR activation. Increasing amounts of RXR partially reversed the inhibition in a reciprocal manner; PPAR also inhibited TR activation. Results with heterodimerization-deficient TR and PPAR mutants further confirmed that interaction between PPAR and TR signaling systems is indirect. These results suggest that a convergence of the peroxisome proliferator and T_3 signaling pathways occurs through their common interaction with the heterodimeric partner RXR.**

Several structurally diverse compounds, designated as peroxisome proliferators, induce peroxisome proliferation and selectively increase the transcription of genes encoding the peroxisomal fatty acid β-oxidation enzymes (1). A receptor-based mechanism for the pleiotropic responses induced by peroxisome proliferators has been confirmed by the identification of peroxisome proliferator-activated receptor (PPAR) isoforms belonging to the nuclear hormone receptor superfamily (2). Nuclear hormone receptors regulate gene expression by binding to specific response elements in the promoter regions of target gene(s) (3). On the basis of structural homologies, these receptors have been divided into two subfamilies (4). One subfamily includes receptors for steroid hormones (glucocorticoids, progestins, androgens, estrogens, and mineralocorticoids), and the other consists of the 3,3′,5-triiodo-L-thyronine (T_3) receptor (TR), retinoic acid receptor (RAR), retinoid X receptor (RXR), and vitamin D_3 receptor (VDR) (4). PPARs have features similar to the second subfamily of nuclear hormone receptors in that they (*i*) form heterodimers with RXR and (*ii*) recognize a direct repeat motif of hexamer half-sites (AGGTCA), termed peroxisome proliferator response elements (PPREs), that are separated by 1 or 2 bp (5). At least three major PPAR isoforms (α, β, γ), which may be activated by structurally distinct peroxisome proliferators and mediate transcriptional activation of genes encoding the peroxisomal β-oxidation system enzymes, have been identified (2, 6).

Tissue and species responses to peroxisome proliferators may depend on pharmacokinetics, the abundance of the PPAR isoforms and their auxiliary proteins, the nature of PPREs in the responsive genes, and, to some extent, hormone levels (2). PPARs regulate target genes by forming PPAR–RXR heterodimers in response to peroxisome proliferators (5). Because VDR, TR, and RAR also heterodimerize with RXR by recognizing direct repeats of AGGTCA half-sites spaced by 3, 4, or 5 bp, respectively (7), it is conceivable that they might interfere with PPAR by competing with the common heterodimerization partner RXR. In the present study we demonstrate, both *in vivo* and *in vitro*, that the transcriptional activation of genes encoding β-oxidation enzymes mediated by PPAR is repressed by T_3 and that TR action can also be inhibited by PPAR. The results provide evidence for interaction between the peroxisome proliferator and T_3 signaling pathways by mutual competition for RXR, their cohet-erodimeric partner.

MATERIALS AND METHODS

Animals. Transgenic mice harboring peroxisomal enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase bifunctional enzyme (HD)–luciferase (Luc) fusion gene (8) were divided into four groups ($n = 3$). The first group was fed a normal diet and injected once daily with 100 μl of vehicle (phosphate-buffered saline containing 0.002% bovine serum albumin). The second group was fed a normal diet and injected daily with 0.45 μg of T_3 contained in 100 μl of vehicle. The third group was fed a diet containing 0.025% ciprofibrate and injected with 0.45 μg of T_3 in 100 μl of vehicle. The fourth group received a ciprofibrate-containing diet and was injected with vehicle alone. Mice were treated for 4 days and killed under ether anesthesia. The liver was excised, assayed for luciferase activity, and analyzed for the expression of endogenous peroxisomal fatty acyl-CoA oxidase (ACOX) and HD mRNAs by Northern blotting (9).

Construction of Reporter Plasmids for Transfection. The HD–Luc construct containing a 3.2-kb promoter region of rat peroxisomal HD has been described (8). A basal reporter construct, TK-Luc, was constructed by insertion of a 242-bp

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: T_3, 3,3′,5-triiodo-L-thyronine; PPAR, peroxisome proliferator-activated receptor; RAR, retinoic acid receptor; TR, T_3 receptor; RXR, retinoid X receptor; VDR, vitamin D_3 receptor; PPRE, peroxisome proliferator response element; HD, peroxisomal enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase bifunctional enzyme; HD-Luc, HD-luciferase transgene; ACOX, peroxisomal fatty acyl-CoA oxidase; TK, thymidine kinase; PAL, palindrome; LAP, inverted palindrome.

‡To whom reprint requests should be addressed.

thymidine kinase (TK) promoter into pGL2-basic (Promega). Two annealed synthetic oligonucleotides, 5′-CTTTCCCGAACGTGACCTTTGTCCTGGTCCCCTTTTGCTA-3′ and 5′-CTCTCCTTTGACCTATTGAACTATTACCTACATTTGA-3′, corresponding to ACOX PPRE and HD PPRE, respectively (10, 11), were inserted in front of the minimal TK promoter to generate ACOX-DR1-TK-Luc and HD-DR2-TK-Luc reporters. Two other reporter constructs, TRE-LAP-TK-Luc and TRE-PAL-TK-Luc, have been described elsewhere (12).

Generation of Receptor Expression Vectors. Full-length rat PPARα cDNA (a gift from Frank Gonzalez, National Institutes of Health) was cloned into the *Bam*HI site of the expression vector pSG5 (Stratagene). Nine mutant PPARs were created by site-directed mutagenesis and cloned into the pSG5 vector (9). A 1.5-kb fragment encoding rat RXRα was obtained by amplification of a rat liver cDNA library using primers of 5′-AATGCGGCCGCTATGCATCACCATCACCATCACATGGACACCAAACATTTCCTG-3′ and 5′-ATTGTCTAGAGCAGCTGTGTCCAGGCGGGG-3′ for 35 cycles. Amplified product was first cloned into pGEMT vector (Promega) and sequenced before subcloning into a *Bam*HI site of pSG5. Human TRα1, TRβ1, mutTRβ1(Δ453–461), and TRβ-L428R cDNAs, subcloned in a Rous sarcoma virus-driven expression vector and pGEM7 plasmid (Promega), have been described (7, 12).

Transient Expression Assays. The HD–Luc construct was used to transfect rat hepatoma H4-II-E-C3 cells, and reporters of ACOX-DR1-TK-Luc, HD-DR2-TK-Luc, TRE-LAP-TK-Luc, and TRE-PAL-TK-Luc were used to transfect monkey CV-1 cells. Cells were cultured in medium containing 10% hormone-depleted fetal calf serum (14) and transfected with plasmids using the calcium phosphate technique (9). After 16 hr, plates were washed with phosphate-buffered saline, and fresh medium containing the indicated ligand was added. After a 40-hr incubation, the cells were processed to assess luciferase activity, and the activity obtained for individual transfections was expressed relative to the β-galactosidase activity obtained for the same preparation of lysate.

Expression of Rat PPARα, Rat RXRα, and Human TRβ1 in Insect Cells. The construction of a recombinant baculovirus expressing rat PPARα with a six-histidine tag at the N-terminal end has been described (13). To generate a recombinant baculovirus expressing RXR or TRβ1, baculovirus transfer vectors harboring rat RXRα and human TRβ1 with a six-histidine tag were used to transfect insect Sf9 cells with linearized *Autographa californica* nuclear polyhedrosis virus DNA to generate recombinant virus. The recombinant proteins were purified using nickel nitrilotriacetic acid-agarose (Qiagen) as described (17). The final concentration of purified receptor protein was adjusted to ≈10 ng/μl and stored at −70°C.

Gel Retardation Assay. Gel mobility shift assays were performed to analyze DNA-binding and dimerization properties of the purified receptors using synthetic oligonucleotides. The double-stranded synthetic oligonucleotides 5′-ACGTTGACCTTTGTCCTGTCGA-3′, 5′-ACGTTGACCTATTGAACTATCGA-3′,5′-AGCTTGACCTGACGTCAGGTCACTCGA-3′, and 5′-AGCTTCAGGTCATGACCTGACTCGA-3′, designated as PPRE-DR1, PPRE-DR2, TRE-LAP, and TRE-PAL, respectively, were end-labeled with [^{32}P]dATP using Klenow DNA polymerase. The labeled oligonucleotides were diluted to ≈16,000 cpm/μl for binding assays. Receptors were incubated with 5 μl of the radiolabeled oligonucleotide (≈80,000 cpm) in a total volume of 30 μl for 20 min at room temperature. The protein–DNA complexes were analyzed by electrophoresis through a 4% polyacrylamide gel using 0.5× TBE (45 mM Tris borate/1 mM EDTA) buffer followed by autoradiography.

RESULTS

***In Vivo* Regulation of HD–Luc by T_3.** In rats and mice, peroxisomal HD and ACOX are highly inducible by peroxisome proliferators (1). PPREs in the promoter region of the rat HD gene have been identified (11), and using this entire promoter sequence (3.2 kb) fused to a luciferase reporter gene, transgenic mice were generated (8). Basal expression of the reporter gene in transgenic livers was at the limit of detection. After a 24-hr treatment with a peroxisome proliferator, luciferase activity was dramatically increased (>1000-fold) (8). Transgenic mice injected with T_3 intramuscularly showed a reduction in ciprofibrate-inducible luciferase activity (Fig. 1*A*). Northern blot analysis indicated that ciprofibrate-induced expression of endogenous ACOX and HD was also inhibited by T_3. T_3 alone had no effect on the expression of either the transgene HD or endogenous ACOX and HD genes (Fig. 1*B*).

Inhibition of Ciprofibrate-Induced HD–Luc Activity by TR *in Vitro*. Transfection of H4-II-E-C3 cells with the HD–Luc construct resulted in a low level of luciferase activity; the activity increased 5- to 10-fold after ciprofibrate treatment (Fig. 2). The role of T_3 repression was examined by cotransfection of expression vectors for TRα1, TRβ1, or a ligand-binding mutant mutTRβ1(Δ453-461), which has 9 aa residues deleted from the C terminus (7). When TRα1, TRβ1, or mutTRβ1 expression vectors were added, the HD–Luc induction was reduced by 64%, 53%, and 58%, respectively. In the presence of 10 nM T_3, a ligand-dependent repression was seen at low receptor concentrations with TRα1 and TRβ1 but not with the mutant mutTRβ1, indicating that the T_3 effect was mediated through functional TR. These results suggest that TR is able to repress PPAR–RXR-mediated transactivation of β-oxidation genes, confirming the *in vivo* results obtained with HD–Luc transgenic mice.

TR Represses PPAR–RXR-Mediated Transactivation in CV-1 Cells. Induction of ACOX and HD is directed by the DNA response element of the TGACCTTTGTCCT (−570 to −558) motif (10) and the TGACCTATTGAACT (−2947 to −2934) motif (11), respectively. PPAR and RXR form heterodimers and regulate target gene expression by binding to the PPRE (5). A typical PPRE consists of two repeated AGGTCA motifs spaced by 1 or 2 bp. To determine if the inhibition of PPAR–RXR-mediated transactivation by TR is through minimal PPREs, oligonucleotides corresponding to the respective PPREs of ACOX and HD were cloned upstream of a TK promoter driving a luciferase reporter, and CV-1 cells were used for transfection assays. As shown in Fig. 3, PPAR–RXR-mediated induction of luciferase activity was stimulated 2-fold by ciprofibrate (from 47% to 100% for ACOX, and from 53% to 113% for HD). The difference in the magnitude of induction by ciprofibrate of

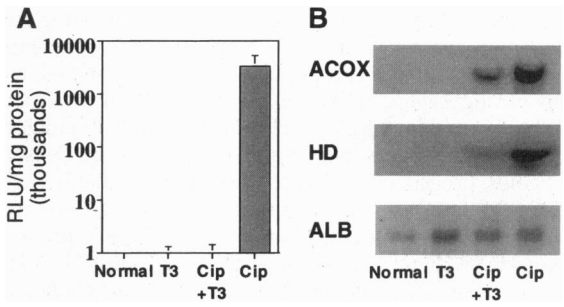

FIG. 1. Inhibition of ciprofibrate (Cip)-induced luciferase activity by T_3 in transgenic mice. (*A*) Luciferase activity in the liver of transgenic mice containing the coding region of luciferase under the control of a 3.2-kb promoter sequence of rat HD gene. (*B*) Northern blot analysis of liver RNA from transgenic mice. Total RNA (20 μg) was probed with rat ACOX and HD cDNA. Rat albumin (ALB) cDNA was used as a control for RNA loading. Error bars indicate standard deviations. RLU, relative light units.

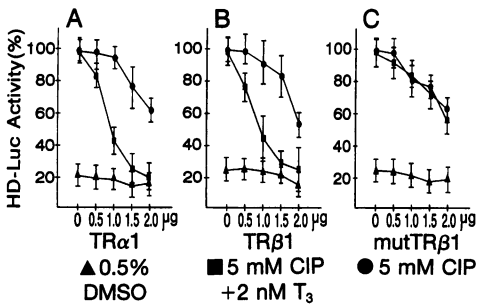

FIG. 2. Repression of PPAR-mediated trans-activation of ciprofibrate by T_3 occurs through functional TR *in vitro*. The same construct used for the *in vivo* experiment was employed to transfect H4-II-E-C3 cells. Each of the transfection experiments contained 2 μg of reporter plasmid and the indicated amounts of TR expression vectors. pSG5 plasmid DNA was included in all transfections to normalize expression vector promoter dosage. The results are normalized to the luciferase activity observed with 2 μg of reporter plasmid alone at 5 mM ciprofibrate. The data represent the mean ± SD of three independent experiments performed in duplicate. DMSO, dimethyl sulfoxide; CIP, ciprofibrate.

luciferase *in vivo* and *in vitro* systems using the HD promoter could be due to differences in activators and suppressors under *in vivo* and *in vitro* conditions. Cotransfection of TR expression vector repressed the basal activity of both reporters, and treatment with T_3 further repressed their activity. TR-mediated repression was partially reversed by transfecting increased amounts of RXR expression vector.

TR Interferes with PPAR–RXR Binding to PPREs. Gel mobility shift assays revealed that TR and PPAR were unable to form homo- or heterodimers binding to either ACOX PPRE-DR1 or HD PPRE-DR2 (Fig. 4, lane 7). TR and RXR formed heterodimers that bound weakly to ACOX PPRE-DR1 and HD PPRE-DR2 (lane 2). This is consistent with other observations that TR–RXR binds very weakly or not at all to the DR1 repeat. When PPAR was added to the TR–RXR mixture, the predominant protein–DNA complex corresponded to the slower migrating PPAR–RXR heterodimer (lanes 13–15). Similarly, the PPAR–RXR DNA complex could also be converted to TR–RXR heterodimers by the inclusion

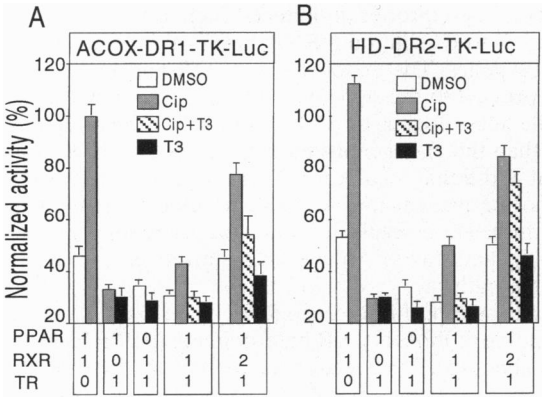

FIG. 3. TR inhibits ciprofibrate-induced activation of PPAR on different PPREs. PPREs of rat ACOX (*A*) and HD (*B*) were fused to a TK minimal promoter linked to a luciferase reporter gene. Reporter plasmids were cotransfected into CV-1 cells with different receptor plasmids as indicated (amounts given are in micrograms). Each transfection contained a total of 5 μg of reporter and receptor plasmid DNA balanced with pSG5 and an additional 0.5 μg of β-galactosidase expression vector pCMVβ as an internal control. Luciferase activity is expressed as a percentage of the normalized response where induced PPAR–RXR activity on ACOX-DRI-TK-Luc in the presence of 5 mM ciprofibrate is arbitrarily set at 100%. DMSO, dimethyl sulfoxide; Cip, ciprofibrate.

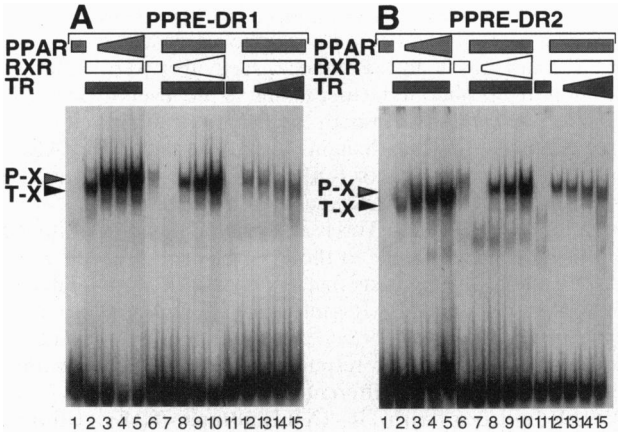

FIG. 4. Interaction of TR with PPAR–RXR binding on PPREs. N-terminal six-histidine-tagged rat PPARα, rat RXRα, and human TRβ1 were expressed in insect Sf9 cells and purified to near homogeneity on nickel nitrilotriacetic acid-agarose columns. ^{32}P-labeled ACOX (*A*) and HD (*B*) PPRE oligonucleotides were incubated with 0, 1, 2.5, and 5 μl of purified receptors as indicated above each lane. P-X, PPAR–RXR complex; T-X, TR–RXR complex. Open and shaded rectangles mean 2.5 μl of receptor.

of increasing amounts of TR. When equal amounts of PPAR and TR were present in the solution, only PPAR–RXR complexes were detected. This was confirmed by using antibodies against PPAR (data not illustrated). These results suggest that inhibition of PPAR–RXR-mediated induction of PPRE-regulated genes by TR occurs through the formation of TR–RXR complexes, which impair the formation and DNA binding of PPAR–RXR to these DR1 and DR2 response elements.

PPAR Inhibits Ligand-Induced Transactivation of TR–RXR on TREs. If the inhibition of PPAR–RXR-mediated induction by TR occurs through formation of competing TR–RXR complexes, then PPAR should also be able to compete with RXR from the TR–RXR complex by forming PPAR–RXR heterodimers. Transfection assays were used to determine whether PPAR inhibits TR activity through RXR. As shown in Fig. 5, when AGGTCA half-sites were arranged in front of a minimal TK promoter in an inverted palindrome (TRE-LAP-TK-Luc) or a palindrome (TRE-PAL-TK-Luc), neither PPAR–RXR nor PPAR–TR was able to transactivate the reporter to a large extent in the presence of either ciprofibrate or T_3. In the presence of T_3,

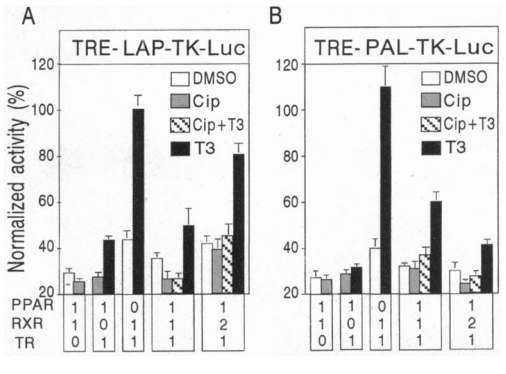

FIG. 5. Ciprofibrate-enhanced PPAR repression of TR activation on TREs. TRE-LAP-TK-Luc (*A*) and TRE-PAL-TK-Luc (*B*) constructs were used to assess the effect of PPAR on TR-mediated expression of TR by PPAR. Transfections were performed as described in Fig. 3 except for the use of different reporter plasmids (amounts given are in micrograms). Luciferase activity is presented as a percentage of the normalized response where induced TR–RXR activity on TRE-LAP-TK-Luc construct in the presence of 2 nM T_3 is arbitrarily set at 100%. DMSO, dimethyl sulfoxide; Cip, ciprofibrate.

there was, as expected, a 2.5- to 3-fold induction with combined transfection of TR and RXR. Nonetheless, in the presence of PPAR, both basal and T_3-induced activities were inhibited. Addition of ciprofibrate further inhibited the level of expression mediated by TR–RXR on both the inverted palindrome (LAP) and palindrome (PAL) elements in the presence of PPAR. A doubling of the amounts of RXR expression vector resulted in partial reversal of the PPAR inhibition on the LAP element but not on the PAL element. This is attributed to the high affinity of PPAR–RXR heterodimer to the PAL element and not to the LAP element. This suggests that the degree of mutual interference between TR and PPAR is influenced not only by the amount of each receptor and the presence or absence of their ligand but also by the nature of the response element for which various heterodimers may have different affinities.

PPAR Interferes with TR–RXR Binding to TREs. Although TRs can bind to some TREs as both monomers and homodimers, the binding is not of high affinity. In contrast, TR–RXR heterodimer binds relatively strongly to a direct repeat (DR4), LAP, or PAL element (7, 12). PPAR did not affect the TR homodimer binding to the LAP element, but it titrated TR away from a TR–RXR complex by forming a PPAR–RXR complex, which migrated to a slightly lesser extent than the TR–RXR complexes (Fig. 6, lanes 3–5). The PPAR–RXR complex could be forced to recombine into a TR–RXR heterodimer by increasing amounts of TR (lanes 13–15).

Heterodimerization-Defective TR and PPAR Mutants Do Not Exhibit Inhibitory Activity. Transfections and DNA-binding assays suggest that the cross talk between PPAR and TR occurs through mutual competition for RXR. To further clarify the role of interaction between PPAR and TR, nine PPAR mutants (K292A, K310A, K327A, K345A, R348A, K349A, K358A, K364A, and R388A) were created with single amino acid substitutions in the ligand-binding domain. Two mutants, K345A and K364A, were found to retain <10% heterodimerization ability with RXR (data not shown). These two PPAR mutants, together with a well-characterized TRβ-L428R mutant impairing heterodmerization with RXR (12), were tested for their actions. The binding of PPAR and RXR to the DR1 element was reduced by the wild-type TR (Fig. 7, lane 3) and was completely abolished in the presence of unlabeled LAP DNA (lane 4). Nonetheless, LAP element stabilized TR–RXR heterodimer and accordingly enhanced TR titrating out RXR from the PPAR–RXR heterodimer. In contrast, the binding of PPAR–RXR complexes to the DR1 element was not affected by the heterodimerization-defective TRβ-L428R mutant (lanes 5 and 6). On the LAP element, unlike the wild-type PPAR (lane 2), both of the PPAR-K345A and PPAR-K364A mutants were unable to dissociate the interaction of RXR–TR complexes (lanes 3 and 4). Transient transfection assays indicated that the TRβ-L428R mutant displayed only 10% of TRβ1 inhibition to PPAR in CV1 cells using the ACOX-DR1-TK-Luc construct in the presence of T_3. PPAR mutants also lost their ability to inhibit TR (data not shown). These results further confirm the finding that the interaction between PPAR and TR occurs through their common heterodimeric partner, RXR.

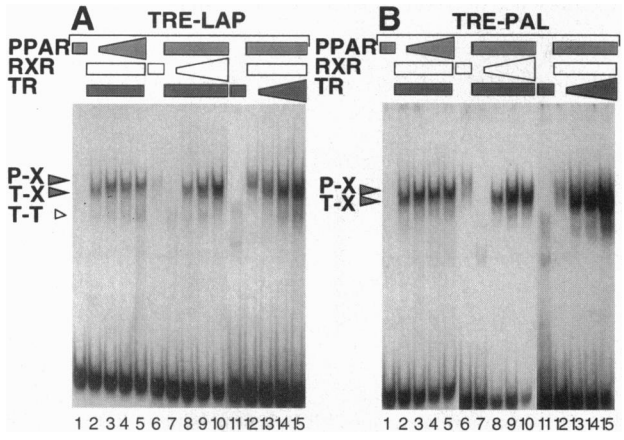

FIG. 6. Effect of PPAR on the binding of TR–RXR to TREs. The inverted palindromic (*A*) and palindromic (*B*) TREs were tested for binding by TR–RXR affected by PPAR. The receptor proteins used for the binding assay were the same as described in Fig. 4 and are indicated above each lane. P-X, PPAR–RXR complex; T-X, TR–RXR complex; T-T, TR–TR complex. Open and shaded rectangles mean 2.5 μl of receptor.

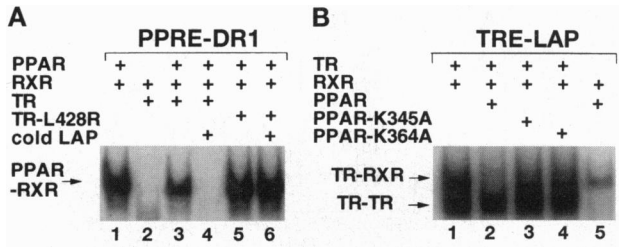

FIG. 7. Interaction properties of the heterodimerization-deficient TR and PPAR mutants. Purified rat RXRα and *in vitro*-translated TRβ1, TR-L428R, PPARα, PPAR-K345A, and PPAR-K364A proteins were incubated with ^{32}P-labeled PPRE-DR1 (*A*) and TRE-LAP (*B*) oligonucleotides as indicated above each lane. A 5-fold excess of unlabeled LAP oligonucleotide (cold LAP) was included in the experiment in *A* as indicated.

DISCUSSION

Peroxisome proliferators and T_3 are important coregulators of genes involved in lipid metabolism, calorigenesis, and in adipocyte differentiation and function. While T_3 and peroxisome proliferators both regulate the processes of lipid metabolism, they have been found to act in either synergistic or opposing manners on specific genes involved in lipid metabolism (15, 16). In transgenic mice, we have demonstrated that a 3.2-kb DNA segment of the 5′-flanking region of the HD gene is capable of conferring responsiveness to peroxisome proliferators (8). In the present study, T_3 was found to inhibit markedly the ciprofibrate-induced increase in the transgene activity as well as in the mRNA levels of endogenous ACOX and HD genes. The endogenous ACOX and HD genes were not repressed to the same extent as the transgene, suggesting that the heterologous rat HD promoter may be more sensitive to T_3 than the endogenous mouse ACOX and HD promoters or that additional regulatory DNA sequences of the HD gene may not be present in the transgene used to generate transgenic mice. These results indicate that peroxisome proliferator signaling pathway(s) can be antagonized *in vivo* by the T_3 signaling pathway.

Peroxisome proliferators regulate gene expression through the binding of PPAR–RXR heterodimers to PPREs present in target genes (5). Transfection assays indicate that the PPAR–RXR complex can respond to both peroxisome proliferators and 9-*cis*-retinoic acid by interacting with PPREs (5). In the absence of RXR, PPAR is unable to bind to PPREs (5, 17). Thus RXR plays a critical role in the peroxisome proliferator-induced signaling pathway. Modulation of PPAR action by other members of the nuclear receptor superfamily might occur at several different levels. Competition could result at the binding level of other nuclear receptors to PPREs that share a motif similar to many other receptor DNA-binding sites. Binding by competing nuclear receptors at PPREs could prevent PPAR action at these sites or possibly allow PPREs to serve as response elements for other superfamily receptors and

their ligands. For example, the chicken ovalbumin upstream promoter transcription factor (COUP-TF), an orphan member of the nuclear hormone receptor superfamily, has been shown to repress hormonal induction of the target genes of VDR, TR, and RAR, as well as PPAR, through competitive binding to various hormone response elements (18, 19). A second form of modulation by other nuclear receptors could be expected to occur at the level of heterodimerization with RXR, which is essential for PPAR action. Since all members of the second subfamily of nuclear hormone receptors are able to form heterodimers with RXR, the quantity and activity of other nuclear receptor signaling pathways that utilize RXR may modulate PPAR action by controlling the availability of a limited amount of RXR protein. Competition at the level of availability of limiting RXR has been demonstrated between the TR, RXR, and RARs (20). In these competitions, the formation of heterodimers between the receptors, or formation of the individual receptor homodimers, is controlled by the presence of the receptor's ligand. We found that an excess of TR exhibited an ability to titrate out RXR from PPAR–RXR complexes by the formation of TR–RXR complexes (Fig. 4). Apparently, the repression of PPAR-responsive genes by TR is through disassociation of the PPAR–RXR complex, preventing PPRE binding by PPAR–RXR. The competition for binding to PPRE by TR–RXR, if any, is not as efficient as COUP-TF (19).

It has been shown that T_3 reduces the formation of TR homodimers and promotes the formation and activation of TR–RXR heterodimers (7, 12). TR-mediated inhibition of PPAR action in our experiments occurred in the presence and absence of T_3, but was more pronounced when ligand was present, suggesting that some enhancement of TR–RXR heterodimerization may be occurring in the presence of ligand. The inhibition of PPAR-mediated transactivation by a dominant-negative mutant RAR 403 recently reported (21) is likely to have occurred through a similar mechanism of RXR sequestration. More importantly, heterodimerization-deficient TR and PPAR mutants were unable to perform the inhibitor action, indicating that heterodimerization with RXR is a key factor in TR–PPAR interactions. It should be noted that because these mutants are defective in heterodimerization, they also bind to DNA poorly. Thus, while their lack of inhibition is most likely due to defective interactions with RXR or other dimeric partners, one cannot exclude an effect at the level of DNA binding. Although PPAR reportedly can form a heterodimer with TR in solution and subsequently affect TR homodimerization on thyroid hormone response elements (22), this effect is unlikely to be as quantitatively important as the highly efficient competition of PPAR for RXR as shown in the present experiments and recently by Meier-Heusler *et al.* (23). The modulation of PPAR transactivation by TR, and possibly other nuclear hormone receptors, suggests that pleiotropic responses induced by peroxisome proliferators may be the result of integrated action of multiple members of the nuclear hormone receptor superfamily.

This work was supported by National Institute of Health Grants R37 GM 23570 and DK42144 and by the Joseph L. Mayberry, Sr., Endowment Fund. P.K. is supported by a fellowship from "schweizerische stiftung für Biologisch-Medizinische Stipendien." L.D.M. was supported by a T.R.A.C. award from Knoll Pharmaceuticals.

1. Reddy, J. K., Goel, S. K., Nemali, M. R., Carrino, J. J., Laffler, T. G., Reddy, M. K., Sperbeck, S. J., Osumi, T., Hashimoto, T., Lalwani, N. D. & Rao, M. S. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 1747–1751.
2. Reddy, J. K. & Mannaerts, G. P. (1994) *Annu. Rev. Nutr.* **14,** 343–370.
3. Parker, M. G. (1991) *Nuclear Hormone Receptors: Molecular Mechanisms, Cellular Functions, Clinical Abnormalities* (Academic, London), pp. 61–82.
4. Issemann, I. & Green, S. (1990) *Nature (London)* **374,** 645–650.
5. Kliewer, S. A., Umesono, K., Noonan, D. J., Heyman, R. A. & Evans R. M. (1992) *Nature (London)* **358,** 771–774.
6. Kliewer, S. A., Forman, B. M., Blumberg, B., Ong, E. S., Borgmeyer, U., Mangelsdorf, D. J., Umesono, K. & Evans, R. M. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 7355–7359.
7. Nagaya, T., Madison, L. D. & Jameson, L. J. (1992) *J. Biol. Chem.* **267,** 13014–13019.
8. Alvares, K., Fan, C., Dadras, S. S., Yeldandi, A. V., Rachubinski, R. A., Capone, J. P., Subramani, S., Iannaccone, P. M., Rao, M. S. & Reddy, J. K. (1994) *Cancer Res.* **54,** 2303–2306.
9. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).
10. Osumi, T., Wen, J. & Hashimoto, T. (1991) *Biochem. Biophys. Res. Commun.* **175,** 866–871.
11. Zhang, B., Marcus, S. L., Sajjadi, F. G., Alvares, K., Reddy, J. K., Subramani, S., Rachubinski, R. A. & Capone, J. P. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 7541–7545.
12. Nagaya, T. & Jameson, L. J. (1993) *J. Biol. Chem.* **268,** 15766–16771.
13. Huang, Q., Alvares, K. Chu, R., Bradfield, C. A. & Reddy, J. K. (1994) *J. Biol. Chem.* **269,** 8493–8497.
14. Samuels, H. H., Stanley, F. & Casanova, J. (1979) *Endocrinology* **105,** 80–85.
15. Loeb, J. N. (1991) in *Werner and Ingbar's The Thyroid*, eds. Braremen, L. E. & Utiger, R. D. (Lippincott, Philadelphia), pp. 124–142.
16. Kawashima, Y. & Kozuka, H. (1985) *Biochim. Biophys. Acta* **834,** 118–123.
17. Gearing, K. L., Gottlicher, M., Teboul, M., Widmark, E. & Gustafsson, J.-A. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 1440–1444.
18. Cooney, A. J., Tsai, S. Y., O'Malley, B. W. & Tsai, M. (1992) *Mol. Cell. Biol.* **12,** 4153–4163.
19. Miyata, K. S., Zhang, B., Marcus, S. L., Capone, J. P. & Rachubinski, R. A. (1993) *J. Biol. Chem.* **268,** 19169–19172.
20. Lehmann, J. M., Zhang, X., Graupner, G., Lee, M. O., Hermann, T., Hoffmann, B. & Pfahl, M. (1993) *Mol. Cell. Biol.* **13,** 7698–7707.
21. Imakado, S., Bickenbach, J. R., Bundman, D. S., Rothnagel, J. A., Attar, P. S., Wang, X., Walczak, V. R., Wisniewski, S., Pote, J., Gordon, J. S., Heyman, R. A., Evans, R. M. & Roop, D. R. (1995) *Genes Dev.* **9,** 317–329.
22. Bogazzi, F., Hudson, L. D. & Nikodem, V. M. (1994) *J. Biol. Chem.* **269,** 11683–11686.
23. Meier-Heusler, S. C., Zhu, X., Juge-Aubry, C., Pernin, A., Burger, A. G., Cheng, S. & Meijer, C. A. (1995) *Mol. Cell. Endocrinol.* **107,** 55–66.