Conjugated linoleic acid is a potent naturally occurring ligand and activator of PPARα

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Abstract We have previously shown that a mixture of dietary conjugated derivatives of linoleic acid (conjugated linoleic acid, CLA) induces peroxisome proliferator-responsive enzymes and modulates hepatic lipid metabolism in vivo. The present studies demonstrate that CLA is a high affinity ligand and activator of peroxisome proliferator-activated receptor α (PPARα) and induces accumulation of PPAR-responsive mRNAs in a rat hepatoma cell line. Using a scintillation proximity assay (SPA), CLA isomers were shown to be ligands for human PPARα with a rank order of potency of (9Z,11E)>(10E,12Z)>(9E,11E)> furan-CLA (IC_{50} values from 140 nM to 400 nM). Levels of acyl-CoA oxidase (ACO), liver fatty acid-binding protein (L-FABP), and cytochrome P450IVA1 (CYP4A1) mRNA were induced by CLA in FaO hepatoma cells. Even though linoleate and CLA were incorporated into lipids of hepatoma cells to the same extent, linoleate had little or no effect on ACO, CYP4A1, or L-FABP mRNA. In agreement with its binding potency, (9Z,11E)-CLA was the most efficacious PPARα activator in the mouse PPARα-GAL4(UAS)$_5$-CAT reporter system. These data indicate that CLA is a ligand and activator of PPARα and its effects on lipid metabolism may be attributed to transcriptional events associated with this nuclear receptor. Also, (9Z,11E)-CLA is one of the most avid fatty acids yet described as a PPARα ligand.—Moya-Camarena, S. Y., J. P. Vanden Heuvel, S. G. Blanchard, L. A. Leesnitzer, and M. A. Belury. **Conjugated linoleic acid is a potent naturally occurring ligand and activator of PPARα. *J. Lipid Res.* 1999.** 40: **1426–1433.**

Supplementary key words ligand affinity • gene expression • lipid metabolism

Conjugated linoleic acid (CLA) refers to a mixture of octadecadienoate derivatives which have a conjugated double-bound system (8–10, 9–11, 10–12, or 11–13 in Z and/or E configurations) (1). CLA is synthesized in vivo (by rumen bacteria) from linoleic acid through oxidative pathways and enzymatic isomerization (2). Recently, it has been reported that CLA may be formed by conversion from dietary *trans*-fatty acids in humans (3). In foods, CLA is found in ruminant meats and dairy products where its content ranges from 2.5 to 15.0 mg/g lipid (1, 4). CLA has unique properties such as anti-cancer (5–8), anti-atherogenic (9), and anti-diabetogenic effects in rodent models (10). Several hypotheses to explain the pleiotropic effects of CLA have been proposed but the mechanisms of action of CLA are not fully understood. Previous studies in our laboratory have shown that dietary CLA decreased the incidence of skin tumors in female SENCAR mice but also caused lower body weight and increased hepatic lipid accumulation when compared to mice fed non-CLA containing diets (7, 11). These observations suggested that CLA plays a significant role in the modulation of lipid metabolism that may explain its anti-carcinogenic, anti-atherogenic, and anti-diabetogenic properties.

The ligand-activated transcription factor, peroxisome proliferator-activated receptor (PPAR), modulates lipid metabolism. Specific PPAR responsive elements have been identified in the regulatory regions of genes encoding lipid metabolizing enzymes, such as ACO, enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase [bi-(tri) functional enzyme], cytochrome P450 4A family (CYP4A), L-FABP, acyl Co-A synthase, lipoprotein lipase, and many others. Three subtypes of PPARs have been found in amphibians, rodents, fish, and humans. PPARα is predominantly expressed in liver, kidney and heart, PPARβ (also called δ) is expressed in almost every tissue, and PPARγ is found almost exclusively in adipose tissue and has been linked to adipocyte differentiation (12). Among the synthetic activators of PPARα, collectively known as peroxisome proliferators for their ability to increase peroxisomes in rodent liver, are the fibrate class of hypolipidemic drugs (i.e., clofibric acid, bezafibrate, or their analogs). Recently it has been reported that the fatty acid derivative leuko-

Abbreviations: CLA, conjugated linoleic acid; PPARα, peroxisome proliferator-activated receptor alpha; ACO, acyl-CoA oxidase; L-FABP, liver fatty acid-binding protein; CYP4A1, cytochrome P450 IVA1; PPRE, PPAR-responsive element; DMSO, dimethyl sulfoxide; SPA, scintillation proximity assay.

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triene B_4 (LTB_4) and the fibrate analogs [4-chloro-6-(2,3-xylidino)-2-pyrimidinylthioacetic acid] (Wy-14,643) and 2-(4-[2-(3-[2,4-difluorophenyl]-1-heptylureido)ethyl]phenoxy)-2-methylbutyric acid (GW2331) bind to PPARα (13, 14). Fatty acids and eicosanoids have been also identified as PPAR ligands (14–16).

CLA has some structural features similar to peroxisome proliferators. Moreover the physiological responses observed in mice (reduced body weights, hepatic lipid accumulation, hypolipidemia) are characteristic of this group of chemicals. These similarities lead us to hypothesize that CLA activates hepatic PPARα. Previously, we had shown that SENCAR mice fed increasing levels of CLA, accumulated several PPAR-responsive enzymes including ACO, L-FABP, and CYP4A1 at mRNA and protein levels (17). The present studies were performed to determine the affinity of several CLA isomers for PPARα, the extent of PPARα activation, and resultant changes in gene expression. These studies are the first to demonstrate that CLA is a ligand and activator of PPARα, and as CLA induces PPAR-responsive genes in cultured cells, this dietary fatty acid may regulate lipid metabolism through a PPARα-mediated mechanism. Interestingly, the most biologically relevant isomer, (9Z,11E)-CLA, is among the most avid PPARα ligands of dietary origin yet described.

MATERIALS AND METHODS

Materials

[^{14}C]CLA and [^{14}C]linoleic acid were from American Radiolabeled Chemicals (St. Louis, MO). A mixture of CLA isomers [41.2% (9Z, 11E)- and (9E,11Z)-CLA, 44.1% (10E,12Z)-CLA, 1.1% (9Z,11Z)-, 9.4% (10Z,12Z)-, 1.3% (9E,11E)-, and (10E, 12E)-CLA, 0.7% linoleate and 2.2% unidentified compound] was from NuChek Prep, Inc. (Elysian, MN). Pure CLA isomers (9Z,11E)-CLA (97% purity), (9E,11E)-CLA (98% purity), (10E, 12Z)-CLA, and the furan form 8-(5-hexyl-2-furyl)-octanoic acid were from Matreya Inc. (Pleasant Gap, PA). Wy-14,643 was purchased from BIOMOL (Plymouth Meeting, PA) and Chemsyn (Lenexa, KS). Bezafibrate and troglitazone were from Sigma (St. Louis, MO) and Parke-Davis (Ann Arbor, MI), respectively. The expression plasmid pcDNA3-mPPARα and psV-GL2-PPRE luciferase reporter plasmid were generously donated by Dr. J. Tugwood (Zeneca Central Toxicology Laboratory, UK). The pSG5-mGAL4-PPARα chimera expression construct and $(UAS)_5$-tk-CAT reporter construct were generated as reported previously (18). The psV-β-galactosidase plasmid was from Promega (Madison, WI). [^{3}H]-GW2331 was prepared as previously described (14).

Ligand binding assays

The binding affinity of several CLA isomers for human PPARα was determined by scintillation proximity assay (SPA) using bacterially expressed PPARα ligand binding domain as previously described for PPARγ (19). The (9Z,11E)-, (10E,12Z)-, (9E,11E)-CLA isomers and the putative furan-CLA metabolite were tested in duplicate as inhibitors of [^{3}H]-GW2331 (14) binding to PPARα.

Reporter gene assays

Mouse PPARα-GAL4/$(UAS)_5$-CAT reporter system. COS-1 cells were co-transfected with GAL4-PPARα, (UAS)5-tk-CAT, and pSV-β-galactosidase (Promega, Madison, WI) as described previously (20). Transfected cells were treated with the test chemicals for 6 h. Chloramphenicol acetyltransferase (CAT) levels in cell lysates were assessed by enzyme-linked immunoabsorbent assay (GIBCO). Data were quantified relative to β-galactosidase activity and expressed as a percent of the DMSO vehicle treated cells.

Full-length mouse PPARα/PPRE-luciferase reporter system. CV-1 cells were co-transfected with $pcDNA_3$-PPARα, psV-GL2-PPRE-luciferase and pSV-β-galactosidase. Cells were transfected using LipofectAMINE™ (GIBCO). Transfected cells were treated for 6 h with the test chemicals. Luciferase and β-galactosidase activities were assayed on cell lysates (Promega, Madison, WI). Data were quantified relative to β-galactosidase activity and expressed as a percent of DMSO vehicle treated cells.

Total RNA and protein extraction

FaO cells were treated with various doses of (9Z,11E)-CLA isomer, linoleate, Wy-14,643 (100 μm) or 0.1% vehicle DMSO. Total RNA was extracted from FaO hepatoma cells using TRI-Reagent® (Molecular Research Center, Cincinnati, OH). For protein analyses, FaO cells were treated for 24 h with various concentrations of (9Z,11E)-CLA (50 μm, 100 μm and 200 μm), linoleate, Wy-14,643, bezafibrate or troglitazone (100 μm) and 0.1% DMSO as control. Cell lysates were obtained by homogenization with lysis buffer (PBS, 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS with 100 μg/ml phenylmethylsulfonyl fluoride, 300 μg/ml aprotinin, and 100 μg/ml sodium orthovanadate) and centrifugation (15,000 *g* for 30 min at 4°C). Total RNA and protein extracts were frozen (−80°C) until analyses were performed.

Quantitative reverse transcriptase-PCR (RT-PCR)

Quantitative RT-PCR using recombinant RNA as an internal standard was performed as reported previously (17, 21) to determine β-actin, ACO, CYP4A1, or L-FABP mRNA levels.

Protein analysis

Protein samples (20 μg) were separated by 12% SDS-PAGE and transferred to nitrocellulose membranes. Sheep anti-rat CYP4A1 or rabbit anti-rat albumin primary antibodies were used, followed by anti-sheep Ig-biotinylated antibody and streptavidin-horseradish peroxidase conjugate (Amersham Life Sciences, Arlington Heights, IL) or anti-rabbit Ig-horseradish peroxidase-linked secondary antibody (Bio-Rad Corp., Richmond, CA). Immunoreactive bands were detected using enhanced chemiluminescence (Amersham Life Sciences, Arlington Heights, IL) and the films were quantified using pdi™ scanning densitometry.

Incorporation of [^{14}C]CLA and [^{14}C]linoleate into FaO hepatoma cells

Cells were incubated with 0.1 μCi/ml in 0.1% bovine serum albumin-containing media of [^{14}C]CLA or [^{14}C]linoleate for 1, 3, 6, 9, 12, or 24 h. Percentage of incorporation into cells was determined by scintillation counting.

Total lipid extraction, neutral and phospholipids fractionation, and fatty acid analyses

FaO cells were incubated with serum-free media for 12 h and treated with vehicle (0.1% DMSO), linoleate or (9Z,11E)-CLA (100 μm) in serum-free media supplemented with 0.01% bovine serum albumin for 12 h. Total lipids were extracted from FaO cell homogenates and analyzed as reported previously (11).

RESULTS

CLA is a ligand for human PPARα. Scintillation proximity assay (SPA) is a novel assay system useful for quantita-

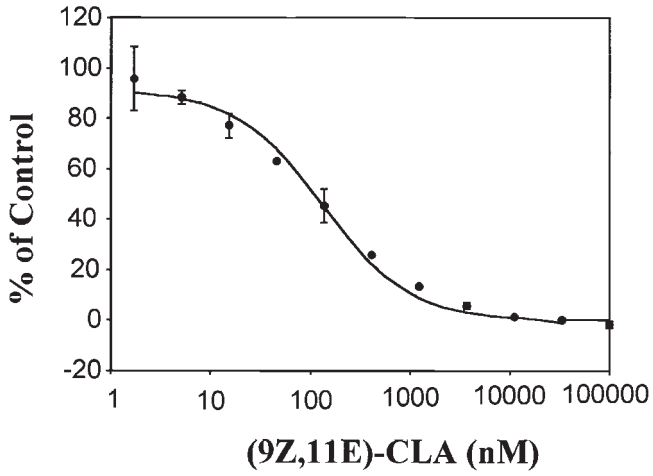

Fig. 1. CLA binds to PPARα. Competitive binding assays were performed by scintillation proximity assay (SPA) for human PPARα ligand binding domain and 10 nm [^{3}H]-GW2331 in the presence of increasing concentrations (up to 100 μm) of non-radioactive (9Z, 11E)-CLA as competitor.

tion of receptor–ligand equilibria. Major advantages of the method are simplicity and speed as separation of receptor-bound from free ligand is not necessary. We have recently reported an SPA for screening potential ligands for PPARγ with the results obtained with SPA showing an excellent correlation with the traditional gel filtration system (19). In addition, it has been previously shown that the fibrate analogue GW2331 is a high affinity ligand for both PPARα and PPARγ (14). We tested whether individual CLA isomers were able to inhibit specific binding of [^{3}H]-GW2331 to PPARα by competitive SPA. **Figure 1** represents a typical inhibition curve obtained for (9Z,11E)-CLA isomer (IC_{50} = 140 ± 90 nm) demonstrating that this isomer is a potent PPARα ligand. The other CLA isomers tested were also potent ligands for PPARα. IC_{50} values for CLA isomers determined by inhibition of [^{3}H]-GW2331 binding were similar and ranged from 140 to 420 nm (**Table 1**). Interestingly, CLA also binds to PPARγ but with lower affinity (IC_{50}s = 5–7 μm) when compared to PPARα (M. A. Belury, unpublished data).

CLA activates mouse PPARα. After determining that CLA is a high affinity ligand for PPARα, we determined the extent that CLA is able to activate PPARα. The GAL4-mPPARα–ligand binding domain (LBD) chimera reporter assay is very responsive to peroxisome proliferators (20) and as an artificial receptor is being used, has little interference from endogenous expression of PPAR subtypes. The isomeric mixture of CLA resulted in a dose-dependent activation of PPARα (5-fold) (**Fig. 2A**). Activation of mPPARα by individual isomers of CLA was also examined and compared to that of Wy-14,643 and perfluorodecanoic acid as positive controls (Fig. 2B). As expected, Wy-14,643 and perfluorodecanoic acid activated mPPARα with a maximal induction of 50- and 4-fold, respectively, above vehicle-treated cells. The (9Z,llE)-CLA isomer was a very efficacious activator of mPPARα in the

TABLE 1. IC_{50} values of PPARα in the competitive binding scintillation proximity assay

Compound	IC_{50}
	nM
(9Z,11E)-CLA	140 ± 90
(10E,12Z)-CLA	200 ± 30
(9E,11E)-CLA	260 ± 40
Furan-CLA	420 ± 20
GW2331	30

Mean and standard deviation are presented (n = 2). Competitive binding assays were performed by scintillation proximity assay (SPA) for human PPARα ligand binding domain and 10 nm [^{3}H]-GW2331 in the presence of increasing concentrations (up to 100 μm) of non-radioactive (9Z,11E)-CLA or GW2331 as competitors.

GAL4 system resulting in a 26-fold increase in activity (Fig. 2B). In regard to other mPPARα activators, treatment with the (9Z,11Z)- and furan-CLA isomers also resulted in substantial increases in reporter expression.

The full-length mouse PPARα/PPRE–luciferase reporter assay is generally less responsive than the GAL4 system described above but is closer to the natural cell environment. This system may suffer from interference by endogenously expressed PPAR. Despite its limitations, the luciferase reporter gene system co-transfected with the full-length mPPARα, was responsive to the activation of mPPARα by CLA and its individual isomers (Fig. 2C). Wy-14,643 activated PPARα with a maximal induction of 7.5-fold over DMSO-treated cells (Fig. 2D). The CLA mixture also activated PPARα to the same extent as the furan-CLA metabolite (2.7- and 2.9-fold induction, respectively). The individual CLA isomers (10E,12Z), (9E,11E), and (9Z,11E) were activators of PPARα (4- to 4.5-fold induction) with no apparent differences in efficacy.

CLA induces mRNA and protein of PPAR-responsive enzymes. The FaO cell line is a well-differentiated subclone derived from the rat hepatoma H4IIEC3 line (22) which expresses both PPARα and PPARβ but very low PPARγ1 (23). To determine the effect of CLA on PPAR-responsive gene expression, we used RT-PCR to quantify mRNA accumulation of ACO, L-FABP, and CYP4A1 (**Fig. 3**). Messenger RNA levels of the housekeeping gene β-actin were found to be similar among treatments and time points (data not shown). FaO cells were incubated with a (9Z,11E)-CLA, linoleate, or Wy-14,643 (100 μm) in DMEM supplemented with fetal bovine serum (Fig. 3A) for 6 and 24 h. ACO mRNA levels showed a slight increase at 6 h for all treatments but were not significantly different from control treated cells ($P > 0.01$). As expected, FaO cells treated with the prototypical peroxisome proliferator, Wy-14,643, for 24 h showed a higher induction (7-fold, $P < 0.01$) of ACO mRNA compared to vehicle-treated cells. The accumulation of ACO mRNA was significantly increased ($P < 0.01$) when FaO cells were treated with 200 μm CLA for 24 h in charcoal-stripped fetal bovine serum-containing media compared to vehicle-treated cells (data not shown).

Levels of L-FABP mRNA were more responsive to CLA and Wy-14,643 (100 μm) than ACO mRNA after 24 h (Fig.

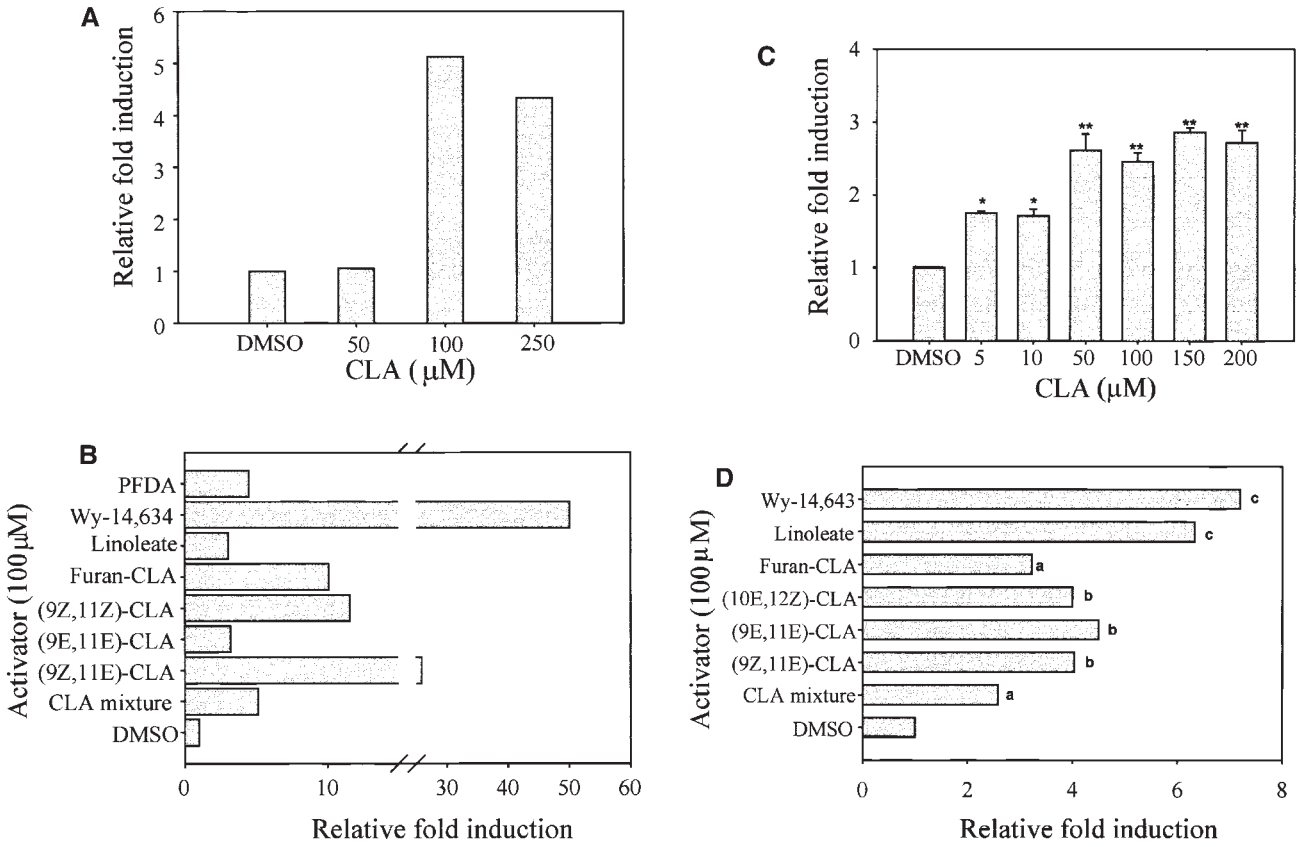

Fig. 2. Activation of mPPARα by CLA and its different geometric isomers. A: Transfected cells were treated for 6 h with increasing concentrations of CLA mixture (0 μm, 50 μm, 100 μm, or 250 μm). CAT / β-galactosidase ratios are expressed relative to vehicle treated cells (0.1% DMSO, n = 3). B: COS-1 cells were transfected with GAL4-mPPARα along with a CAT-reporter and β-galactosidase vectors. Transfected cells were treated for 6 h with the following compounds (100 μm): CLA mixture, (9Z,11E)-CLA, (9E,11E)-CLA, (9Z,11Z)-CLA, 8-(5-hexyl-2-furyl)-octanoic acid (furan-CLA), linoleate, perfluorodecanoic acid (PFDA), or Wy-14,643. CAT / β-galactosidase ratios are expressed relative to vehicle treated cells (0.1% DMSO, n = 3). C: CV-1 cells were co-transfected with pcDNA$_3$-PPARα, psV-GL2-PPRE-luciferase and pSV-β-galactosidase vectors. Transfected cells were treated for 6 h with increasing concentrations of CLA mixture. Luciferase / β-galactosidase ratios are expressed relative to vehicle treated cells (0.1% DMSO). Asterisks denote values that are significantly different from DMSO treated cells ($P < 0.05$, n = 3). D: Transfected cells were treated for 6 h with the following compounds (100 μm): CLA mixture, (9Z,11E)-CLA, (9E,11E)-CLA, (10E,12Z)-CLA, 8-(5-hexyl-2-furyl)-octanoic acid (furan-CLA), linoleate, or Wy-14,643. Luciferase / β-galactosidase ratios are expressed relative to vehicle treated cells (0.1% DMSO). Different letters denote significant differences ($P < 0.05$, n = 3).

3B), although there was no change after 6 h of treatment with CLA or linoleate. CLA induced L-FABP mRNA ($P < 0.05$) 11-fold after 24 h treatment in FaO cells compared to cells treated with vehicle, whereas Wy-14,643 induced the levels of L-FABP mRNA approximately 940-fold ($P < 0.0001$, Fig. 3B). L-FABP mRNA levels for linoleate treated cells were slightly higher than control cultures ($P < 0.05$).

As shown in Fig. 3C, CYP4A1 mRNA was elevated after 6 h of treatment with CLA and Wy-14,643 (34-fold and 68-fold, respectively). After 24 h the levels of CYP4A1 mRNA were similar for CLA and Wy-14,634-treated cells (9-fold, $P < 0.05$). Linoleate (a CLA parent compound and ligand for PPARα) did not have any effect on CYP4A1 mRNA accumulation at any time point studied.

To determine whether the changes in CYP4A1 mRNA by CLA were associated with enhanced protein expression, Western blot analysis was performed. The dramatic effect of CLA on CYP4A1 protein levels paralleled mRNA levels. CYP4A1 protein levels were increased by (9Z,11E)-CLA in a dose-dependent manner (**Fig. 4A**). As expected, Wy-14,643 (a PPARα selective activator) and bezafibrate (a non-subtype selective PPAR activator) induced the accumulation of this protein (Fig. 4B). Troglitazone (a PPARγ selective activator) did not cause any change in CYP4A1 protein corroborating the low expression of PPARγ in FaO cells. Linoleate was able to increase CYP4A1 protein to a lesser extent than CLA (2.5- versus 5-fold). Albumin levels were not affected by CLA ($P > 0.05$).

CLA and linoleate are incorporated into FaO hepatoma cells to the same extent**.** Because of the striking difference between CLA and the parent compound linoleate on affecting PPAR-responsive gene expression, we compared their incorporation into FaO cells and into neutral and phospholipid fractions. The maximal uptake of [^{14}C]CLA and [^{14}C]linoleate occurred by 9 h (**Fig. 5**) without significant change after 12 and 24 h ($P > 0.01$). Incorporation of [^{14}C]CLA or [^{14}C]linoleate in FaO cells was similar at all time points determined. As shown in **Table 2**, linoleate and (9Z,11E)-CLA were incorporated at the same extent into the neutral and phospholipid fractions. The percent-

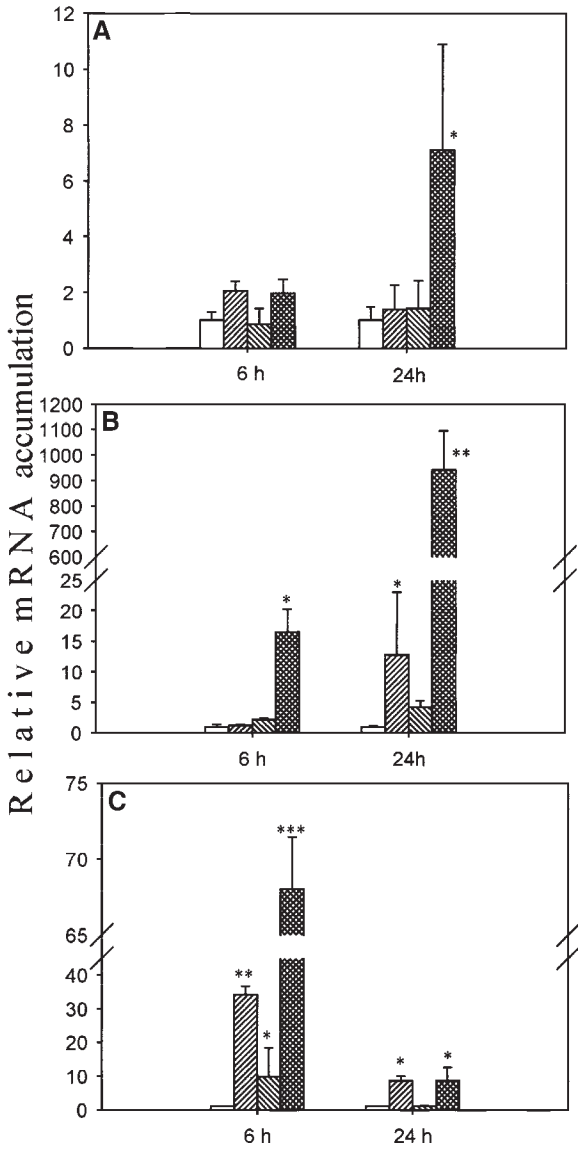

Fig. 3. Effect of CLA on (A) ACO (B) L-FABP, and (C) CYP4A1 mRNA accumulation in FaO hepatoma cells. Cells were incubated for 6 and 24 h with DMSO (□), 100 μm CLA (▨), 100 μm linoleate (▧), or 100 μm Wy-14,643 (⊠). Data are expressed relative to vehicle treated cells (0.1% DMSO). Total RNA was isolated and RT-PCR was performed as described in Materials and Methods. Asterisks designate values that are significantly different among treatments (* and **$P < 0.05$, ***$P < 0.0001$, n = 6–9).

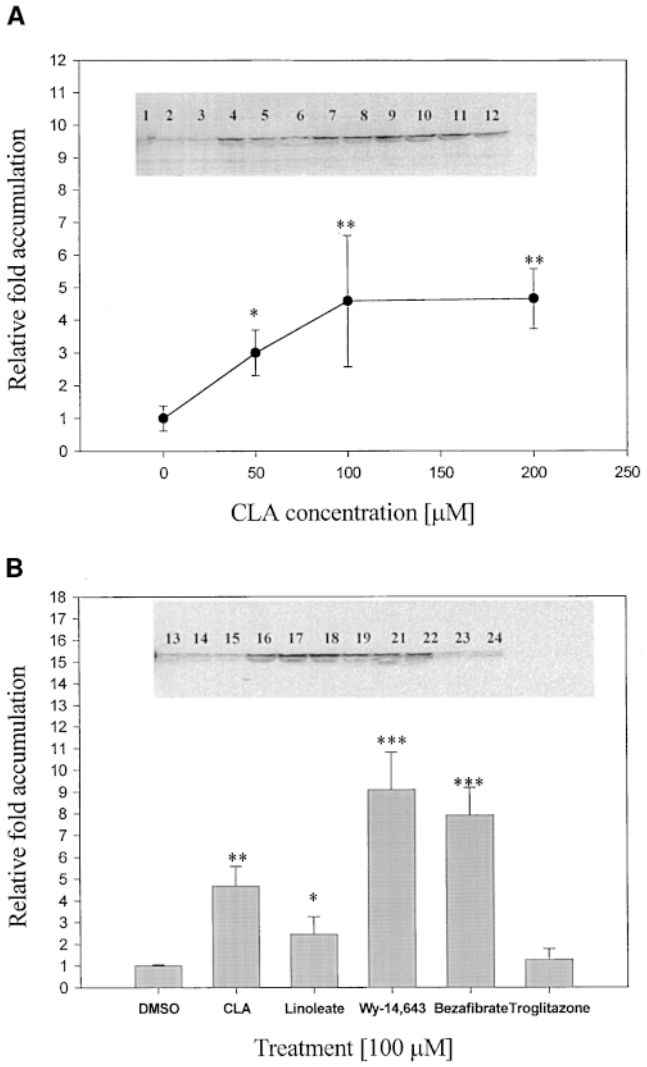

Fig. 4. Effect of CLA on CYP4A1 protein accumulation in FaO hepatoma cells. A: Inset, FaO cells were treated for 24 h with increasing concentrations of (9Z,11E)-CLA: Lanes 1–3, DMSO; 4–6, 50 μm; 7–9, 100 μm; and 10–12, 200 μm. A: Densitometric analysis for CYP4A1 bands. Data (mean ± SD) are presented after normalization with albumin and relative to DMSO-treated cells. Asterisks denote significant differences among treatments (n = 3, $P < 0.05$). B: Inset, FaO cells were treated for 24 h with: lanes 13–15, 100 μm linoleate; 16–18, 100 μm Wy-14,643; 19–21, 100 μm bezafibrate; and 22–23, 100 μm troglitazone. B: Densitometric analysis for CYP4A1 bands. Data (mean ± SD) are presented after normalization with albumin and relative to DMSO-treated cells. Asterisks denote significant differences among treatments (n = 3, $P < 0.05$).

age of incorporation of equimolar mixture of CLA isomers [(9Z,11E)-, (10E,12Z)-, and (9E,11E)-] were also similar in the neutral and phospholipid fractions. These data indicate that the increased efficacy of CLA compared to linoleic at inducing gene expression is due to differences in affinity for PPARα (IC_{50}s = 140 nm versus 1.1 μm, present study and ref. 24) and not due to pharmacokinetic differences.

DISCUSSION

One of the potential mechanisms of CLA modulation of cancer, atherosclerosis, and diabetes may be through its potent effect on lipid transport and metabolism in vivo (9, 10, 25). In SENCAR mice, we found that CLA supported decreased body weights, increased hepatic lipid accumulation (11), and increased accumulation of lipid metabolizing enzymes (ACO, L-FABP and CYP4A1) (17). These proteins are regulated, in part, by PPARα, the pivotal transcription factor for lipid metabolism in the liver (15, 26, 27).

Searching for the involvement of CLA in modulating lipid metabolism, we found that several of the CLA isomers studied bound to PPARα with submicromolar IC_{50}s (140 nm to 400 nm). Although one cannot directly com-

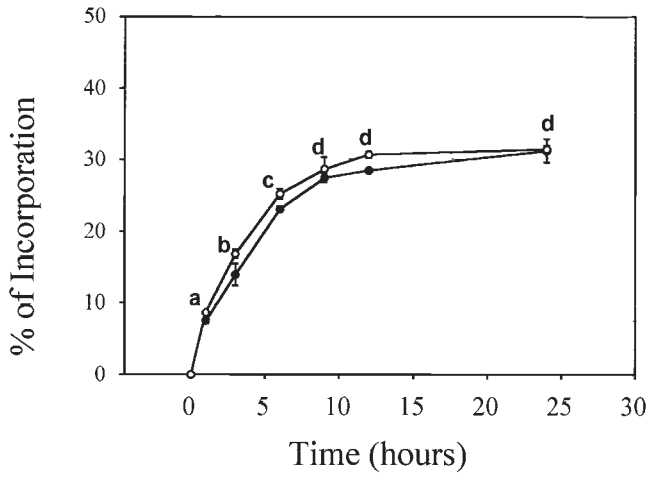

Fig. 5. Incorporation of [^{14}C]CLA and [^{14}C]linoleate into FaO hepatoma cells. Cells were incubated with 0.1 μCi/ml 0.1% BSA-containing media of [^{14}C]CLA (○) or [^{14}C]linoleate (●) for 1, 3, 6, 9, 12, or 24 h. After the time period indicated, percentage of incorporation into cells was determined by scintillation counting.

pare the IC_{50}s obtained in different studies due to different methodologies and/or receptor constructs utilized, CLA isomers appear to be among the most avid fatty acid ligands of PPARα reported to date (c.f., the eicosanoid derivative leukotriene B_4, 8(S)-hydroxyeicosatetraenoic, and the prostaglandin I_2 analog carbaprostacyclin which bind PPARα with IC_{50}s of 90 nm, 100 nm, and 500 nm, respectively) (14, 15). In particular, the (9Z,11E)- is the CLA isomer with the highest affinity (IC_{50} = 140 nm) in the present study. Interestingly, linoleate (CLA parent compound) has lower affinity for PPARα (IC_{50} = 1.1 ± 0.14 μm; 24) when compared to CLA isomers under the same conditions of analysis.

In agreement with the binding data, we found that several CLA isomers activated PPARα with the (9Z,11E)- isomer being the most efficacious mPPARα-GAL4 activator, whereas linoleate activated the chimeric construct weakly. Activation of PPARα by long chain fatty acids has been demonstrated previously, with polyunsaturated being slightly better activators than saturated fatty acids (28, 29). The difference in binding affinity to PPARα and cellular activity (140 nm vs. 50 μm) is not surprising based on the number of potential metabolic pathways and other factors related to the disposition of CLA in cells, such as incorporation into membrane phospholipids, binding to cytosolic proteins (i.e., L-FABP), and catabolism via β-oxidation (28, 30). In particular, the incorporation of CLA into the FaO cells in the present study was 30%.

According to our binding and transcriptional activation data, it is not surprising that CLA and linoleate have different abilities to induce L-FABP and CYP4A1 mRNA (Figs. 3B and 3C), and these differences are not attributed to preferential incorporation into liver cells or lipid fractions (Table 2). Different potencies of various fatty acids (at 320 μm) on the induction of L-FABP mRNA and protein have been reported previously in FaO cells (31). The potency of linoleate to induce L-FABP mRNA in the present study was comparable to that reported in the Meunier-Durmort et al. study (31) (3.1-fold vs. 3.8-fold, respectively). Interestingly, the linoleate conjugated isomer (9Z,11E-CLA) studied here was a more potent inducer (11-fold) than its parent compound; moreover, the CLA potency obtained was comparable to the PPARα activator ETYA (31). Although the differences in potency in the Meunier-Durmort et al. report (31) were attributed to the length of the fatty acid chain when octanoic (no effect), linoleic (3.8-fold), arachidonic (5.0-fold) acids, and the PPAR activator ETYA (14-fold) were compared,

TABLE 2. Incorporation of CLA isomers into rat hepatoma FaO cells

	Total Lipid Extraction (% fatty acid)				
Treatment	(9Z,12Z)-LA	(9Z,11E)-CLA[a]	(10E,12Z)-CLA	(9E,11E)-CLA	20:4 n6 Arachidonic Acid
DMSO	1.80	0.14	0.00	0.00	5.74
Mixed CLA[b]	1.74	5.57	4.57	2.91	4.95
(9Z,11E)-CLA	1.42	18.82	0.00	0.09	4.53
(9Z,12Z)-linoleate	20.98	0.09	0.00	0.00	5.20
	Neutral lipid fraction (% fatty acid)				
Mixed CLA	0.93	10.81	9.16	7.42	2.44
(9Z,11E)-CLA	0.86	28.28	0.00	0.31	2.41
(9Z,12Z)-LA	28.83	0.07	0.00	0.00	5.31
	Phospholipid fraction (% fatty acid)				
Mixed CLA	3.22	7.23	5.96	5.13	2.21
(9Z,11E)-CLA	3.45	19.78	0.16	0.00	2.89
(9Z,12Z)-LA	23.84	0.34	0.00	0.00	6.11

Cells were treated with vehicle or fatty acid (100 μm) for 12 h. Extracted lipids were quantified by gas chromatography as described in Experimental Procedures.

[a] (9Z,11E)- and (9E,11E)-CLA isomers were not resolved from each other with the chromatographic conditions used.

[b] (9Z,11E)-CLA, (10E,12Z)-CLA, and (9E,11E)-CLA were mixed in equimolar concentration (100 μm final concentration).

our results suggest that the spatial and positional double bond system present in CLA may favor a conformational change important for the binding and activation of PPARα.

Based on the current knowledge of peroxisome proliferator-mediated gene expression and our findings, we can speculate that peroxisome proliferators and CLA share a mechanism to explain their hypolipidemic and hypocholesterolemic effects. At the extracellular level, the hypolipidemic effect induced by peroxisome proliferators, may be due to an increased hydrolysis by lipoprotein lipase of triglycerides derived from chylomicrons and very low density lipoprotein particles (32). Evidence suggests that the plasma triglyceride clearance processes are regulated by peroxisome proliferators and, in some steps, directly by hepatic PPARα as lipoprotein lipase expression is induced by PPAR activators (32). Inside the cell, free fatty acids (FFAs) are transported by FABP. This binding protein is also up-regulated through a PPAR-mediated mechanism (23, 33) with one functional PPAR response element (PPRE) regulating its expression (34). FFAs regulate other lipid-metabolizing enzymes of the peroxisome, microsome, or cytosol via activation of PPARα. In peroxisomes, the rate-limiting enzyme for β-oxidation ACO and the enzyme responsible for the second and third step in β-oxidation enoyl-CoA hydratase-3-hydroxyacyl-CoA dehydrogenase have functional PPREs (35, 36). Other lipid metabolizing enzymes regulated via PPAR are also found in mitochondria (acyl-CoA synthase, medium chain acyl-CoA dehydrogenase, β-hydroxy-β-methylglutaryl-CoA synthase, reviewed in 32), in microsomes (CYP4A, ω-hydroxylase, 37) and in cytosol (phosphoenol pyruvate carboxy kinase, reviewed in 32).

These are the first findings to demonstrate that (9Z,11E)-CLA is a high affinity ligand and activator for PPARα. Taken together, these data suggest that CLA regulates lipid metabolism through binding and activation of PPARα. Therefore, CLA may be beneficial for those metabolic disorders related to impaired lipid metabolism, such as non-insulin-dependent diabetes mellitus, atherosclerosis, obesity, and cancer.

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