Structurally diverse peroxisome proliferators and related compounds that have been demonstrated to induce the ligand-dependent transcriptional activation function of mouse peroxisome proliferator-activated receptor α (mPPARα) in transfection experiments were tested for the ability to induce conformational changes within mPPARα in vitro. YY-14,643, 5,8,11,14-eicosatetraynoic acid, LY-171883, and clofibrate acid all directly induced mPPARα conformational changes as evidenced by a differential protease sensitivity assay. Carboxyl-terminal truncation mutagenesis of mPPARα differentially affected the ability of these ligands to induce conformational changes suggesting that PPAR ligands may make distinct contacts with the receptor. Direct interaction of peroxisome proliferators and related compounds with, and the resulting conformational alteration(s) in, mPPARα may facilitate interaction of the receptor with transcriptional intermediary factors and/or the general transcription machinery and, thus, may underlie the molecular basis of ligand-dependent transcriptional activation mediated by mPPARα.

Peroxisome proliferator-activated receptors (PPARs) are members of a large family of ligand-inducible transcription factors that includes receptors for retinoids, vitamin D, and thyroid and steroid hormones (1–5). The mammalian PPAR family is composed of at least three genetically and pharmacologically distinct subtypes, PPARα, -γ, and -δ (reviewed in Ref. 6). Murine PPAR α (mPPARα) was originally isolated from a mouse liver cDNA library by Issemann and Green (7) who demonstrated that the receptor was activated in transfection experiments by a group of compounds known to induce peroxisome proliferation in rodents. A number of structurally diverse compounds have subsequently been demonstrated to activate PPARα in transient transfection experiments. Particularly noteworthy among these compounds are: 1) lipids such as arachidonic acid (8–11) and its synthetic analog 5,8,11,14-eicosatetraynoic acid (ETYA, Refs. 9, 11–13), 8-[S]-hydroxyeicosatetraenoic acid (14), a lipoxigenase metabolite of arachidonic acid, and linoleic acid (8–11, 14, 15); 2) fibric acid anti-hyperlipidemic drugs (YY-14,643, clofibrate acid, gemfibrozil, ciprofibrate acid; Refs. 10, 16, 17) that represent a class of therapeutic agents useful in the treatment of hypertriglyceridemia (18); and 3) a leukotriene D4 antagonist, LY-171883 (19). Many of these compounds, together with phthalate ester plasticizers (di(2-ethylhexyl)-phthalate) and herbicides (2,4,5-trichlorophenoxyacetic acid), are known collectively as peroxisome proliferators (reviewed in Ref. 20). While chemically distinct, most of these compounds have been demonstrated to induce proliferation of peroxisomes leading to hepatic hyperplasia and hepatocarcinogenesis in many species (20). Peroxisome proliferator-induced alteration of hepatocyte phenotype is believed to result from activation of PPARα and subsequent modulation of gene expression downstream of this nuclear receptor (reviewed in Refs. 6, 20; see below). The central role of PPARα in xenobiotic-induced peroxisomal proliferation was recently demonstrated by the absence of hepatomegaly and peroxisome proliferation in mice null for expression of this gene (21).

PPARs modulate expression of target genes by binding to response elements comprised of a degenerate direct repeat of the hexameric nucleotide sequence, TGACCT, separated by one base pair (DR1). PPAR has been shown to bind cognate response elements with high affinity only in the context of a heterodimeric complex with the retinoid X receptor (RXR, Refs. 11, 17, 22–24). PPAR-RXR heterodimeric complexes appear to be responsive to both PPAR activators and 9-cis-retinoic acid, the endogenous ligand for RXR (11, 17, 22–24).

PPAR response elements (PPREs) have been identified in the 5′ regions of several mammalian genes coding for proteins involved in lipid metabolism such as acyl-CoA oxidase (17, 25), bifunctional enzyme (26, 27), malic enzyme (16), liver fatty acid binding protein (28), 3-hydroxy-3-methylglutaryl-CoA synthase (15), and cytochrome P450 fatty acid ω-hydroxylase (29). Such findings indicate a prominent regulatory role for the PPAR receptor family in lipid metabolism and homeostasis. In addition, overexpression of PPARs and -γ in cultured fibroblasts and subsequent exposure to PPAR ligands has been shown to confer adipogenicity (30, 31), further illustrating the central regulatory role of PPAR family members in lipid homeostasis.

In contrast to many other receptors in the retinoid/thyroid hormone receptor superfamily, functional domains of PPARs...
and critical amino acid residues within such putative domains have not been extensively characterized. Two previous studies with PPARα have identified: 1) a Glu282 to Gly point mutation in mPPARα that ameliorates transcriptional responses to WY-14,643 and ETYA (13), and 2) a Leu443 to Arg point mutation in human PPARα (hPPARα) that abolishes heterodimerization with RXR (32). The present studies were undertaken to identify mPPARα carboxyl-terminal receptor regions that are important for both ligand responsiveness and heterodimerization with mRXRα and to determine if structurally diverse PPAR ligands induce similar conformational changes within mPPARα. To our knowledge, these studies provide the first direct biochemical evidence demonstrating that peroxisome proliferators induce conformational changes within mPPARα ligand-induced stabilization of particular mPPARα conformational states likely underlies the molecular basis for the ability of these compounds to activate the receptor and to modulate expression of mPPARα target genes including those implicated in peroxisome proliferation.

MATERIALS AND METHODS

Plasmids and Receptor Constructs—Full-length mPPARα (7) was kindly provided by Drs. S. Green and J. Tugwood (Macclesfield, UK) and was used as a template for the polymerase chain reaction during construction of all PPAR mutants described herein. Full-length mouse RXRα (mRXRα, Ref. 33) and pGEX-ct (34) were kind gifts from Drs. Ph. Kastner and P. Champon (Strasbourg) and Dr. W. Dougherty (Oregon State University), respectively.

A full-length mPPARα amino-terminal truncation mutant was constructed by polymerase chain reaction using a 5′ primer (ML023) that introduced an EcoRI site, a favorable Kozak sequence, and a TNF b chain ribosomal-binding site immediately upstream of the natural initiator methionine and a 3′ primer that introduced a BamHI site 3′ of the mPPARα natural stop codon. The resulting fragment was appropriately digested and subcloned into the eukaryotic expression vector, pTL1 (33), yielding PPARAB. PPARAB is transcribed/translated in vitro at least 10-fold more efficiently than full-length receptor and exhibits DNA binding and heterodimerization activities that are indistinguishable from full-length receptor (data not shown). The carboxyl-terminal truncation mutants, PPARΔAB/Δ448 and PPARΔAB/Δ425 (Fig. 1A), were prepared by polymerase chain reaction using ML023 as the 5′ primer and a 3′ primer that introduced stop codons at positions 448 and 425, respectively, preceding BamHI site. Both of the resulting fragments were appropriately digested and subcloned into pTL1 as described above. PPARAB, PPARΔAB/Δ448, and PPARΔAB/Δ425 were transcribed/translated in vitro with equal efficiencies (data not shown).

GST-mRXRα was prepared by polymerase chain reaction amplification of full-length mouse mRXRα using a 5′ primer that introduced a HincII site downstream of the carboxyl-terminal truncation mutant primer that introduced an EcoRI site 3′ of the natural stop codon of mRXRα. The resulting fragment was appropriately digested and subcloned into a pEBEcoRI/DIG fusion vector (pGEX-ct).

In Vitro Transcription/Translation—Proteins were prepared by in vitro transcription/translation using rabbit reticulocyte lysate as described previously (3, 33). Translation reactions were carried out in the presence of [35S]methionine for production of radioactively labeled proteins used in DPSAs and GST-pull down experiments, whereas receptor proteins used in electrophoretic mobility shift assays were translated in the presence of unlabeled methionine. Unprogrammed lysates were generated identically using equal amounts of linearized pTL1 in place of receptor-coding templates.

Electrophoretic Mobility Shift Assays (EMSA)—Two probes were used in these studies as follows. DR1, 5′-cagGACcGctGTGACcctacctgcG-3′ (APO-PCPRE, 5′-ctctgcggagctGACcCttGtcacctgcG-3′). One strand of each probe is shown for clarity, and the directly repeated motifs are indicated in uppercase letters and by underlining. Both DR1 (35, 36) and ACO-PPRE (25) probes have been described previously.

Receptor proteins (10 and 20 fmol of PPARAB and mRXRα, respectively) were preincubated on ice for 15 min prior to addition of a mix containing ~500,000 cpm of Klenow end-25labeled DR1 or ACO-PPRE probes. Components of the probe mix were (in nm) HEPES-NaOH, pH 7.5, 10; EDTA, 1; dithiothreitol, 1; and NaCl, 150. The mix was supplemented with 10% glycerol, 1 μg/ml bovine serum albumin, and poly[d(I:C)] (2 μg/tube). The amount of lysate in each binding reaction was held constant by addition of unprogrammed reticulocyte lysate. Samples were loaded on a 5% polyacrylamide gel, electrophoresed, and gels were dried and subjected to autoradiography as described previously (33, 36).

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RESULTS

Carboxyl-terminal truncation mutants of PPARAB were constructed to define regions of the receptor required for interaction with RXRα and to determine if diverse ligands require distinct mPPARα structural features. Based on the crystal structures of RXRα (37, 38) and retinoic acid receptor γ (RARγ, Ref. 38) LBDs and the predicted structural similarities, these recombinant to mPPARα (39) and data not shown) two PPARΔAB carboxyl-terminal truncation mutants were prepared as follows: 1) PPARΔAB/Δ448 that lacks a portion of putative helix H11 and all of helix H12, and 2) PPARΔAB/Δ425 that lacks putative helices H10-H12 (see Fig. 1A). Because both carboxy-terminal truncation mutants lack the core of the putative ligand-dependent transcriptional activation function (AF-2, Ref. 39),...
neither would be expected to activate transcription in a ligand-dependent manner.

**mPPARα Carboxyl-terminal Truncation Mutants Define PPAR-RXR Heterodimerization Interface**—EMSAs were conducted to compare the ability of PPARαAB, PPARαAB/448, and PPARαAB/425 to bind two degenerate DR1 probes: a DR1 retinoid responsive element described previously (35) and a peroxisome proliferator-activated response element (PPRE) identified in the promoter region of the rat acyl-CoA oxidase gene (ACO-PPRE) that confers peroxisome proliferator inducibility on this gene (25). While none of the PPAR receptor proteins and an immobilized GST protein (Fig. 2, lanes 4 and 6) interacted with GST-mRXR on either probe (Fig. 2, lanes 8 and 16), mRXR homodimeric complexes have previously been demonstrated to bind DR1 response elements (36, 40–43), and indeed such complexes are observed in our binding assays on the DR1 but not the ACO-PPRE probe (Fig. 2, compare lanes 2 and 10). The efficiency of mRXR-mPPARαAB/448 complex formation on both probes was reduced approximately 2-fold relative to that of mRXR-mPPARαAB, suggesting that mPPARα residues 448–468 contribute to the stability of the heterodimeric complex but are not absolutely required for complex formation and DNA binding. However, truncation of an additional 23 mPPARα carboxyl-terminal residues (amino acids 425–468; PPARαAB/425) abolished the ability of the receptor to interact with mRXRs on either probe (Fig. 2, lanes 8 and 16).

Protein-protein interaction experiments were carried out to investigate the ability of PPARα carboxyl-terminal truncation mutants to interact with RXR independently of DNA binding. GST-mRXR fusion protein, immobilized on glutathione-Sepharose, was used in standard GST pull-down experiments for this purpose. In vitro translated [35S]-PPARαAB and [35S]-PPARαAB/448 both interacted with GST-mRXR (Fig. 3, lanes 4 and 5) while an interaction between [35S]-PPARαAB/425 and GST-mRXR was not detected (Fig. 3, lane 6). The efficiency of [35S]-PPARαAB/448 interaction with GST-mRXR was reduced approximately 2-fold relative to that of [35S]-PPARαAB with GST-mRXR in agreement with DNA binding experiments described above. No interactions between any of the PPAR receptor proteins and an immobilized GST protein (Fig. 3, lanes 7–9) or glutathione-Sepharose alone were observed (data not shown). Additionally, results from experiments conducted in the presence of unlabeled response elements, identical to those used in DNA binding assays (see above), were indistinguishable from those described above (data not shown).

**The Sensitivity of mPPARα to Chymotryptic Digestion Is Altered by Interaction with Ligands That Activate the Receptor**—We have adapted a differential protease sensitivity assay (DPSA, Ref. 36) for use with [35S]-PPARαAB to address the possibility that peroxisome proliferators and related compounds (see Fig. 1B) interact directly with and alter the protease sensitivity of the receptor. Digestion of [35S]-PPARαAB with increasing concentrations of chymotrypsin in the presence of 100 μM LY-171883, ETYA, or WY-14,643 (Fig. 4A, lanes 11–13, 14–16, and 17–19, respectively) resulted in the appearance of protease-resistant fragments of approximately 33, 31, and 27 kDa, referred to hereafter as PFF3, PFF31, and PFF27, respectively. Clofibrate acid and clofibrate, when examined at concentrations of 1 μM, resulted in very weak signals (data not shown); therefore, these PPAR ligands were examined at concentrations of 1 mM. While clofibrate acid clearly induced forma-
Induction of Proteolytic Fragments Is Dependent on Ligand Concentration—DPSAs were conducted using $^{35}S$-PPAR$\Delta$AB at a constant chymotrypsin concentration and increasing concentrations of PPAR ligands (WY-14, 643, ETYA, LY-171883, CFA; see Fig. 1B) to determine the dependence of PF33, PF31, and PF27 on ligand concentration. Induction of all proteolytic fragments from $^{35}S$-PPAR$\Delta$AB was clearly ligand-dependent in all cases (Fig. 5A–D), and the relative potencies with which these compounds induced $^{35}S$-PPAR$\Delta$AB conformational change in vitro was generally consistent with previously reported transcriptional activation studies (Refs. 7, 13, 19; see “Discussion”).

**PPAR Activator-induced Alteration in Chymotryptic Sensitivity of mPPAR Is Not Altered by Heterodimerization with mRXR**—Because PPARs has been demonstrated to heterodimerize with RXR (10, 11, 22, 23; see Figs. 2 and 3), DPSAs were conducted to examine the effects of heterodimerization with mRXR on the induction of $^{35}S$-PPAR$\Delta$AB proteolytic fragments by WY-14, 643. DPSAs, in the presence of unprogrammed lysate or *in vitro* translated mRXR, were carried out at a constant protease concentration and increasing concentrations of WY-14, 643. Interaction with mRXR did not alter the protease sensitivity of unliganded $^{35}S$-PPAR$\Delta$AB (Fig. 6A, compare lanes 2 and 6) or $^{35}S$-PPAR$\Delta$AB (Fig. 6B, compare lanes 2 and 6). In addition, the concentration dependence of WY-14, 643 on the induction of proteolytic fragments derived from either receptor did not differ noticeably in the presence of mRXR (compare lanes 2–5 with lanes 6–9 of Fig. 6A and B, respectively). Similar results were observed for both $^{35}S$-PPAR$\Delta$AB and $^{35}S$-PPAR$\Delta$AB when using the PPAR ligands clofibrate acid, clofibrate, LY-171883, ETYA, and LY-171883, and ETYA (data not shown). Moreover, the rank order of efficacy of the five compounds tested for induction of PFs within PPAR$\Delta$AB and PPAR$\Delta$AB did not differ from that stated above (data not shown). Therefore, heterodimerization with mRXR does not appear to influence, positively or negatively, the capacity of mPPARs to bind PPAR activators and undergo ligand-induced conformational changes. $^{35}S$-PPAR$\Delta$AB was not examined in these experiments due the inability of this receptor mutant to interact with mRXR (Figs. 2 and 3) or bind ligand (Fig. 4C).

**DISCUSSION**

Our results suggest that the extreme carboxyl-terminal amino acids of mPPARs are required for formation of PPAR-RXR heterodimeric complexes both in solution and bound to DR1 and ACO-PPRE probes. This finding is in agreement with a previous study that characterized a hPPAR$\alpha$ point mutation (Leu$^{43}$Arg corresponding to the same residue in mPPARs) which abolished heterodimerization with RXR (32) thus illustrating a critical role for this region (which is deleted in PPAR$\Delta$AB) specifies the importance of mPPARs to interact with RXR and binding to DR1 and ACO-PPRE probes, albeit at a 2-fold decreased efficiency as compared...
pared with a receptor protein with an intact carboxyl terminus (PPAR\(\Delta AB\)). Considered together, these results suggest that the mPPAR\(\alpha\) dimerization interface contains at least Leu\(^{433}\), which is 100% conserved across all PPAR subtypes (32, data not shown), and extends through at least Ile\(^{447}\). In addition to heterodimerizing with RXR, PPARs have been reported to interact with thyroid hormone receptor (Ref. 45), and more recently, Miyata et al. (46) reported that mPPAR\(\alpha\) interacts with a third member of the nuclear receptor superfamily, the orphan receptor LXR\(\alpha\). Therefore, it appears that there may be phys-

Fig. 4. Ligand-induced mPPAR\(\alpha\) conformational change. A. \(^{35}\)S-PPAR\(\Delta AB\) subjected to DPSA. \(^{35}\)S-PPAR\(\Delta AB\) (~10 fmol) was preincubated for 30 min at room temperature with either vehicle (lanes 1–4), 1 mM clofibrate acid (CFA, lanes 5–7), 1 mM clofibrate (CLO, lanes 8–10), 100 \(\mu\)M LY-171883 (lanes 11–13), 100 \(\mu\)M ETYA (lanes 14–16), or 100 \(\mu\)M WY-14,643 (lanes 17–19) before addition of chymotrypsin (final concentrations of 75, 150, and 300 \(\mu\)g/ml, respectively, in lanes 2–4, 5–7, 8–10, 11–13, 14–16) or water (lane 1). Proteolytic digestions were carried out at room temperature for 20 min, after which time samples were denatured and electrophoresed on a 12.5% SDS-polyacrylamide gel. Gels were processed as described under “Materials and Methods.” B. \(^{35}\)S-PPAR\(\Delta AB/\Delta 448\) subjected to DPSA. Preincubations, electrophoresis, and gel processing were carried out as described in A. Final concentrations of chymotrypsin were 20, 50, and 100 \(\mu\)g/ml, respectively, in lanes 2–4, 5–7, 8–10, 11–13, 14–16. C. \(^{35}\)S-PPAR\(\Delta AB/\Delta 425\) subjected to DPSA. Preincubations, protease concentrations, electrophoresis, and gel processing were carried out as described in B. Arrows throughout the figure indicate positions of proteolytic fragments and migration of Bio-Rad prestained low molecular mass standards. Note that unproteolyzed receptor preparations incubated with vehicle alone (lane 1) were indistinguishable from those incubated with all ligands tested (data not shown). Clofibrate acid and clofibrate are abbreviated as CFA and CLO, respectively.
iologically relevant cross-talk between PPARs and signaling pathways mediated by other nuclear receptors. It will be of interest to determine if other nuclear receptors interact with PPARs through distinct or common heterodimeric protein interfaces and if these protein-protein interactions and/or the functional capacities of the involved receptors are allosterically regulated by DNA binding as previously demonstrated for other nuclear receptors (47, 48).

PPARs, like other receptor proteins within the nuclear receptor superfamily, exhibit a conserved subdivision of receptor regions referred to as A/B, C, D, and E/F (49, reviewed in Refs. 2–5). Experiments conducted with various chimeric receptor proteins composed of putative PPARα ligand binding domains (LBDs) fused to heterologous DNA binding domains from estrogen (7, 50) and glucocorticoid (8) receptors, bacterial tetracycline repressor (14), and GAL4 (44, 51, 52) have demonstrated the requirement for a large portion of the carboxyl terminus of PPARs (D and E/F regions as defined in Ref. 7) for ligand-responsive transcriptional activation.

PPAR activating ligands constitute a chemically diverse group of compounds in which the most obvious common structural elements are an acidic group (free carboxyl group, a metabolically labile derivative thereof, or a bioisostere such as a tetrazole or sulfonamide moiety) and a π electron-rich region (aromatic ring or series of alkenes or alkynes) (53). When considering the structural diversity exhibited by these compounds, it seems possible that the molecular determinants of mPPARα interaction with each ligand or class of ligands may be distinct. Indeed, our results indicate that distinct mPPARα regions are required for responsiveness to different PPAR activators. While PPARAB is responsive to WY-14,643, ETYA, LY-171883, clofibric acid, and clofibrate, as detected by DPSAs, deletion of mPPARα residues 448–468 (PPARAB/D448) severely compromises responsiveness to ETYA but not other PPAR ligands. The distal carboxyl-terminal amino acids of mPPARα that are deleted in PPARAB/D448 correspond to part (H12) of the region that has been proposed to stabilize ligand-receptor interactions with hRARγ by functioning as a “lid” on the ligand binding cavity (38, 39). The greatly reduced efficacy with which ETYA induced PPARAB/D448 conformational change relative to that of PPARAB suggests that the hydrophobicity of putative H12 may play a critical role in the stabilization of ETYA binding, perhaps by stabilizing an extended conformation of this compound. Truncation of mPPARα residues 425–468 (PPARAB/D425) gave rise to a receptor protein which was slightly responsive to WY-14,643 but unresponsive to all other PPAR ligands examined. In addition to deletion of putative helix H12, PPARAB/D425 also lacks putative helices H10 and H11, encompassing a region that has been proposed to form one side of the nuclear receptor ligand binding pocket (39), which may explain the inactivity of this mutant in DPSAs. However, we cannot presently rule out the possibility that the inactivity of PPARAB/D425 is due to improper protein folding and/or detrimental structural distortions outside the deleted region. Nonetheless, it is clear that mPPARα residues 448–468 are important for ligand binding and/or conformational change induced by ETYA while being dispensable for responsiveness to other PPAR ligands support-
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Figure 6. Effect of heterodimerization with RXR on ligand-induced PPAR conformational change. A. 35S-PPARΔAB subjected to DPSA in the presence of vehicle (lanes 2 and 6) or increasing concentrations of 15-deoxy-Δ12,14-prostaglandin J2 (12, 52) has been shown to bind directly to the ligand binding domain of the mouse PPARα. Direct binding of any compounds to PPARα subtypes, however, has not been demonstrated. Issmann et al. (7) specifically report the lack of [3H]nafenopin binding by mPPARα. It has been hypothesized that distinct mPPARα receptor regions may be required for interaction with structurally dissimilar PPAR activators.

Recently, several synthetic antiangiogenic thiazolidinediones (44, 51, 52) and 15-deoxy-Δ12,14-prostaglandin J2 (44, 52) have been shown to bind directly to the ligand binding domain of the mouse PPARα. Direct binding of any compounds to PPARα subtypes, however, has not been demonstrated. Issmann et al. (7) specifically report the lack of [3H]nafenopin binding by mPPARα. mPPARα and its carboxy-terminal truncation mutants were used suggest an alternative, species-specific receptor activity, cell-specific factors, and/or mechanism(s) other than direct binding of this arachidonic acid analog to the receptor.

It has been hypothesized that Hsp72, which has been demonstrated to interact directly with rat PPARα, may bind PPAR activators and, in turn, allosterically activate the associated receptor protein (65). Presently, we cannot exclude this possibility; however, results from DPSAs in which mPPARα carboxy-terminal truncation mutants were used suggest an alternative molecular mechanism. For example, deletion of 21 mPPARα carboxy-terminal amino acids (PPARΔA/Δ448) compromises the responsiveness of the receptor to ETYA but not other PPAR ligands. If Hsp72 binds these ligands directly and allosterically transduces a signal that alters the conformation of mPPARα, it seems unlikely that this process would be attenuated by carboxy-terminal truncation of mPPARα unless the deleted amino acids are required for mPPARα-Hsp72 interaction. In such a case, one would expect this truncation to abolish responsiveness to all ligands. Of course, it is possible that some ligands selectively interact with mPPARα, Hsp72, or both proteins in the context of an mPPARα-Hsp72 complex. The latter possibility would be reminiscent of eddysonic receptor in which eddysonic binding activity is associated with a complex of eddysonic receptor and ultraspireacle (61). In any event, ligand-induced conformational change likely underlies the molecular basis of ligand activation of the putative mPPARα transcription activation function, AF-2, and identification of ligand-induced mPPARα proteolytic fragments will be of critical importance to our understanding of the dynamic process of PPAR activation by ligands and interaction of liganded receptor with putative transcriptional intermediary factors.

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Note Added in Proof—Consistent with some of the data presented herein, Devchand et al. recently published results demonstrating direct binding of [3H]leukotriene B4 to a bacterially expressed and purified, GST-Xenopus PPARα fusion protein and inhibition of this binding by unlabeled 15-deoxy-Δ12,14-prostaglandin J2 (44, 52). An excellent model for the interaction of PPARα with a ligand is generally consistent with previously reported transcriptional activation studies utilizing mPPARα: 15-deoxy-Δ12,14-prostaglandin J2 (44, 52) was a sub-stantially weaker ligand than 15-deoxy-Δ12,14-prostaglandin J2 (44, 52) in the in vitro studies employing PPARΔAB described herein (Fig. 5). Hsu and co-workers (11) also reported that ETYA was approximately 10-fold weaker than 15-deoxy-Δ12,14-prostaglandin J2 (44, 52) as an activator of mPPARα in transient transfection experiments, suggesting that the enhanced potency of ETYA reported by Keller and co-workers (11) may be conferred by species-specific receptor activity, cell-specific factors, and/or mechanism(s) other than direct binding of this arachidonic acid analog to the receptor.

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