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Biochemistry

Fatty acids and retinoids control lipid metabolism through activation of peroxisome proliferator-activated receptor–retinoid X receptor heterodimers

(peroxisome proliferator-activated receptor response element/retinoid X receptor response element/acyl-CoA oxidase gene/nuclear hormone receptors)

HANSJÖRG KELLER*, CHRISTINE DREYER†, JEFFREY MEDIN‡, ABDERRAHIM MAHFOUDI*, KEIKO OZATO‡, AND WALTER WAHLI*§

*Institut de Biologie animale, Université de Lausanne, Bâtiment de Biologie, CH-1015 Lausanne, Switzerland; †Max-Planck-Institut für Entwicklungsbiologie, D-7400 Tübingen, Germany; and ‡Laboratory of Molecular Growth Regulation, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892

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ABSTRACT **The nuclear hormone receptors called PPARs (peroxisome proliferator-activated receptors α, β, and γ) regulate the peroxisomal β-oxidation of fatty acids by induction of the acyl-CoA oxidase gene that encodes the rate-limiting enzyme of the pathway. Gel retardation and cotransfection assays revealed that PPARα heterodimerizes with retinoid X receptor β (RXRβ; RXR is the receptor for 9-*cis*-retinoic acid) and that the two receptors cooperate for the activation of the acyl-CoA oxidase gene promoter. The strongest stimulation of this promoter was obtained when both receptors were exposed simultaneously to their cognate activators. Furthermore, we show that natural fatty acids, and especially polyunsaturated fatty acids, activate PPARs as potently as does the hypolipidemic drug Wy 14,643, the most effective activator known so far. Moreover, we discovered that the synthetic arachidonic acid analogue 5,8,11,14-eicosatetraynoic acid is 100 times more effective than Wy 14,643 in the activation of PPARα. In conclusion, our data demonstrate a convergence of the PPAR and RXR signaling pathways in the regulation of the peroxisomal β-oxidation of fatty acids by fatty acids and retinoids.**

Peroxisome proliferator-activated receptors (PPAR) are nuclear hormone receptors activated by substances including fibrate hypolipidemic drugs, phthalate ester plasticizers, and herbicides that cause peroxisome proliferation in the liver (1, 2). So far three PPAR receptors (α, β, and γ) have been described in *Xenopus* (2), one in mouse (1), and one in rat (3). These receptors are transcription factors that control the peroxisomal β-oxidation pathway of fatty acids through regulation of the acyl-CoA oxidase gene that encodes the rate-limiting enzyme of the pathway (2, 4). Thus, PPARs play an important role in lipid metabolism.

Structural analysis of the PPARs revealed that they belong to the nuclear hormone receptor subgroup, which comprises receptors for all-*trans*-retinoic acid (RAR), 9-*cis*-retinoic acid (retinoid X receptor; RXR), thyroid hormone, vitamin D, and several orphan receptors. All of these receptors recognize the canonical DNA response sequence AGGTCA and accordingly possess the same P-box amino acid sequence in the first zinc finger of their DNA-binding domain (5). We and others have identified a PRAR response element (PPRE) in the acyl-CoA oxidase promoter (2, 4). This response element contains a direct repeat of the AGGTCA motif with one intervening nucleotide, which is called DR-1. Interestingly, the RXR response element (RXRE) in the promoter of the cellular retinol-binding protein type II gene contains also DR-1 elements (6). Thus, the convergence of the PPAR and RXR signaling pathways in the transcriptional regulation of the acyl-CoA oxidase gene was an interesting hypothesis to test. Further indications of a coupling between PPARs and RXRs came from the recently demonstrated induction of the acyl-CoA oxidase gene by retinoic acid in cultured rat hepatocytes (7) and the observation of heterodimerization of RXR with other members of the nuclear hormone receptor superfamily (8–15).

β-Oxidation of long-chain fatty acids is an essential process in lipid metabolism. Its disruption, which occurs in disorders such as Zellweger syndrome and adrenoleukodystrophy (16), leads to a lethal accumulation of very long-chain fatty acids in the blood. To further our understanding of the hormonal control of the peroxisomal β-oxidation by PPARs and possibly by RXRs, we searched for physiologically occurring activators of PPARα. Fatty acids were possible candidates for this role, since high-fat diets have been reported to stimulate β-oxidation (17). In this paper, we show that PPARα and RXRβ heterodimerize and that they cooperatively stimulate the acyl-CoA oxidase gene promoter. Furthermore, we show that physiological concentrations of fatty acids, and especially polyunsaturated fatty acids (PUFA), activate *Xenopus laevis* PPARα (xPPARα) to the same extent as the xenobiotic peroxisome proliferator Wy 14,643. Finally, the synthetic arachidonic acid (AA) analogue 5,8,11,14-eicosatetraynoic acid (ETYA) was found to fully activate xPPARα at a concentration 1/100th that of Wy 14,643.

MATERIALS AND METHODS

Immunoprecipitations. Nuclear extracts containing baculovirus recombinant mouse RXRβ (mRXRβ) were prepared from infected Sf9 cells as described (18), except for the additional inclusion of protease inhibitors. *In vitro* translated and ^{35}S-labeled xPPARα was combined with 1 μg of mRXRβ or control baculovirus extract and anti-mRXRβ antiserum (19) in 100 μl of buffer A [20 mM Hepes, pH 7.9/50 mM NaCl/1 mM EDTA/5% (vol/vol) glycerol/0.05% Triton X-100] and allowed to associate overnight at 4°C. Samples were then added to prewashed protein A-agarose beads (Boehringer Mannheim) and incubated at 4°C for 2 hr with rocking. The beads were collected by centrifugation and

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Abbreviations: AA, arachidonic acid; ETYA, 5,8,11,14-eicosatetraynoic acid; NDGA, nordihydroguaiaretic acid; PPAR, peroxisome proliferator-activated receptor; PPRE, PPAR response element; PUFA, polyunsaturated fatty acid(s); RAR, all-*trans*-retinoic acid receptor; RXR, retinoid X receptor (for 9-*cis*-retinoic acid); RXRE, RXR response element; mRXRβ, mouse RXRβ; xPPARα, *Xenopus laevis* PPARα; CAT, chloramphenicol acetyltransferase.

§To whom reprint requests should be addressed.

washed twice with buffer A containing 0.05% bovine serum albumin followed by a single washing in buffer A alone. The beads were collected and boiled in 50 μl of SDS loading buffer for 2 min and pelleted by centrifugation for 4 min, and the resulting supernatant was analyzed by SDS/PAGE.

Gel Retardation Assays. Five microliters of *in vitro* translated xPPARα and 2 μl of nuclear extract containing baculovirus-expressed recombinant mRXRβ (see above) or mock controls were incubated on ice for 15 min in buffer containing 10 mM Tris·HCl (pH 8.0), 40 mM KCl, 0.05% (vol/vol) Nonidet P-40, 5% glycerol, 1 mM dithiothreitol, and 0.1 μg of poly(dI·dC) (Pharmacia). For competition experiments, 40 ng of ACO-A or ACO-B double-stranded oligonucleotides (2) were also included during preincubation. [ACO is a reporter plasmid containing the 5′ flanking region of the acyl-CoA oxidase gene in front of the chloramphenicol acetyltransferase (CAT) gene; ACO-A and ACO-B are synthetic oligonucleotides with sequences of the two enhancer regions of the acyl-CoA oxidase gene promoter.] Then, 1 μl of ACO-A double-stranded oligonucleotide (1 ng/μl), labeled with ^{32}P by fill-in with Klenow polymerase, was added, and the incubation was continued for 10 min at room temperature before samples were electrophoresed on a 5% polyacrylamide gel in 0.5× TBE buffer (45 mM Tris/45 mM boric acid/1 mM EDTA) at 4°C. For antibody supershift assays, anti-xPPARα (unpublished data) or preimmune serum as a control was added to the samples after the incubation with the ACO-A probe, and incubation was continued for another 10 min at room temperature followed by gel electrophoresis on a 3.5% polyacrylamide gel.

Transfections. The xPPARα expression vector and the reporter plasmids ACO-A.TK.CAT, ACO-G.CAT, and G.CAT, as well as the mRXRβ expression vector pRSV-H-2RIIBP have been described (2, 20); G refers to the β-globin gene promoter and TK refers to the thymidine kinase gene promoter. Cotransfections of CV-1 cells were performed as described for HeLa cells (2), except for the application of a glycerol shock (15%) for 2 min before the activators were added. As an internal control, a luciferase expression plasmid (21), which does not respond to the activators used, was also cotransfected, and luciferase activities were used to normalize CAT activities. Charcoal-treated serum, which was depleted of fatty acids (22), was used in transfection experiments. Activators were added to the cell culture medium either in ethanol solutions in the case of free acids or in 10% ethanol/0.2% $NaHCO_3$ in the case of sodium salts. Final ethanol concentrations in the cell culture medium were ≤0.1% to avoid negative effects on the cells. All fatty acids used were from Sigma and were stored at −20°C under argon. 9-*cis*-Retinoic acid was obtained from M. Klaus at Hoffmann–La Roche (Basel).

RESULTS

Heterodimerization of PPARα with RXRβ. Using a coimmunoprecipitation assay, we observed that ^{35}S-labeled PPARα, when mixed with unlabeled RXRβ, is specifically precipitated by anti-mRXRβ serum (Fig. 1, lane 2). No coimmunoprecipitation of PPARα was observed with preimmune serum in the presence of RXRβ (lane 3) or with anti-RXRβ serum in the absence of RXRβ (lane 4), confirming the specificity of the interaction between xPPARα and mRXRβ. In agreement with this result, chemical crosslinking experiments also revealed specific heterodimer formation between xPPARα and mRXRβ (data not shown), demonstrating that xPPARα binds to mRXRβ in solution.

With this evidence for an association between xPPARα and mRXRβ in solution, we next performed gel mobility-shift experiments to analyze whether xPPARα–mRXRβ heterodimers bind to the recently identified PPRE of the acyl-CoA oxidase gene (2, 4). mRXRβ and xPPARα alone did not bind significantly to the PPRE within the ACO-A probe (Fig. 2, lanes 3 and 4). However, incubation of the ACO-A probe with a mixture of PPARα and RXRβ resulted in a prominent complex (lane 5). The specificity of this complex was demonstrated by competition with a 40-fold excess of unlabeled ACO-A oligonucleotide (lane 6), whereas a 40-fold excess of ACO-B oligonucleotide, which does not contain a PPRE (2), did not lead to disappearance of the complex (lane 7). The

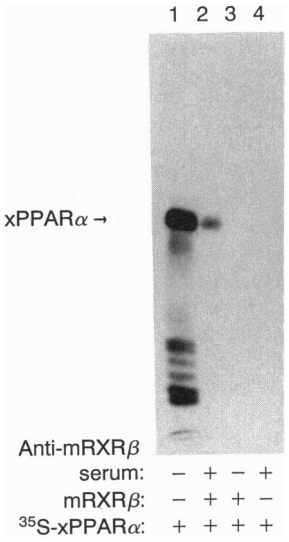

FIG. 1. Formation of PPARα–RXRβ heterodimers: coimmunoprecipitation. An equal amount of *in vitro* translated and ^{35}S-labeled xPPARα (^{35}S-xPPARα) as shown in lane 1, was incubated with baculovirus-expressed mRXRβ (lanes 2 and 3) or control nuclear extract (lane 4) and was subjected to coimmunoprecipitation with anti-mRXRβ serum (lanes 2 and 4) or preimmune serum (lane 3) followed by SDS/PAGE analysis of the precipitated material.

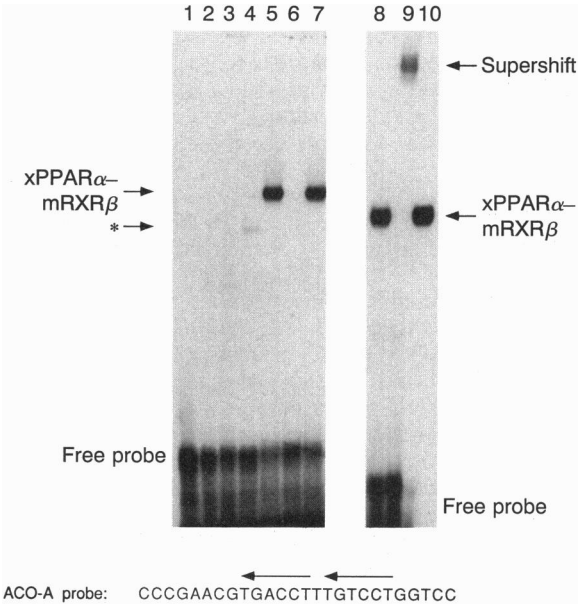

FIG. 2. Formation of PPARα–RXRβ heterodimers: gel retardation assay. *In vitro* synthesized xPPARα and baculovirus-expressed mRXRβ or mock controls were incubated in the presence of the PPRE-containing ^{32}P-labeled ACO-A probe, and protein–DNA complexes were analyzed by electrophoresis on a 5% polyacrylamide gel. In the case of antibody-induced supershifts, samples were analyzed by electrophoresis on a 3.5% polyacrylamide gel. Lanes: 1, free probe; 2, mock baculovirus wild-type nuclear extract (bac-WT) and mock reticulocyte lysate (RL); 3, baculovirus-expressed mRXRβ and RL; 4, bac-WT and *in vitro* translated xPPARα; 5, baculovirus-expressed mRXRβ and *in vitro* translated xPPARα; 6, competition of the mRXRβ–xPPARα complex as in lane 5 with a 40-fold excess of unlabeled ACO-A oligonucleotide; 7, same as lane 6, but competition with ACO-B oligonucleotide which does not contain a PPRE; 8–10, antibody supershift assay of the mRXRβ–xPPARα complex: mRXRβ–xPPARα complex as in lane 5 (lane 8), supershift of the mRXRβ–xPPARα complex with anti-xPPARα (lane 9), but not with preimmune serum (lane 10).

nature of the minor band indicated by an asterisk in lane 4 is unknown. It could represent weak binding of xPPARα to the probe as a monomer, a homodimer, or a heterodimer with an insect cell nuclear protein such as Usp, the homologue of RXR. The presence of PPARα in the PPARα–RXRβ complex was confirmed by a specific anti-xPPARα-induced supershift (lane 9), whereas preimmune serum had no effect (lane 10). In agreement with the fact that nuclear hormone receptors bind as dimers to response elements consisting of two half sites (5), we conclude that PPARα and RXRβ heterodimerize in solution and bind synergistically as heterodimers to the PPRE.

This observation and the similarity of the PPRE and the RXRE of the cellular retinol-binding protein type II gene promoter (8) led us to examine whether there is a functional interaction of PPARα and RXRβ in transcriptional activation of the PPRE-containing ACO-G.CAT reporter gene. The ACO-G.CAT plasmid contains the acyl-CoA oxidase gene promoter sequence from −471 to −1273 in front of the rabbit β-globin basal promoter-controlled CAT gene. The PPRE is located between −578 and −553 within this promoter region (2, 4). PPARα and RXRβ expression plasmids, either alone or combined, were cotransfected with the ACO-G.CAT reporter plasmid into CV-1 cells, and CAT assays were performed after induction in the presence or absence of the specific activator for each receptor (100 μM Wy 14,643 for PPARα and 1 μM 9-*cis*-retinoic acid for RXRβ). The highest stimulation of the ACO-G.CAT reporter plasmid was observed by cotransfection of the PPARα and RXRβ expression plasmids in the presence of the two activators, indicating that optimal cooperation between both signaling pathways depends on the simultaneous activation of both receptors (Fig. 3). Compared with the effect of the two individual receptors, the combined effect of both receptors on transcriptional induction was additive. It is noteworthy that induction by PPARα transfected alone occurred also with the RXR ligand 9-*cis*-retinoic acid, and conversely, stimulation by RXRβ, albeit weaker, was observed with Wy 14,643.

These results are compatible with the involvement of endogenous CV-1 cell RXR and PPAR activities through heterodimerization with the introduced PPARα and RXRβ receptors, respectively. The presence of a low level of endogenous receptors in these cells was further supported by a 2-fold receptor-independent, but activator-dependent, stimulation of ACO.G-CAT, but not of G-CAT, as observed previously in HeLa cells (2). Furthermore, expression of the two receptors alone or in combination in the absence of inducers led to an increase in activity, indicating a low level of constitutive receptor activity or the presence of a weak unidentified endogenous activator. Effects similar to those seen with the ACO.G-CAT reporter gene were observed with the ACO-A.TK.CAT reporter gene containing one copy of the PPRE in front of the thymidine kinase gene promoter (2), indicating that the functional interaction between PPARα and RXRβ does indeed occur through the PPRE (data not shown). Taken together, these results show that the PPAR and RXR signaling pathways converge in the regulation of the acyl-CoA oxidase promoter.

Fatty Acids Activate PPARα. In our search for endogenous activators of xPPARα, two reasons prompted us to test whether fatty acids activate PPARα: (*i*) known potential ligands of PPAR, such as fibrate hypolipidemic drugs, present an amphipathic structure similar to fatty acids—e.g., having a free carboxyl group and a lipophilic moiety—and (*ii*) high dietary fat intake and certain fatty acid analogues induce the peroxisomal β-oxidation of fatty acids (23, 24). For these experiments, HeLa cells were cotransfected with the xPPARα expression plasmid and the ACO-A.TK.CAT reporter plasmid. Subsequently, various fatty acids were added to the culture medium to a final concentration of 50 μM, and CAT activities were determined. All of the PUFAs tested activated

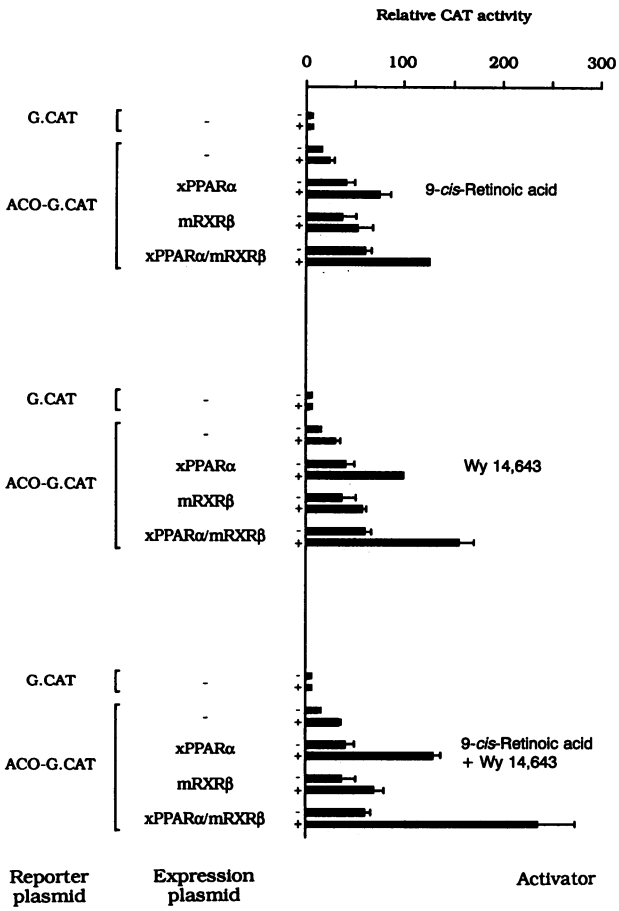

FIG. 3. Transcriptional activation of the acyl-CoA gene promoter by xPPARα and mRXRβ. CV-1 cells were cotransfected with the expression vectors for xPPARα and mRXRβ and the reporter plasmids ACO-G.CAT and G.CAT as indicated. ACO-G.CAT contains the acyl-CoA oxidase gene promoter from −471 to −1273 in front of the rabbit β-globin basal promoter-driven CAT gene, whereas G.CAT, which is used as control, is the same construct without the acyl-CoA oxidase gene promoter sequences (2, 4). After treatment with the indicated activators (+)—Wy 14,643 (100 μM) or 9-*cis*-retinoic acid (1 μM) or both—or with solvent (ethanol) as a control (−), CAT assays were performed, and the results were normalized arbitrarily to the activity observed by PPARα in the presence of 100 μM Wy 14,643, which was taken as 100%. The mean values of three independent experiments with the corresponding standard deviations are shown.

PPARα by 4- to 8-fold (Fig. 4)—i.e., to the same extent as Wy 14,643, which is the most potent activator known so far (1, 2). No significant difference in the activation of xPPARα was observed between the ω-6 fatty acids (AA and linoleic acid) and ω-3 fatty acids (docosahexaenoic, eicosapentaenoic, and linolenic acids), which represent the two classes of essential PUFAs (25). In contrast, the monounsaturated fatty acids tested displayed a wide range of effectiveness in the activation of PPARα. Whereas petroselinic acid activated PPARα with a similar efficiency as PUFAs, oleic acid and elaidic acid were less potent, and the very long-chain fatty acids erucic acid and nervonic acid did not activate PPARα. Since most of the naturally occurring fatty acids have double bonds in the cis configuration, it is interesting that elaidic acid, which has a trans double bond, activated PPARα to the same level (about 2.5-fold) as did its natural cis homologue oleic acid. Moreover, the saturated fatty acid lauric acid activated PPARα only weakly, and the dicarboxylic fatty acid dodecanedioic acid did not activate PPARα. As a control, triiodothyroacetic acid, which is also an amphipathic molecule but not a fatty acid and

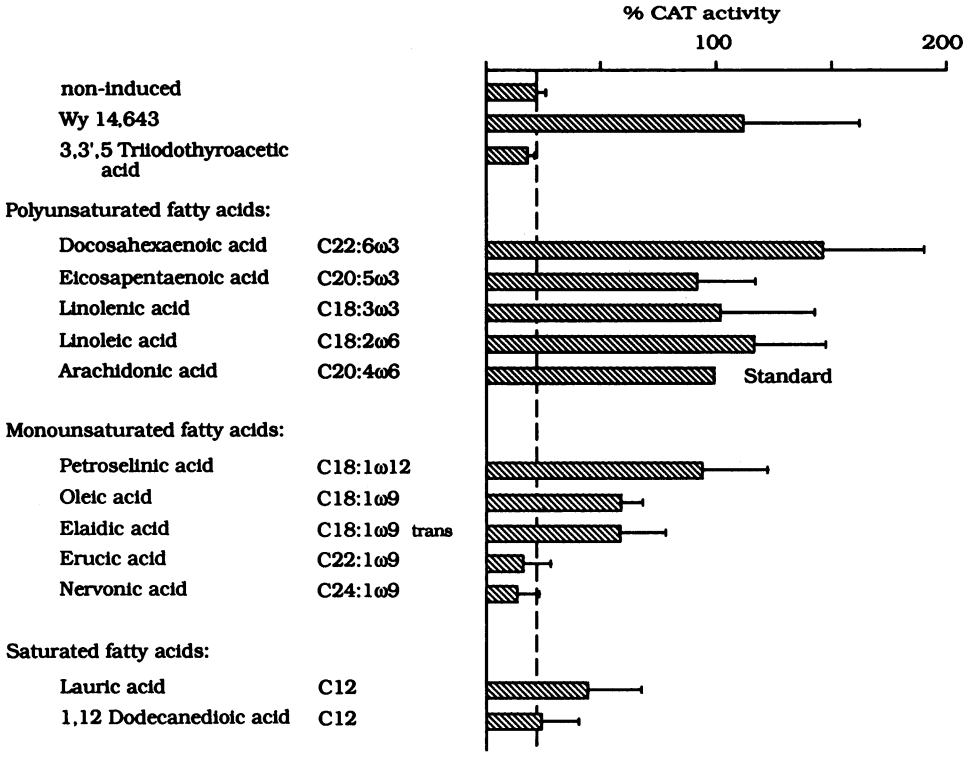

FIG. 4. Activation of xPPARα by fatty acids. The xPPARα expression vector and ACO-A.TK.CAT reporter plasmid were cotransfected into HeLa cells. Subsequently, activators or solvent as a control was added to the cell culture medium, and CAT activity was assayed; 100% CAT activity was taken arbitrarily as the CAT activity observed with 50 μM AA (all additives were at 50 μM). Basal level of CAT activity is indicated by the dashed line. Experiments were done at least in triplicate, and the mean values with the corresponding standard deviations are shown. Fatty acids are listed by their trivial name, and their structure is indicated by the ω nomenclature, which shows from left to right the number of carbon atoms, the number of double bonds, and the location of the first double bond counting from the ω (end) carbon of the carbohydrate chain (25). Most naturally occurring fatty acids have double bonds in the cis configuration. Thus, the only exception, elaidic acid, is labeled "trans."

which activates the thyroid hormone receptor, also did not activate PPARα. Furthermore, activation of the ACO-A.TK.CAT reporter plasmid by fatty acids was dependent upon the presence of PPARα and a reporter plasmid without the PPRE was not induced by fatty acid-activated PPARs (data not shown). The activator-independent transcriptional activity of xPPARα (see above) was not due to residual fatty acids possibly present in the cell culture medium, since this activity was also observed in the absence of 10% fetal calf serum in the culture medium.

ETYA, a Synthetic AA Analogue, Is a 100-fold More Potent Activator of xPPARα Than AA or Wy 14,643. Since AA is the precursor for the synthesis of eicosanoids such as prostaglandins, thromboxanes, lipoxins, and leukotrienes, which are implicated in various cell-specific signaling events, we tested whether the activation of xPPARα by AA was due to AA itself or to an AA metabolite. Three pathways are involved in the production of the eicosanoids mentioned above, the cyclooxygenase, lipoxygenase, and epoxygenase pathway (26). Commonly used specific blockers of these pathways are aspirin and indomethacin for the cyclooxygenase pathway, nordihydroguaiaretic acid (NDGA) for the lipoxygenase pathway, and metyrapone for the epoxygenase pathway (27). Activation of xPPARα by 10 μM AA in transfection experiments (data not shown) was not blocked or significantly inhibited by 100 μM aspirin, 10 μM indomethacin, 10 μM NDGA, or 10 μM metyrapone. Consistently, the prostaglandins PGD_2, PGE_2, and $PGF_{2\alpha}$ (10 μM each), and the hydroperoxyeicosatetraenoic acids (HPETE) 5-, 8-, 12- and 15-HPETE (0.6 μM each) did not activate xPPARα (data not shown). Surprisingly, ETYA, a blocker of lipoxygenases and cyclooxygenases, fully activated xPPARα at a concentration of only 1 μM, and the dose–response curve revealed an ED_{50} of 200 nM, which is lower by a factor of about 100 than those for Wy 14,643 and AA (Fig. 5).

DISCUSSION

Interaction of PPARα and RXRβ Signaling Pathways. Transfection experiments with xPPARα and RXRβ demonstrated that the receptors cooperatively activate the acyl-CoA oxidase promoter through the PPRE. This is consistent with the observation that retinoic acid (9), most likely by isomerization to 9-*cis*-retinoic acid (28), and fatty acids induce the acyl-CoA oxidase gene *in vivo*. While it is known that 9-*cis*-retinoic acid binds to RXR and thereby converts it into an active transcription factor, we do not know whether fatty acids work in a similar way and bind directly to PPARs. Our transfection experiments indicate that the strongest activation of the acyl-CoA oxidase gene requires PPARα and RXRβ in the activated state. However, a slight cooperative stimulation was also observed in the absence of activators. Although *in vitro* gel retardation assays indicated a synergistic binding of PPARα–RXRβ heterodimers to the PPRE, it is not clear from the transfection experiments whether there is also a preferential or even exclusive formation of PPARα–RXRβ heterodimers on the PPRE *in vivo* because the transcriptional activation observed by PPARα and RXRβ is additive. Furthermore, the fact that the acyl-CoA oxidase

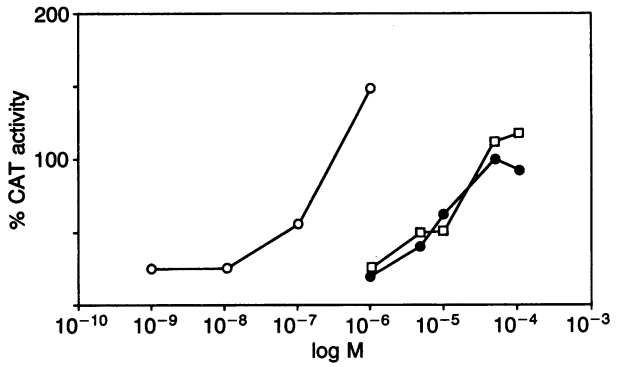

FIG. 5. Activation of xPPARα: ETYA (○), AA (●), and Wy 14,643 (□) dose–response curves. Activation of xPPARα by increasing concentrations of ETYA, AA, or Wy 14,643 was assayed in cotransfection experiments as described in Fig. 4. Higher concentrations of activators than those shown were cytotoxic or led to complete detachment of the cells. Mean values of at least three independent experiments are shown.

gene promoter is activated by transiently expressed PPARα and RXRβ alone may suggest that homodimers are also transcriptionally active. However, since endogenous PPAR and RXR are present in CV-1 cells, as is indicated by the low level of activation of the acyl-CoA oxidase gene promoter in the absence of transfected receptors and by the apparently ubiquitous expression of the two receptors (2, 30), we believe that heterodimers are in fact responsible for the transcriptional activation observed. Ultimately, transfection experiments with dominant negative PPAR and RXR mutants that still form heterodimers but do not stimulate transcription or transfection experiments with cells deficient in endogenous PPAR and RXR are needed to answer the question of which PPAR–RXR species are functionally relevant *in vivo*.

Regulation of the Peroxisomal β-Oxidation by Fatty Acids. Based on structural and functional considerations, we have tested several natural fatty acids for activation of PPARα. Stimulation of xPPARα was strongest in the presence of PUFAs, followed by monounsaturated fatty acids and saturated fatty acids. Similar observations have also been made with a chimeric receptor containing the transactivation and DNA-binding domains of the glucocorticoid receptor and the ligand-binding domain of the rat PPAR (3). However, in contrast to these results, we found that PUFAs activated the genuine xPPARα receptor as efficiently as the most potent peroxisome proliferator, Wy 14,643. This may be due to differences between the full-length and the artificial chimeric receptors, to the different transfection assay systems applied, or to species differences. Interestingly, the very long-chain monounsaturated fatty acids nervonic acid and erucic acid, which exert a negative effect on the peroxisomal β-oxidation system (31), did not activate xPPARα. Dietary fatty acids occur in a great variety as saturated and unsaturated fatty acids. In contrast to the saturated and monounsaturated fatty acids, PUFAs are absolutely necessary for the growth and health of animals and humans. According to their origin from linolenic or linoleic acid, PUFAs are classified into ω-3 and ω-6 PUFAs as defined by the location of the first double bond from the end of the terminal methyl group of the carbohydrate chain (25). Great interest in ω-3 and ω-6 PUFAs has recently arisen because of their beneficial role in the prevention of atherosclerosis due to their effect on lowering triglyceride and cholesterol plasma concentrations (32, 33). We show now that ω-3 and ω-6 PUFAs are potent activators of xPPARα and, thus, the degradation of fatty acids via peroxisomal β-oxidation. This represents a positive feedback regulation and may explain the hypolipidemic effect of PUFAs at the molecular level.

ETYA is a structural analogue of AA in which four alkyne bonds replace the four alkene bonds present in AA. ETYA has been synthesized as a candidate hypocholesterolemic drug, and, indeed, inhibition of cholesterol biosynthesis and reduction of serum cholesterol concentration has been observed. However, ETYA has not been introduced as hypocholesteremic drug because of side effects (29). We show now that ETYA is a potent activator of xPPARα and, based on dose–response curves, that it is 100 times more effective than Wy 14,643 or AA. Comparison of the ED_{50} of ETYA with the ED_{50}s of retinoids activating transiently expressed RAR or RXR in CV-1 cells (28) suggests the possibility that ETYA may be a high-affinity ligand of xPPARα. Alternatively, ETYA could induce the formation or release of endogenous ligands because of its high metabolic stability. Indeed, metabolic studies of ETYA in rats revealed only partial ω- and β-oxidation of this compound, and all of the triple bonds in the molecule remained intact (29). Along the same line, ETYA blocks several AA-metabolizing enzymes such as lipoxygenase and cycloxygenase by acting as a false substrate (27), and it has been reported that 1 μM ETYA led to total inhibition of prostaglandin release from isolated perfused rabbit heart (29). Ultimately, binding studies will be required to determine whether ETYA is a high-affinity ligand of xPPARα. In conclusion, regulation of the expression of genes involved in lipid metabolism by nutrients such as PUFAs is of great physiological and clinical importance, and it will require the identification of further PPAR activators and target genes to elucidate the complete role of PPARs in the hormonal control of lipid metabolism.

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1. Issemann, I. & Green, S. (1990) *Nature (London)* **347**, 645–650.
2. Dreyer, C., Krey, G., Keller, H., Givel, F., Helftenbein, G. & Wahli, W. (1992) *Cell* **68**, 879–887.
3. Göttlicher, M., Widmark, E., Li, Q. & Gustafsson, J.-A. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 4653–4657.
4. Tugwood, J. D., Issemann, I., Anderson, R. G., Bundell, K. R., McPheat, W. L. & Green, S. (1992) *EMBO J.* **11**, 433–439.
5. Wahli, W. & Martinez, E. (1991) *FASEB J.* **5**, 2243–2249.
6. Mangelsdorf, D. J., Umesono, K., Kliewer, S. A., Borgmeyer, U., Ong, E. S. & Evans, R. M. (1991) *Cell* **66**, 555–561.
7. Hertz, R. & Bar-Tana, J. (1992) *Biochem. J.* **281**, 41–43.
8. Yu, V. C., Delsert, C., Andersen, B., Holloway, J. M., Devary, O. V., Naar, A. M., Kim, S. Y., Boutin, J. M., Glass, C. K. & Rosenfeld, M. G. (1991) *Cell* **67**, 1251–1266.
9. Zhang, X. K., Hoffmann, B., Tran, P. B. V., Graupner, G. & Pfahl, M. (1992) *Nature (London)* **355**, 441–446.
10. Marks, M. S., Hallenbeck, P. L., Nagata, T., Segars, J. H., Appella, E., Nikodem, V. M. & Ozato, K. (1992) *EMBO J.* **11**, 1419–1435.
11. Kliewer, S. A., Umesono, K., Mangelsdorf, D. J. & Evans, R. M. (1992) *Nature (London)* **355**, 446–449.
12. Leid, M., Kastner, P., Lyons, R., Nakshatri, H., Saunders, M., Zacharewski, T., Chen, J.-Y., Staub, A., Garnier, J.-M., Mader, S. & Chambon, P. (1992) *Cell* **68**, 377–395.
13. Bugge, T. H., Pohl, J., Lonnoy, O. & Stunnenberg, H. G. (1992) *EMBO J.* **11**, 1409–1418.
14. Widom, R. L., Rhee, M. & Karathanasis, S. K. (1992) *Mol. Cell. Biol.* **12**, 3380–3389.
15. Kliewer, S. A., Umesono, K., Mangelsdorf, D. J., Dyck, J. A. & Evans, R. M. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 1448–1452.
16. Goldfischer, S. & Reddy, J. K. (1984) *Int. Rev. Exp. Pathol.* **26**, 45–84.
17. Osmundsen, H., Bremer, J. & Pedersen, J. I. (1991) *Biochim. Biophys. Acta* **1085**, 141–158.
18. Schreiber, E., Matthias, P., Müller, M. M. & Schaffner, W. (1989) *Nucleic Acids Res.* **17**, 6419.
19. Marks, M. S., Levi, B.-Z., Segars, J. H., Driggers, P. H., Hirschfeld, S., Nagata, T., Appella, E. & Ozato, K. (1992) *Mol. Endocrinol.* **6**, 219–230.
20. Nagata, T., Segars, J. H., Levi, B.-Z. & Ozato, K. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 937–941.
21. DeWet, J. R., Wood, K. V., DeLuca, M., Helinski, D. R. & Subramani, S. (1987) *Mol. Cell. Biol.* **7**, 725–737.
22. Chen, F. R. (1967) *J. Biol. Chem.* **242**, 173–181.
23. Berge, R. K., Aarsland, A., Kryvi, H., Bremer, J. & Aarsaether, N. (1989) *Biochim. Biophys. Acta* **1004**, 345–356.
24. Lock, E. A., Mitchell, A. M. & Elcombe, C. R. (1989) *Annu. Rev. Pharmacol. Toxicol.* **29**, 145–163.
25. Johnston, P. V. (1991) in *Encyclopedia of Human Biology*, ed. Dulbecco, R. (Academic, San Diego), Vol. 3, pp. 567–580.
26. Smith, W. L. (1989) *Biochem. J.* **259**, 315–324.
27. Rainsford, K. D. (1988) in *Prostaglandins: Biology and Chemistry of Prostaglandins and Related Eicosanoids*, ed. Curtis-Prior, P. B. (Churchill Livingstone, London), pp. 52–68.
28. Heyman, R. A., Mangelsdorf, D. J., Dyck, J. A., Stein, R. B., Eichele, G., Evans, R. M. & Thaller, C. (1992) *Cell* **68**, 397–406.
29. Tobias, L. D. & Hamilton, J. G. (1979) *Lipids* **14**, 181–193.
30. Mangelsdorf, D. J., Borgmeyer, U., Heyman, R. A., Zhou, J. Y., Ong, E. S., Oro, A. E., Kakizuka, A. & Evans, R. M. (1992) *Genes Dev.* **6**, 329–344.
31. Flatmark, T., Christiansen, E. N. & Kryvi, H. (1983) *Biochim. Biophys. Acta* **753**, 460–466.
32. Hayes, K. C. & Khosla, P. (1992) *FASEB J.* **6**, 2600–2607.
33. Nestel, P. J. (1990) *Annu. Rev. Nutr.* **10**, 149–167.