Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 11012–11016, November 1994
Biochemistry

The peroxisome proliferator-activated receptor regulates mitochondrial fatty acid oxidative enzyme gene expression

(fatty acid oxidation/mitochondrial enzymes/nuclear hormone receptor/transcriptional regulation)

TOD GULICK*†, SHARON CRESCI†‡, TERESA CAIRA†‡, DAVID D. MOORE*, AND DANIEL P. KELLY‡§¶

*Department of Molecular Biology, Massachusetts General Hospital and Genetics Department, Harvard Medical School, Boston, MA 02114; and Departments of ‡Medicine and §Molecular Biology and Pharmacology, Washington University School of Medicine, St. Louis, MO 63110

Communicated by William H. Daughaday, August 2, 1994

ABSTRACT **Medium-chain acyl-CoA dehydrogenase (MCAD) catalyzes a pivotal reaction in mitochondrial fatty acid (FA) β-oxidation. To examine the potential role of FAs and their metabolites in the regulation of MCAD gene expression, we measured MCAD mRNA levels in animals fed inhibitors of mitochondrial long-chain FA import. Administration of carnitine palmitoyltransferase I inhibitors to mice or rats resulted in tissue-limited increases in steady-state MCAD mRNA levels. HepG2 cell cotransfection experiments with MCAD promoter reporter plasmids demonstrated that this was a transcriptional effect mediated by the peroxisome proliferator-activated receptor (PPAR). The activity mapped to a nuclear receptor response element that functioned in a heterologous promoter context and specifically bound immunoreactive PPAR in rat hepatic nuclear extracts, confirming an *in vivo* interaction. PPAR-mediated transactivations of this promoter and element were also induced by exogenously added FA and fibric acid derivatives. Induction of PPAR transactivation by perturbation of this discrete metabolic step is unusual and indicates that intracellular FA metabolites that accumulate during such inhibition can regulate MCAD expression and are likely candidates for PPAR ligand(s). These results dictate an expanded role for the PPAR in the regulation of FA metabolism.**

The flavoenzyme medium-chain acyl-CoA dehydrogenase [MCAD; acyl-CoA:(acceptor) 2,3-oxidoreductase; EC 1.3.99.3] is one of four different chain-length-specific enzymes that catalyze the initial reaction in the mitochondrial fatty acid (FA) β-oxidation cycle (1, 2). Substrates for MCAD include medium-chain length (C_6–C_{12}) acyl-CoA thioesters derived from (*i*) medium-chain FAs that enter mitochondria by diffusion, (*ii*) products of mitochondrial β-oxidation of saturated and unsaturated long-chain FAs, and (*iii*) products of peroxisomal β-oxidation of long-chain and very long-chain FAs. Because these diverse pathways of FA oxidation converge at this point, MCAD catalyzes a pivotal step in cellular FA metabolism.

Expression of MCAD is highly regulated by a variety of conditions that alter substrate availability and tissue energy demands. MCAD mRNA and enzyme are expressed predominantly in tissues that use FAs preferentially as energy substrates, including heart, liver, slow-twitch skeletal muscle, and kidney (3). Hepatic and cardiac MCAD mRNA are markedly upregulated in concert with mitochondrial FA oxidation rates during the postnatal period (3). Induction of MCAD expression is also seen in these tissues during periods of fasting, when cellular energy requirements are met primarily by FA oxidation, an effect that has been attributed to transcriptional modulation (4).

The mechanisms involved in regulation of cellular FA oxidation at the transcriptional level are largely unknown. We have explored the regulation of MCAD promoter activity and have identified sequences that confer both basal (5) and nuclear hormone receptor-mediated activities (6–8). As a further step in delineating the mechanisms involved in control of MCAD expression and to segregate the influences of fasting, hormones, and substrate availability, we sought to modulate intracellular FA metabolism while controlling for other parameters. We used inhibitors of carnitine palmitoyltransferase I (CPT-I) (EC 2.3.1.21), the enzyme that catalyzes the CoA–carnitine exchange necessary for mitochondrial import of long-chain FAs (2). We show that CPT-I inhibition leads to induction of MCAD mRNA *in vivo* and that this effect is mediated at the transcriptional level by the peroxisome proliferator-activated receptor (PPAR) (9), a nuclear receptor previously regarded as a regulator of peroxisomal β-oxidative (10–13) and cytochrome P450 (14) enzyme gene targets.

METHODS

RNA Blot Analysis. Adult male Sprague–Dawley rats were gavage-fed every 12 hr with L-(+)-*p*-hydroxyphenylglycine (500 mg/kg; oxfenicine; Sigma) in 0.5% methyl cellulose or vehicle alone. Adult male Swiss–Webster mice were fed every 24 hr with 2-[5-(4-chlorophenyl)pentyl]-oxirane-2-carboxylic acid (10 mg/kg; POCA; Byk-Guiden Pharmazeutika) in dimethyl sulfoxide (DMSO) or vehicle alone. Total cellular RNA isolation and Northern blot analysis were done as described (3) by using probes derived from clones encoding rat MCAD (3) and β-actin (15).

Plasmid Construction. *Reporter plasmids.* pTKLUC and TKGH have been described (16). MCADLUC[−1054] was constructed by replacing the herpes simplex virus thymidine kinase (TK) promoter fragment of pTKLUC with a human MCAD gene promoter fragment (5) extending from −1054 to +190 relative to the transcription start site (= +1). Promoter fragments for the MCAD promoter deletion series plasmids were isolated by digesting MCADLUC[−1054] with *Bst*EII (−707), *Rsa* I (−376), or *Dsa* I (partial, −302); reaction with T4 DNA polymerase in the presence of dNTPs to generate blunt double-stranded DNA; heat inactivation; and digestion with *Hin*dIII. Fragments were recloned into pTKLUC digested with *Sma* I and *Hin*dIII, replacing the TK promoter sequence. $[NRRE\text{-}1]_3$TKLUC was made by cloning direc-

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: ARP-1, apolipoprotein regulatory protein 1; COUP-TF, chicken ovalbumin upstream promoter transcription factor; CPT-I, carnitine palmitoyltransferase I; DMSO, dimethyl sulfoxide; FA, fatty acid; GH, growth hormone; HNF-4, hepatocyte nuclear factor 4; MCAD, medium-chain acyl-CoA dehydrogenase; NRRE-1, nuclear receptor response element 1; POCA, 2-[5-(4-chlorophenyl)-pentyl]-oxirane carboxylic acid; PPAR, peroxisome proliferator-activated receptor; RAR, all-*trans*-retinoic acid receptor; RXR, 9-*cis*-retinoic acid receptor; TK, thymidine kinase.

†T.G., S.C., and T.C. contributed equally to this work.
¶To whom reprint requests should be addressed.

tionally trimerized oligonucleotides corresponding to nuclear receptor response element 1 (NRRE-1) (6) into the *Bam*HI- and *Bgl* II-digested pTKLUC polylinker.

Mammalian expression vectors. Vectors CDMhmCOUP-TF and CDMrHNF-4 have been described (8), as have vectors CDM, CDMhMB67, CDMhRARα, CDMhRXRα, and CDM-rTRβ (CDM62) (16); CDMfluhBD73 (17); and pNGFI-B wild type (18). The PPAR open reading frame was cloned from a mouse liver cDNA library using the PCR with primers based on the published cDNA sequence (9): (forward) 5'-cccgcgccATGGTGGACACAGAGAGCCCC-3' and (reverse) 5'-gcgcccgcggccgctttaGTACATGTCTCTGTAGATCTC-3', in which uppercase letters depict PPAR open reading frame residues. The *Nco* I- and *Not* I-digested product was cloned into a modified CDM vector to yield CDMmPPAR. Vector CDMhARP-1 was constructed similarly by using a PCR product generated with primers: (forward) 5'-cccgcgccATGGCAATGGTAGTCAGCACGTGG-3' and (reverse) 5'-gcgcccgcggccgctttaTTGAATTGCCATATACGGCCAGT-3', based on the human apolipoprotein regulatory protein 1 (ARP-1) sequence (19). Sequences were verified using the dideoxynucleotide chain-termination reaction technique.

Cell Culture Transfection. HepG2 cells were maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% (vol/vol) fetal calf serum, 2 mM glutamine, and gentamicin (25 μg/ml). For transfection experiments, cells grown to 60–80% confluence in 12-well plates were preincubated overnight in DMEM supplemented with 5% (vol/vol) charcoal-stripped fetal calf serum (DMEM5st) (6, 16). Cells were transfected using a modification of the diethylaminoethyl–dextran technique (20) with each well receiving 2 μg of plasmid TKGH to control for transfection efficiency and/or stimulus effects on the TK promoter, 2 μg of reporter, and 0.1–0.5 μg of receptor expression vector as indicated. After transfection, cells were incubated for 12–18 hr in DMEM5st before a culture medium change to DMEM5st supplemented with activators or DMSO vehicle. Cells were incubated 24–30 hr further before harvesting. POCA, ciprofibrate, clofibrate, and gemfibrozil were dissolved in DMSO at 10 mM, 100 mM, 1 M, and 1 M, respectively, and diluted to indicated concentrations in DMEM5st. Oleic and linoleic acids were diluted to 250 mM in DMSO and diluted further to indicated concentrations in 37°C culture medium followed by sonication and sterile filtration. Palmitic acid was compexed to albumin (1:1) by slowly adding a 20 mM palmitic acid solution to a 20% albumin solution at 37°C and diluted further to indicated concentrations in 37°C culture medium. Luciferase assays were done by using a Monolight 2010 luminometer with an assay kit (Promega) as directed by the manufacturer, and values were corrected for medium human growth hormone (GH) concentration as determined by RIA with a Nichols Institute (San Juan Capistrano, CA) kit.

Electrophoretic Mobility-Shift Assays. Hepatic nuclear protein extract preparation and mobility-shift assays were done as described (7). In antibody supershift assays, 2 μl of anti-PPAR antiserum αFP_2 (21) or preimmune serum was preincubated with the extract for 10 min before addition of reaction mixture and probe.

FA Oxidation Studies. Cells (10^5) were seeded into 25-cm^2 flasks. At 40% confluence, medium supplemented with [1-^{14}C]palmitate (200 nCi/ml; 1 Ci = 37 GBq) or [1-^{14}C]acetate (200 nCi/ml) and POCA (25 μM), gemfibrozil (100 μM), or DMSO vehicle was added to flasks that were sealed after securing a 1 × 1.5 inch (1 inch = 2.54 cm) piece of Whatman no. 1 filter paper to a center well. After 4 hr, duplicate flasks for each of the six conditions were analyzed. $^{14}CO_2$ was liberated from the culture medium by acidification with 2 ml of 6 M HCl and collected overnight on filter paper alkalinized with 250 μl of 2 M NaOH. $^{14}CO_2$ was measured by scintillation counting of the filters.

RESULTS

Tissue-Limited MCAD mRNA Levels Increase *in Vivo* in Response to CPT-I Inhibition. The fasted state in animals is associated with mobilization of stored lipid and an increase in FA flux through the mitochondrial β-oxidation cycle. These processes are mediated by the shunting of peripherally stored lipids to liver and by counterregulatory hormone-mediated inhibition of acetyl-CoA carboxylase (2). This latter process reduces cytosolic malonyl-CoA concentration, thereby relieving inhibition of CPT-I and allowing import of long-chain fatty acyl moieties into mitochondria where oxidative phosphorylation-coupled FA oxidation occurs. Thus, hormonal influences and substrate availability act in concert to regulate hepatic FA catabolism.

Previous studies have shown that MCAD transcription is upregulated in fasted animals (4), and our preliminary results indicated that MCAD mRNA levels increase in cultured cells incubated in medium with high FA concentration. These results suggested that MCAD gene expression might be regulated by intracellular FAs or FA metabolites by a mechanism distinct from counterregulatory hormonal influences. We investigated this hypothesis in animal and cell culture studies using the pharmacological inhibitors of CPT-I, oxfenicine and POCA, while controlling for other variables that exert effects during the fasting response.

Administration of oxfenicine, an agent that preferentially inhibits CPT-I in the heart (22), to adult male rats for 7 days resulted in a marked increase in cardiac MCAD mRNA levels compared with control animals (Fig. 1*A*). POCA, which inhibits CPT-I in multiple tissues (23), also produced this effect in a time- and inhibitor concentration-dependent manner in mice (Fig. 1*B*). POCA-mediated MCAD mRNA increases occurred in murine liver and heart, whereas no effect was exhibited in brain. Fasting-mediated upregulation of MCAD mRNA was additive with the POCA effect (data not shown). Thus, inhibition of mitochondrial long-chain FA import produces increases in steady-state MCAD mRNA levels in tissues that have a high capacity for FA oxidation. We used cultured cells to explore the molecular basis of this phenomenon.

The PPAR Transactivates the MCAD Promoter and Transduces Stimulation by CPT-I Inhibition. The effect of CPT-I inhibition on MCAD transcription was evaluated in cultured cell transfection experiments. A reporter plasmid containing human MCAD gene 5' flanking sequence extending from −1054 to +190 relative to the transcription start site fused to a luciferase reporter (MCADLUC[−1054]) was transfected into HepG2 hepatocytes, followed by incubation in the presence or absence of 25 μM POCA. Because maximal

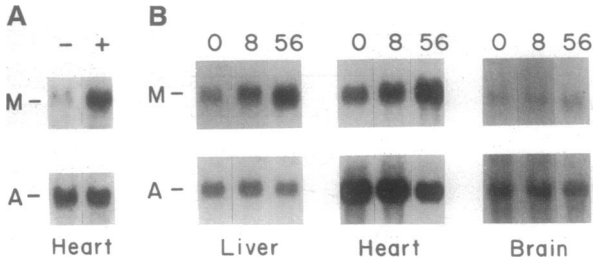

FIG. 1. CPT-I inhibition produces time-dependent, tissue-restricted induction of MCAD mRNA. (*A*) Representative Northern blot analysis of heart RNA isolated from rats that received oxfenicine (+) or aqueous vehicle (−) every 12 hr for 7 days. Each lane contains 15 μg of total RNA. Blots were sequentially hybridized with rat MCAD (M) and β-actin (A) cDNA probes. (*B*) Northern blot analysis of RNA isolated from tissues of POCA-treated mice harvested at indicated times (hours) after the first dose. POCA was administered at time 0 and every 24 hr thereafter.

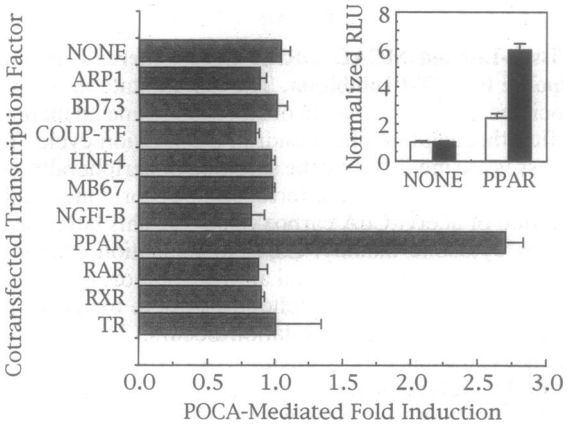

FIG. 2. POCA-mediated MCAD promoter stimulation is transduced by the PPAR. HepG2 cells were cotransfected with vector MCADLUC[−1054] and expression plasmids for the indicated transcription factors and incubated in medium in the absence or presence of 25 μM POCA. Luciferase activities in cell extracts harvested 48 hr after transfection were determined, normalized for transfection efficiency, and expressed as a mean ratio of activity (presence/absence of POCA) ± SD. Results are characteristic of three independent experiments. (Inset) Normalized MCADLUC[−1054] activities in the presence (shaded bars) and absence (open bars) of 25 μM POCA with and without vector CDMmPPAR cotransfection. RLU, relative light units; BD73, (17); MB67, (16); NGFI-B, nerve growth factor-induced clone B; TR, thyroid hormone receptor.

responses to signaling pathways are exhibited with transcription factor overexpression, we screened candidate transducers of the POCA effect in HepG2 cells by cotransfection with expression plasmids for these factors. Among others, we tested members of the nuclear hormone receptor superfamily, including the thyroid hormone receptor, the all-*trans*-retinoic acid receptor (RAR), the 9-*cis*-retinoic acid receptor (RXR), and several orphan receptors known to be expressed in a tissue pattern that resembles that of POCA-mediated MCAD mRNA induction. Each of these factors is known to regulate the expression of gene products that participate in lipid metabolism. The retinoid (6, 8) and orphan receptors hepatocyte nuclear factor 4 (HNF-4) (7) and chicken ovalbumin upstream promoter transcription factor (COUP-TF) (8) have been shown by us to regulate MCAD promoter activity.

In these studies, POCA-mediated activity was seen exclusively in cells cotransfected with CDMmPPAR, an expression vector for the PPAR (Fig. 2). POCA mediated a 2.7-fold induction of transcription superimposed on 2.3-fold constitutive PPAR activity (Fig. 2 *Inset*). No significant POCA-mediated stimulation of promoter activity was exhibited in cells in which the thyroid hormone, retinoid, or orphan receptors were overexpressed (Fig. 2). The tissue-limited pattern of POCA-induced MCAD mRNA induction (Fig. 1*B*) correlates with that reported for PPAR expression (9), supporting its role in mediating the transcriptional response to CPT-I inhibition *in vivo*.

In parallel experiments, inhibition of long-chain FA oxidation rates in HepG2 cells exposed to CPT-I inhibitors was confirmed. Release of $^{14}CO_2$ from cells incubated in medium supplemented with [1-^{14}C]-palmitate was inhibited 91% by 25 μM POCA, from 540 pmol per flask·min to 47 pmol per flask·min. POCA did not significantly alter the catabolism of [1-^{14}C]acetate, confirming the specificity of inhibition by POCA to long-chain FA oxidation. Together with the results of the transcriptional activation studies, these results corroborate the *in vivo* effects and suggest that CPT-I inhibition produces increases in steady-state MCAD mRNA levels via induction of PPAR-mediated transactivation of the MCAD promoter through generation of an intracellular extramitochondrial FA metabolite ligand. Moreover, in cultured cell experiments, this effect is manifest in a system in which endocrine hormonal and nutrient influences are controlled.

The PPAR Regulates MCAD at NRRE-1. The MCAD promoter PPAR-responsive element was mapped to a region between −376 and −302 relative to the transcription start site using MCAD promoter 5′ deletion series reporters in cotransfected HepG2 cells (Fig. 3*A*). This segment contains the pleiotropic NRRE-1 with four hexameric sequences that match the RGGTCA consensus for class II nuclear receptors. The function of NRRE-1 as a PPAR response element was confirmed by using a reporter containing three copies of the element cloned upstream of a minimal herpes simplex virus TK promoter fused to luciferase ([NRRE-1]$_3$TKLUC). In these experiments, CDMmPPAR produced 4.6-fold constitutive activation of [NRRE-1]$_3$TKLUC and mediated 17.4- and 18.5-fold stimulation by 25 μM POCA and 100 μM ciprofibrate, respectively (Fig. 3*B*). Oxfenicine produced a similar, but less pronounced, activation of PPAR compared with POCA (data not shown). Inductions by POCA and clofibrate were concentration-dependent and saturable, with EC_{50} values of 30 and 200 μM, respectively (data not shown). Because NRRE-1 binds and is regulated by HNF-4, COUP-TF, ARP-1, and RXR/RAR (6–8), we retested these recep-

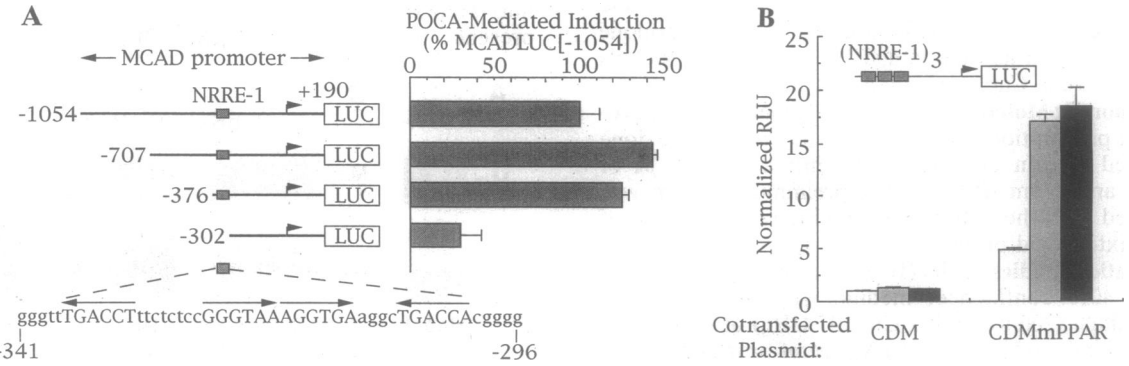

FIG. 3. PPAR MCAD promoter activation maps to NRRE-1. (*A*) HepG2 cells cotransfected with CDMmPPAR and MCADLUC reporters were incubated in the presence or absence of 25 μM POCA or DMSO vehicle. POCA inductions (± SE) are normalized to that seen with vector MCADLUC[−1054] (= 100%). MCAD promoter reporter constructs and the location of the transcription start site (arrowheads) are depicted at left of the corresponding data. NRRE-1 is denoted by a box, and its sequence is shown at bottom; the location and orientation of potential receptor binding sites are indicated by arrows. LUC, luciferase. (*B*) Mean [NRRE-1]$_3$TKLUC activities (± SD) in cells cotransfected with either vector CDM or vector CDMmPPAR and incubated in medium supplemented with control vehicle (open bars), POCA (light shaded bars), or ciprofibrate (dark shaded bars). Data are normalized to the activity of [NRRE-1]$_3$TKLUC cotransfected with CDM in the presence of vehicle alone (= 1.0). RLU, relative light units.

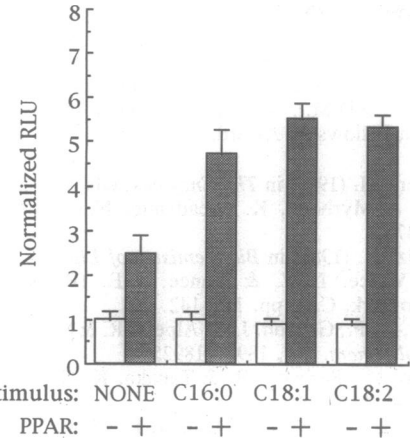

FIG. 4. PPAR activation of the MCAD promoter is stimulated by FAs. MCADLUC[−1054] activity in cells cotransfected with vector CDM (open bars) or vector CDMmPPAR (shaded bars) and cultured in medium supplemented with 125 μM palmitic acid (C16:0), 250 μM oleic acid (C18:1), or 125 μM linoleic acid (C18:2). Activities were normalized (= 1.0) to that of cells cotransfected with vector CDM and incubated in unsupplemented medium. Results are means (± SD) and are representative of three independent experiments. RLU, relative light units.

tors for ability to transduce stimulation of $[NRRE\text{-}1]_3$-TKLUC by CPT-I inhibition and confirmed that POCA-mediated transcriptional stimulation was dependent on and specific for PPAR expression and the presence of NRRE-1.

FAs Stimulate PPAR-Mediated MCAD Transcription. Because inhibition of CPT-I results in obstruction of long-chain FA entry into mitochondria (2) with the presumed accumulation of extramitochondrial long-chain FA metabolites, we examined the influence of exogenous administration of long-chain FAs on MCAD promoter activity. HepG2 cells were cotransfected with vectors CDMmPPAR and MCAD-LUC[−1054] and incubated in medium with or without palmitic, oleic, or linoleic acids. The PPAR effected a 2.5-fold constitutive activation of MCADLUC[−1054], which was augmented to 4.7-, 5.4-, and 5.2-fold by 125 μM palmitic, 250 μM oleic, and 125 μM linoleic acids, respectively (Fig. 4). Exogenous FA administration had no effect on promoter activity in cells not transfected with the PPAR expression vector. In PPAR-cotransfected cells, $[NRRE\text{-}1]_3$TKLUC demonstrated culture medium palmitate concentration and oleate concentration dependence, with EC_{50} values of 50 μM (data not shown). These results are consistent with previous findings of straight long-chain FA stimulation of PPAR-mediated transactivation of other target elements (24, 25). Thus, the PPAR mediates induction of MCAD transcription in response to either exogenous FA loading or inhibition of fatty acyl mitochondrial import.

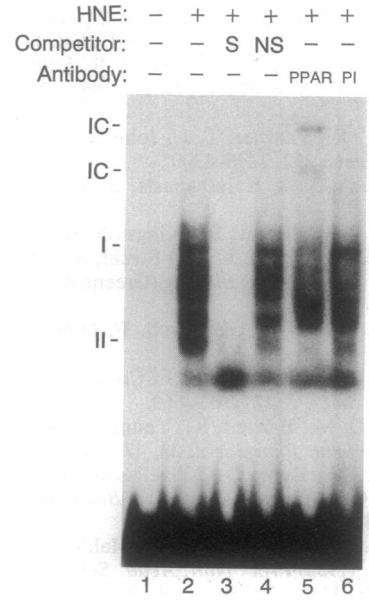

FIG. 5. Hepatic nuclear extract (HNE) immunoreactive PPAR binds MCAD NRRE-1. Electrophoretic mobility-shift assay of binding reactions including a radiolabeled MCAD NRRE-1 oligonucleotide (7) and rat hepatic nuclear protein extract (1 μg). Immune complexes (ICs) indicate specific anti-PPAR–PPAR–NRRE-1 complexes. I and II indicate specific PPAR–NRRE-1 complexes that diminish with antibody incubation. The contents of each lane are indicated at top. S, specific competition with 100-fold molar excess of unlabeled NRRE-1; NS, nonspecific competition with 100-fold molar excess of an unrelated size-matched unlabeled oligonucleotide; PPAR, anti-PPAR; PI, preimmune serum.

Immunoreactive PPAR in Hepatic Nuclear Extract Binds MCAD NRRE-1. PPAR binding to NRRE-1 was confirmed in electrophoretic mobility-shift assays with rat liver nuclear protein extract. As shown in Fig. 5, several prominent specific NRRE-1–protein complexes were formed in mobility-shift assays with reactions containing nuclear extract and a radiolabeled NRRE-1 probe. The least mobile of these (complex I) comigrated with that formed with an acyl-CoA oxidase PPAR response element (11) oligonucleotide probe using the same nuclear extract (data not shown). Coincubation with a PPAR-specific antibody, but not preimmune serum, produced two new bands of much lower mobility (IC bands) and diminution of two specific complexes (I and II), confirming that PPAR was a component of these NRRE-1-bound complexes. The specific binding of rat hepatic nuclear PPAR to the MCAD NRRE-1 oligonucleotide indicates that this element is a likely target for the PPAR *in vivo*.

DISCUSSION

This work demonstrates that expression of the gene encoding MCAD, a pivotal enzyme in mitochondrial FA β-oxidation, is regulated by the PPAR. Furthermore, induction of PPAR activity is produced by specific inhibitors of long-chain FA mitochondrial entry, by exogenous administration of FAs, and by prototypical peroxisome proliferators. Accordingly, this is a mechanism whereby the transcription of a nuclear gene encoding a mitochondrial FA oxidation enzyme is regulated in response to extramitochondrial fatty acyl intermediate concentrations.

The PPAR is a member of the nuclear receptor superfamily that was originally cloned from a liver cDNA library derived from rodents fed peroxisome proliferator agents (9). PPAR-responsive elements have been identified in several gene promoters, including those of each of the three enzymes of the peroxisomal β-oxidation cycle (11–13), the cytochrome P450 4A6 enzyme (14), and the rat liver FA binding protein (29). Cytochrome P450 4A1 has also been implicated as a target for this factor (26). The products of each of these genes are involved in extramitochondrial FA catabolism, but the present work describes a PPAR gene target for a mitochondrial enzyme.

PPAR induction by pharmacological manipulation of a specific metabolite step, obstruction of mitochondrial long-chain FA import by CPT-I inhibition, is distinctive. The induction is probably exerted by a metabolite of accumulated intracellular long-chain FAs or fatty acyl thioesters. We have recently noted that ciprofibrate and POCA at near-saturating concentrations were no more effective than either stimulus alone in activating the PPAR (data not shown). Lack of additive or synergistic PPAR induction suggested that peroxisome proliferators might exert effects on PPAR activity

through a mechanism that involves CPT-I inhibition. However, fibric acid derivatives did not affect long-chain FA oxidation rates. Thus, it is likely that CPT-I inhibition and conventional peroxisome proliferators produce PPAR induction via a common saturable extramitochondrial FA metabolic pathway. Further, our findings together with previous results (27) imply that neither mitochondrial nor peroxisomal FA oxidation are required for PPAR activator/ligand generation.

The major catabolic pathways for FAs in the context of deficient mitochondrial β-oxidation are peroxisomal β-oxidation and P450 ω-oxidation. This latter process produces incompletely catabolized FA intermediates such as dicarboxylic acids (2). Although previous reports have indicated that exogenously administered dodecanedioic acid is inactive in stimulating PPAR-mediated transactivation (24, 25), the induction of the PPAR with CPT-I inhibition indicates that P450 metabolites should not be excluded as candidate PPAR ligands. Support for this hypothesis includes the observation that the gene encoding P450 4A1, the rate-limiting enzyme in ω-oxidation, is upregulated during CPT-I inhibition, and direct inhibition of 4A1 impairs PPAR activator-dependent induction of responsive gene mRNA accumulation (26). Alternatively, neutral acyl steroid esters formed from accumulated thioesters are candidate PPAR ligands. Such compounds conform to the general structure and hydrophobicity typical of nuclear receptor ligands. The use of inhibitors of CPT-I or pharmacological agents that inhibit specific catabolic steps of native and CoA-activated fatty acyl metabolites offers promise as a general strategy in the investigation of the identity of PPAR activator/ligand(s).

The results of our cotransfection and mobility-shift assays implicate MCAD NRRE-1 as a strong functional PPAR response element. Most previously defined PPAR response elements consist of direct repeats of the RGGTCA hexamer consensus sequence with 1-bp separation (DR1) (11–14). In contrast, NRRE-1 is a complex element containing multiple potential hexamer-binding sites (Fig. 3*A*) in a specific arrangement that does not include a DR1 motif. Our mobility-shift assays demonstrated that two distinct NRRE-1–PPAR protein complexes were recognized by the PPAR-specific antibody (Fig. 5). These complexes may represent different PPAR isoforms, alternative heterodimer or homodimer pairings, DNA-bound monomers, or higher order oligomeric complexes. We propose that the specific structure of NRRE-1 permits competitive and cooperative interactions between various nuclear receptors at this element to provide tissue-specific, hormone-, and fatty acyl metabolite-sensitive modulation of MCAD gene expression.

Our findings indicate a more general role for the PPAR in the regulation of FA metabolism. Transcriptional regulation of a mitochondrial oxidative enzyme gene by the PPAR is of major importance because the mitochondrial FA oxidation pathway is the primary source of cellular energy in many tissues. The critical role played by FA oxidation in energy production is underscored by the severe and often fatal consequences of genetic defects in MCAD and other FA oxidative enzymes (28). We speculate that genes encoding additional enzymes in the mitochondrial β-oxidation pathway may be similarly regulated. The PPAR, as an inducible transcription factor, may represent an ideal target for the development of therapeutic strategies for inborn errors in FA oxidation once a cognate ligand is identified.

Note Added in Proof. During review of this manuscript, Rodriguez *et al.* (30) reported the regulation of mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase gene by the PPAR.

We are grateful to Dr. Arnold Strauss for critical reading of the manuscript. We thank Dr. Michael Arand for the anti-PPAR antiserum, Dr. Thomas Raya for several oxfenicine-treated rat tissues, James Baake for advice on oxidation studies, and Dr. Jeffrey Milbrandt for the NGFI-B expression vector. D.P.K. is a Lucille P. Markey Scholar. This work was supported by the Markey Foundation and National Institutes of Health Grants DK45416 (D.P.K.) and DK43382-02 (D.D.M.). S.C. is the recipient of an American Heart Association Fellowship Award.

1. Beinert, H. (1963) in *The Enzymes*, eds. Boyer, P. D., Lardy, H. J. & Myrbock, K. (Academic, New York), Vol. 7, pp. 447–476.
2. Schulz, H. (1985) in *Biochemistry of Lipids and Membranes*, eds. Vance, D. E. & Vance, J. E. (Benjamin/Cummings, Menlo Park, CA), pp. 116–142.
3. Kelly, D. P., Gordon, J. I., Alpers, R. & Strauss, A. W. (1989) *J. Biol. Chem.* **264**, 18921–18925.
4. Nagao, M., Parimoo, B. & Tanaka, K. (1993) *J. Biol. Chem.* **268**, 24114–24124.
5. Zhang, Z., Kelly, D. P., Kim, J. J., Zhou, Y., Ogden, M. L., Whelan, A. J. & Strauss, A. W. (1992) *Biochemistry* **31**, 81–89.
6. Raisher, B. D., Gulick, T., Zhang, Z., Strauss, A. W., Moore, D. D. & Kelly, D. P. (1992) *J. Biol. Chem.* **267**, 20264–20269.
7. Carter, M. E., Gulick, T., Raisher, B. D., Caira, T., Ladias, J. A. A., Moore, D. D. & Kelly, D. P. (1993) *J. Biol. Chem.* **268**, 13805–13810.
8. Carter, M. E., Gulick, T., Moore, D. D. & Kelly, D. P. (1994) *Mol. Cell. Biol.* **14**, 4360–4372.
9. Issemann, I. & Green, S. (1990) *Nature (London)* **347**, 645–650.
10. Dreyer, C., Krey, G., Keller, H., Givel, F., Helftenbein, G. & Wahli, W. (1992) *Cell* **68**, 879–887.
11. Tugwood, J. D., Issemann, I., Anderson, R. G., Bundell, K. R., McPheat, W. L. & Green, S. (1992) *EMBO J.* **11**, 433–439.
12. 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.
13. Kliewer, S. A., Umesono, K., Noonan, D. J., Heyman, R. A. & Evans, R. M. (1992) *Nature (London)* **358**, 771–774.
14. Muerhoff, A. S., Griffin, K. J. & Johnson, E. F. (1992) *J. Biol. Chem.* **267**, 19051–19053.
15. Ponte, P., Gunning, P., Blau, H. & Kedes, L. (1983) *Mol. Cell. Biol.* **3**, 1783–1791.
16. Baes, M. I., Gulick, T., Choi, H.-S., Martinoli, M.-G., Simha, D. & Moore, D. D. (1994) *Mol. Cell. Biol.* **14**, 1544–1552.
17. Dumas, B., Harding, H. P., Choi, H.-S., Lehmann, K., Chung, M., Lazar, M. A. & Moore, D. D. (1994) *Mol. Endocrinol.* **8**, 996–1005.
18. Wilson, T. E., Fahrner, T. J., Johnston, M. & Milbrandt, J. (1991) *Science* **252**, 1296–1300.
19. Ladias, J. A. A. & Karathanasis, S. K. (1991) *Science* **251**, 561–565.
20. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K., eds. (1993) *Current Protocols in Molecular Biology* (Greene & Wiley, New York), pp. 9.2.1–9.2.6.
21. Gebel, T., Arand, M. & Oesch, F. (1992) *FEBS Lett.* **309**, 37–40.
22. Stephens, T. W., Higgins, A. J., Cook, G. A. & Harris, R. A. (1985) *Biochem. J.* **227**, 651–660.
23. Turnbull, D. M., Bartlett, K., Younan, S. I. & Sherratt, H. S. (1984) *Biochem. Pharmacol.* **33**, 475–481.
24. Gottlicher, M., Demoz, A., Svensson, D., Tollet, P., Berge, R. K. & Gustaffson, J.-A. (1994) *Biochem. Pharmacol.* **46**, 2177–2184.
25. Keller, H., Dreyer, C., Medin, J., Mahfoudi, A., Ozato, K. & Wahli, W. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 2160–2164.
26. Kaikaus, R. M., Chan, W. K., Lysenko, N., Ray, R., Ortiz de Montellano, P. R. & Bass, N. (1993) *J. Biol. Chem.* **268**, 9593–9603.
27. Gottlicher, M., Widmark, E., Li, Q. & Gustafsson, J.-A. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 4653–4657.
28. Stanley, C. A., Hale, D. E., Coates, P. M., Hall, C. L., Corkey, B. E., Yang, W., Kelley, R. I., Gonzales, E. L., Williamson, J. R. & Baks, L. (1983) *Pediatr. Res.* **17**, 877–884.
29. Issemann, I., Prince, R., Tugwood, J. & Green, S. (1992) *Biochem. Soc. Trans.* **20**, 824–827.
30. Rodriguez, J. C., Gil-Gomez, G., Hegarat, F. G. & Haro, D. (1994) *J. Biol. Chem.* **269**, 18767–18772.