Fatty Acids Activate Transcription of the Muscle Carnitine Palmitoyltransferase I Gene in Cardiac Myocytes via the Peroxisome Proliferator-activated Receptor α*

(Received for publication, April 17, 1998, and in revised form, June 12, 1998)

Jon M. Brandt‡‡, Fatima Djouradian, and Daniel P. Kelly**‡‡

From the Center for Cardiovascular Research, Departments of Pediatrics, Medicine, and **Molecular Biology & Pharmacology, Washington University School of Medicine, St. Louis, Missouri 63110 and **INSERM U319, Université Paris 7, 75251 Paris Cedex 05, France

To explore the gene regulatory mechanisms involved in the metabolic control of cardiac fatty acid oxidative flux, the expression of muscle-type carnitine palmitoyltransferase I (M-CPT I) was characterized in primary cardiac myocytes in culture following exposure to the long-chain mono-unsaturated fatty acid, oleate. Oleate induced steady-state levels of M-CPT I mRNA 4.5-fold. The transcription of a plasmid construct containing the human M-CPT I gene promoter region fused to a luciferase gene reporter transfected into cardiac myocytes, was induced over 20-fold by long-chain fatty acid in a concentration-dependent and fatty acyl-chain length-specific manner. The M-CPT I gene promoter fatty acid response element (FARE-1) was localized to a hexameric repeat sequence located between 775 and 763 base pairs upstream of the initiator codon. Cotransfection experiments with expression vectors for the peroxisome proliferator-activated receptor α (PPARα) demonstrated that FARE-1 is a PPARα response element capable of conferring oleate-mediated transcriptional activation to homologous or heterologous promoters. Electrophoretic mobility shift assays demonstrated that PPARα bound FARE-1 with the retinoid X receptor α. The expression of M-CPT I in hearts of mice null for PPARα was approximately 50% lower than levels in wild-type controls. Moreover, a PPARα activator did not induce cardiac expression of the M-CPT I gene in the PPARα null mice. These results demonstrate that long-chain fatty acids regulate the transcription of a gene encoding a pivotal enzyme in the mitochondrial fatty acid uptake pathway in cardiac myocytes and define a role for PPARα in the control of myocardial lipid metabolism.

Mammalian cardiac energy substrate utilization rates are regulated during development and in response to physiologic and pathophysiologic stimuli. During the fetal period, glucose serves as the chief myocardial substrate (1). Following birth, myocardial energy is produced primarily via mitochondrial β-oxidation of long-chain fatty acids (2, 3). During the development of cardiac hypertrophy in rodents and humans, fatty acid oxidation (FAO) rates decrease and glucose utilization increases (4–11): a reversion to the fetal energy metabolic program. Recent studies have demonstrated that the expression of genes encoding FAO enzymes is regulated, at the transcriptional level, in parallel with fatty acid utilization rates during development and in the hypertrophied and failing heart (12–14). The gene regulatory mechanisms governing cardiac fatty acid utilization have not been delineated; however, recent studies have focused on the gene encoding medium-chain acyl-CoA dehydrogenase have implicated nuclear receptors and members of the Sp transcription factor family in the metabolic control of FAO enzyme gene expression (13–16).

Carnitine palmitoyltransferase I (CPT I; palmitoyl-CoA: carnitine O-palmitoyltransferase; EC 2.3.1.21) catalyzes the initial reaction in the mitochondrial import of long-chain fatty acids, a tightly regulated step in the cellular fatty acid utilization pathway (17, 18). The activity of CPT I is an important determinant of cellular fatty acid oxidative flux. CPT I catalyzes the transfer of a long-chain fatty acyl group from coenzyme A to carnitine. A specific translocase (carnitine-acylcarnitine carrier) located in the inner mitochondrial membrane delivers long-chain acylcarnitines into the mitochondrial matrix where they are re-esterified to acyl-thioesters by carnitine palmitoyltransferase II (CPT II). Acyl-thioesters in the mitochondria undergo β-oxidation generating reducing equivalents used to produce ATP via oxidative phosphorylation. Recent studies have demonstrated that CPT I exists as two isoforms encoded by separate genes: liver-type (L-CPT I or CPT IA), a hepatic-enriched, ubiquitously expressed protein (19, 20) and muscle-type (M-CPT I or CPT IB), which is expressed abundantly in heart, skeletal muscle, and brown adipose tissue (21–23). CPT I activity is inhibited by the reversible binding of malonyl-CoA, the first committed intermediate in the pathway of fatty acid synthesis (17, 18). Malonyl-CoA is proposed to inhibit hepatic fatty acid oxidation during periods of fatty acid synthesis. Much less is known about the regulation of CPT I activity in heart. The IC50 of M-CPT I for malonyl-CoA is approximately 100-fold lower than that of L-CPT I (24), yet the malonyl-CoA concentration in liver and heart is similar (25). These observations have led to speculation that control of M-CPT I activity occurs, at least in part, via malonyl-CoA independent mechanisms.

We hypothesized that long-chain fatty acids regulate M-CPT

* This work was supported in part by National Institutes of Health Grants DK45416 and HL58493. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ Supported by an individual National Research Service Award grant from the NHLBI, National Institutes of Health.

‡‡ Established Investigator of the American Heart Association. To whom correspondence should be addressed; Center for Cardiovascular Research, Washington University School of Medicine, 660 S. Euclid Ave., Campus Box 5086, St. Louis, MO 63110; Tel.: 314-362-8908; Fax: 314-362-0186; E-mail: dkelly@imgate.wustl.edu.

1 The abbreviations used are: FAO, fatty acid oxidation; CPT, carnitine palmitoyltransferase; M, muscle type; L, liver type; FARE-1, fatty acid response element-I; PPAR, peroxisome proliferator-activated receptor; BSA, bovine serum albumin; PCR, polymerase chain reaction; bp, base pair(s); RXR, retinoid X receptor; TK, thymidine kinase; EMSA, electrophoretic mobility shift assay.
I gene expression. In this study we demonstrate that expression of the M-CPT I gene is regulated in cardiac myocytes, at the transcriptional level, by long-chain fatty acids via the peroxisome proliferator-activated receptor α (PPARα). Our results suggest a mechanism for the control of myocardial fatty acid utilization at the mitochondrial import step, by long-chain acyl substrate levels.

MATERIALS AND METHODS

Primary Rat Neonatal Cardiac Myocyte Cell Culture—Cardiac myocytes were prepared from 1-day-old Sprague-Dawley rat pups as described (13). In brief, hearts were removed and minced in 0.2% collagenase (Wako Chemicals, Richmond, VA); the cells were pooled in Dulbecco’s modified Eagle’s medium containing 10% horse serum, 5% fetal calf serum (Sigma) and subjected to differential plating (1 h) to reduce fibroblast contamination. Nonadherent cells (enhanced cardiocyte fraction) were plated to 50% confluence on 60-mm diameter dishes pre-treated with collagen (Sigma). After 24 h the cell medium was switched to serum-free Dulbecco’s modified Eagle’s medium containing 0.10 mM 5-bromo-2′-deoxyuridine (Sigma), 10 μg/ml insulin (Sigma), 10 μg/ml transferrin (Sigma), and 1 mg/ml fatty acid-free BSA (Sigma).

Preparation of Fatty Acid and Etomoxir Solutions—Oleate was diluted to 20 mM in water preheated to 70 °C. NaOH (1 N) was added dropwise to the solution until it was completely neutralized (Sigma). The sodium salts of oleate were redissolved and standardized in water preheated to 70 °C. Oleate (Sigma) was added to the saline solution to give a final oleate concentration of 4.7 mM. The sodium salts of etomoxir (Research Biochemicals International) was diluted in water to a concentration of 100 mM. The sodium salt of etomoxir (Sigma) and oleate were added to the cell medium 16 h after transfection. The cells were harvested 24 h later.

Luciferase activities were determined by the standard luciferin-ATP assay, and β-galactosidase activity was measured by the Galacto-Light chemiluminescence assay (Tropix, Bedford, MA) in an Analytical Luminescence Monolight 2010 luminometer.

Electrophoretic Mobility Shift Assays (EMSAs)—EMSAs were performed as described (16). The pT7lac-RXRα bacterial expression vector was generously provided by Dr. Tod Gulick (Harvard University). Nuclear extracts (10 μg) were loaded by SDS-PAGE and transferred to a nitrocellulose membrane. Membranes were incubated with a 10 mM solution of etomoxir (Sigma) or vehicle (BSA alone) for 90 h in serum-free medium. In some wells, 1 μg of pCDM.PPAR, a mammalian expression vector containing a mouse PPARα cDNA (30), or 1 μg of pCDM– , the expression vector backbone lacking the PPARα cDNA, was added. Oleate, etomoxir, and vehicle were added to the cell medium 16 h after transfection. The cells were harvested 24 h later.

RESULTS

Long-chain Fatty Acids Induce Expression of the M-CPT I Gene—To determine whether M-CPT I gene expression is regulated by long-chain fatty acids in cardiac myocytes, M-CPT I mRNA levels were delineated in primary ventricular myocytes in culture following exposure to oleate (C18:1). For these experiments, myocytes isolated from 1-day-old rat ventricle were exposed to 0.5 mM oleate complexed to bovine serum albumin (BSA) or vehicle (BSA alone) for 90 h in serum-free medium. Mean M-CPT I mRNA levels were 4.5-fold higher in cardiac myocytes exposed to oleate compared with vehicle-treated cells (p < 0.01; Fig. 1). Expression of the genes encoding several mitochondrial β-oxidation cycle enzymes including very-long-chain and medium-chain acyl-CoA dehydrogenase also increased in the presence of oleate (data not shown). The level of β-actin mRNA, a control for loading, was not different in vehicle compared with oleate-treated cells. Similarly, the expression of the mRNA encoding ATPase subunit e, a nuclear encoded mitochondrial protein, was not affected by exposure to oleate indicating that the regulatory effect does not involve all nuclear genes encoding mitochondrial proteins.

Oleate Induces Transcriptional Activity of a Human M-CPT I Gene Promoter-Reporter Construct—To determine whether the oleate-induced increase in M-CPT I gene expression occurs at the transcriptional level, the oleate experiments were repeated with cardiac myocytes transiently transfected with a plasmid containing the human M-CPT I gene promoter region fused to a luciferase reporter gene. A 1013-bp fragment of M-CPT I gene 5′-flanking DNA was cloned by PCR amplification of a BAC

The results showed that the expression of M-CPT I gene was significantly higher in oleate-treated cells compared with vehicle-treated cells. The reporter plasmid containing the M-CPT I gene promoter region was transfected into cardiac myocytes, and the luciferase activity was measured. The luciferase activity was significantly higher in oleate-treated cells compared with vehicle-treated cells. The results confirmed that the M-CPT I gene expression is regulated by long-chain fatty acids in cardiac myocytes.
subclone template containing the human M-CPT I gene (see “Materials and Methods”). The human M-CPT I gene 5′-flanking DNA contains two untranslated exons, 1A and 1B, that extend from 746 to 633 and 523 to 470 base pairs upstream of the start codon, respectively (31, 32). The 5′ ends of exons 1A and 1B function as independent transcription start sites (32). The M-CPT I gene 5′-flanking region (from 1025 to 12 base pairs 5′ of the start codon adenine) was cloned upstream of the luciferase gene in a promotorless reporter plasmid to generate MCPT.Luc.1025 (Fig. 2A). The basal transcriptional activity of MCPT.Luc.1025 in rat cardiac myocytes was significantly higher (over 15-fold) than that of the promotorless vector backbone (data not shown). Oleate markedly induced the transcriptional activity of MCPT.Luc.1025 (approximately 25-fold; Fig. 2A).

The dose dependence of the oleate response was delineated by repeating the cardiac myocyte transfection experiments in serum-deprived medium containing 50, 250, or 500 μM oleate versus vehicle alone. As shown in Fig. 2B, the transcriptional activity of MCPT.Luc.1025 increases with increasing oleate concentration. To determine whether the fatty acid-induced transcription of the M-CPT I gene is acyl chain length-specific, dose-response experiments were repeated using decanoate (C10:0) and hexanoate (C6:0). Compared with the oleate response, the activity of MCPT.Luc.1025 was induced only modestly by decanoate and was not affected by hexanoate at any of the concentrations tested (Fig. 2B). Taken together, these data indicate that M-CPT I gene transcription is activated by long-chain fatty acids in a concentration-dependent and acyl chain length-specific manner. These results are consistent with the role of M-CPT I in the mitochondrial import of long-chain fatty acids.

The M-CPT I Gene Fatty Acid-responsive Region Contains a Putative Nuclear Receptor Binding Site Composed of an Imperfect Direct Repeat—Cardiac myocyte transfections were repeated with a MCPT.Luc deletion series to localize the oleate-responsive region within the human M-CPT I gene promoter region. Luciferase reporter plasmids containing M-CPT I 5′-flanking DNA extending from 1025 (MCPT.Luc.1025), 915 (MCPT.Luc.915), 781 (MCPT.Luc.781), and 724 (MCPT.Luc.724) bp upstream of the M-CPT I start codon adenine were used for these experiments. The oleate response was preserved with the MCPT.Luc.915 and MCPT.Luc.781 constructs but was absent with MCPT.Luc.724 (Fig. 3A). The basal transcriptional activity of MCPT.Luc.724 was approximately 50% lower than that of MCPT.Luc.781 but still significantly greater than that of the promotorless, reporter vector backbone indicating that the lack of an oleate response is not due to the loss of promotor function. Thus, the M-CPT I gene 5′-flanking region between −781 and −724 contains a fatty acid response element.

DNA sequence analysis of the M-CPT I gene promoter oleate-responsive region revealed the presence of an imperfect hexameric repeat separated by a single nucleotide (DB-1; TGACCTTTTCCCT−763). The antisense sequence of each half-site of the putative fatty acid-responsive element (Fig. 3B) conforms to the consensus (A/G/G/G/T/G/NA) for binding class II members of the nuclear receptor superfamily. The transfect-
PPARα Regulates Cardiac Fatty Acid Oxidation

The M-CPT I Gene Promoter Element, FARE-1, Is a PPARα Response Element—Previous studies have shown that DR-1 elements serve as binding sites for PPARα-RXR heterodimers (33). Given the results of previous studies demonstrating that PPARα regulates the transcription of genes encoding other mitochondrial and peroxisomal fatty acid oxidation enzymes (33), we speculated that this receptor was a candidate for the fatty acid-mediated control of M-CPT I gene expression. Indeed, PPARα was shown to be activated by fatty acids (34, 35). To determine whether FARE-1 could confer PPARα responsiveness to a heterologous promoter, two copies of FARE-1 were cloned upstream of the herpes simplex virus TK promoter fused to a luciferase reporter gene (MCPT(FARE)2TKLuc). Cotransfection studies were performed with MCPT(FARE)2TKLuc and pCDM.PPAR in the presence and absence of oleate or etomoxir (Fig. 4B). Neither oleate nor etomoxir alone activated MCPT(FARE)2TKLuc. Overexpression of PPARα resulted in a 5-fold activation of MCPT(FARE)2TKLuc activity with an additional 1.6–2-fold induction with addition of oleate or etomoxir (total activation 8–10-fold; Fig. 4B). When a point mutation identical to that present in MCPT.Luc.781 m1 was introduced into both copies of FARE-1 in the context of TKLuc (MCPT(FAREm1)2TKLuc), PPARα responsiveness was abolished (Fig. 4B). Taken together, these results define FARE-1 as a PPARα responsive element.

PPARα-RXRα Heterodimers Bind FARE-1—PPARα binds cognate DNA elements as a heterodimer with RXR (36). EM-SAs were performed to characterize the interaction of PPARα-RXRα heterodimers with the PPARα-responsive element, FARE-1. EMSA was performed with a radiolabeled FARE-1 oligonucleotide probe, RXRα (produced by overexpression in bacteria), and PPARα produced by in vitro coupled reticulocyte lysate transcription/translation. The FARE-1 probe formed a light complex with PPARα alone and no complex with RXRα alone (Fig. 5, lanes 2 and 3). A prominent FARE-protein complex formed when both PPARα and RXRα were added to the incubation (Fig. 5, lane 4). Competition experiments performed with a molar excess of specific (FARE-1) or an unrelated, size-matched, double-stranded non-specific oligonucleotide confirmed that the prominent complex of lowest mobility formed with PPARα and RXRα represented a specific DNA-protein interaction (Fig. 5, lanes 5–7). Antibody
PPARα Regulates Cardiac Fatty Acid Oxidation

The energy substrate preference of the mammalian heart is tightly controlled during development and in response to diverse physiologic and pathophysiologic conditions (2–11). The fetal heart produces energy primarily through catabolism of glucose and lactate (1). Following birth, the mammalian heart switches to fatty acids as the chief energy substrate. Compared with glucose, the oxidation of fatty acids provides more ATP per mole of substrate, albeit at the expense of increased oxygen consumption. Thus, fatty acid oxidation provides a greater capacity for energy production to meet the physiologic demands imposed on the postnatal mammalian heart. During cardiac hypertrophy and in the failing heart, the myocardium reverts to the fetal energy substrate utilization pattern using glucose as the chief energy substrate (4–11). Previous studies have shown that expression of nuclear genes encoding mitochondrial fatty acid β-oxidation cycle enzymes is regulated at the transcriptional level in parallel with fatty acid utilization rates during development and in the hypertrophied and failing heart (12–14, 39). Thus, the capacity for myocardial fatty acid oxidation is dictated, at least in part, via transcriptional regulatory recognitions of PPARα and RXRα were present in the FARE-1 protein complex. The FARE-1 protein complex was supershifted by either a polyclonal antibody that recognizes mouse PPARα (37) or an anti-RXRα antibody, whereas addition of preimmune sera did not alter its mobility (Fig. 5, lanes 8–10). Taken together, these findings demonstrate that, as reported for other PPARα response elements, PPARα and RXRα bind FARE-1 as a heterodimer.

PPARα Regulates FAO Enzyme Gene Expression in Vivo—To determine the importance of PPARα in the regulation of cardiac fatty acid oxidative enzyme gene expression in vivo, M-CPT I gene expression was characterized in mice null for PPARα (PPARα−/−; Ref. 28) and compared with age-matched controls (PPARα+/+). Previous studies have shown that mitochondrial and peroxisomal fatty acid β-oxidation cycle enzyme gene expression is reduced in the liver of PPARα−/− mice (28, 38). Northern blot studies demonstrated that steady-state levels of M-CPT I mRNA are significantly lower (by 51 ± 9%) in the hearts of adult PPARα−/− mice compared with controls (Fig. 6A). The studies were repeated following a 5-day administration of etomoxir, a known activator of PPARα. As expected, etomoxir induced myocardial expression of M-CPT I mRNA in PPARα+/+ mice (194 ± 15 versus 100 ± 7). In contrast, the PPAR activator did not induce M-CPT I gene expression in the hearts of PPARα−/− mice (64 ± 10 versus 58 ± 10) (Fig. 6B). These results confirm the role of PPARα as an activator of M-CPT I gene expression in heart in vivo.

**DISCUSSION**

FIG. 4. The human M-CPT I gene promoter fatty acid response element (FARE-1) mediates transcriptional activation by PPARα. A, the homologous promoter reporter plasmid MCPT.Luc.781 (solid bars) and the mutated promoter reporter plasmid MCPT.Luc.781m1 (hatched bars) were transfected into HepG2 cells and incubated in the presence of oleate (250 μM), etomoxir (40 μM), or vehicle with or without cotransfected pCDM.PPAR as indicated. The bars represent mean (± S.E.) luciferase activity (RLU) normalized (±1.0) to the activity of the respective target plasmid cotransfected with the expression vector backbone, pCDM(-). B, the homologous promoter reporter plasmid MCPT(FARE)TKLuc (solid bars) and the mutated PPAR reporter MCPT(FAREm1)TKLuc (hatched bars) were transfected into HepG2 cells in the presence of oleate (250 μM), etomoxir (40 μM), or vehicle with or without cotransfected pCDM.PPAR as indicated. The bars represent mean (± S.E.) luciferase activity (RLU) normalized (±1.0) to the activity of the respective target plasmid cotransfected with pCDM(-). The RLU values shown in A and B were corrected for transfection efficiency using the activity of cotransfected pRSVβ-Gal and represent the mean of at least two independent experiments performed in triplicate.

**FIG. 5. PPAR-RXR heterodimers bind FARE-1.** Autoradiograph of EMSA performed with a 32P-labeled FARE-1 oligonucleotide probe, RXRα overexpressed in bacteria, and PPARα produced by coupled in vitro transcription/translation (see “Materials and Methods”). Competition (Comp) experiments were performed with 50- and 100-fold molar excess of unlabeled FARE-1 DNA (ramp) and 100-fold molar excess of unlabeled, size-matched unrelated competitor oligonucleotide DNA (N.S.) as indicated at the top. Antibodies (Ab) used include preimmune sera (P.I.), anti-PPARα antisera (PPAR) and anti-RXRα antisera (RXR). N.S. on the left denotes nonspecific complexes. I.C. denotes the supershifted immune complexes.

PPARα (PPARα−/−; Ref. 28) and compared with age-matched controls (PPARα+/+). Previous studies have shown that mitochondrial and peroxisomal fatty acid β-oxidation cycle enzyme gene expression is reduced in the liver of PPARα−/− mice (28, 38). Northern blot studies demonstrated that steady-state levels of M-CPT I mRNA are significantly lower (by 51 ± 9%) in the hearts of adult PPARα−/− mice compared with controls (Fig. 6A). The studies were repeated following a 5-day administration of etomoxir, a known activator of PPARα. As expected, etomoxir induced myocardial expression of M-CPT I mRNA in PPARα+/+ mice (194 ± 15 versus 100 ± 7). In contrast, the PPAR activator did not induce M-CPT I gene expression in the hearts of PPARα−/− mice (64 ± 10 versus 58 ± 10) (Fig. 6B). These results confirm the role of PPARα as an activator of M-CPT I gene expression in heart in vivo.

**DISCUSSION**

The energy substrate preference of the mammalian heart is tightly controlled during development and in response to diverse physiologic and pathophysiologic conditions (2–11). The fetal heart produces energy primarily through catabolism of glucose and lactate (1). Following birth, the mammalian heart switches to fatty acids as the chief energy substrate. Compared with glucose, the oxidation of fatty acids provides more ATP per mole of substrate, albeit at the expense of increased oxygen consumption. Thus, fatty acid oxidation provides a greater capacity for energy production to meet the physiologic demands imposed on the postnatal mammalian heart. During cardiac hypertrophy and in the failing heart, the myocardium reverts to the fetal energy substrate utilization pattern using glucose as the chief energy substrate (4–11). Previous studies have shown that expression of nuclear genes encoding mitochondrial fatty acid β-oxidation cycle enzymes is regulated at the transcriptional level in parallel with fatty acid utilization rates during development and in the hypertrophied and failing heart (12–14, 39). Thus, the capacity for myocardial fatty acid oxidation is dictated, at least in part, via transcriptional regulatory
mechanisms. In this report we describe a mechanism for the induction of M-CPT I gene expression by long-chain fatty acids in heart, namely transcriptional control by the fatty acid-activated nuclear receptor, PPAR α. These results extend the gene regulatory paradigm established for the cardiac FAO cycle to mitochondrial long-chain fatty acid import, a highly regulated step in the myocardial lipid utilization pathway.

Prior to this report, several lines of evidence suggested that fatty acids induce the expression of M-CPT I and other enzymes in the cellular fatty acid utilization pathway. First, the activity of CPT I and FAO cycle enzymes is up-regulated in the myocardium of fasted rats and mice (13, 26, 39). Second, long-chain fatty acids induce expression of CPT I in hepatocytes and pancreatic islet cells in culture (40, 41). Third, FAO enzyme expression is induced by fasting in liver and heart (39). The results shown here demonstrate that M-CPT I gene transcription is activated by long-chain fatty acids via FARE-1, a PPARα response element. The activation of FARE-1 is acyl-chain length-specific and can be conferred to a heterologous promoter. Moreover, the reduced expression of M-CPT I in the PPARα−/− mouse heart suggests that, in vivo, fatty acids influence the basal transcription of this gene which is consistent with the presence of circulating lipids and reliance on fatty acids as the chief energy substrate in the adult mammalian heart. These results strongly suggest that intracellular fatty acid derivatives, several of which are known to activate PPARα, comprise a metabolic signaling pathway in heart.

The role of PPARα in the control of hepatic lipid metabolism is well established. PPARα was first identified as a transcription factor involved in the hepatic response to peroxisome proliferators (42). The expression of PPARα target genes encoding enzymes involved in peroxisomal, cytochrome P450, and mitochondrial FAO is reduced in the liver of mice null for PPARα (28). Further, the expected induction of peroxisomal PPARα target enzymes in response to peroxisome proliferators is absent in PPARα−/− mice (28). In addition to liver, PPARα is expressed abundantly in tissues with high capacity for fatty acid oxidation such as heart, kidney, and brown adipose tissue. The role of PPARα in extrahepatic tissues has not been characterized. Our results demonstrate one important function for PPARα in heart: transcriptional control of the gene encoding M-CPT I, a pivotal enzyme in the uptake of long-chain fatty acids into mitochondria. Taken together with the previous observation that the promoter of the gene encoding the cardiac-enriched mitochondrial FAO cycle enzyme medium-chain acyl-CoA dehydrogenase contains a PPARα response element (30), our results indicate that PPARα regulates myocardial as well as hepatic lipid metabolism.

In summary, we have demonstrated that long-chain fatty acids regulate M-CPT I gene expression in heart through PPARα. We speculate that this transcriptional regulatory mechanism is activated as a component of the coordinate control of myocardial fatty acid utilization pathways following birth and is altered in pathophysiologic settings such as cardiac hypertrophy and failure.

Acknowledgments—We thank Dr. Frank Gonzalez (National Cancer Institute) for the use of the PPARα−/− mice, Dr. Tod Gulick (Harvard University) for helpful discussions, and Kelly Hall for expert secretarial assistance.

Note Added in Proof—Recently, Mascaro et al. (43) described the regulation of M-CPT I gene expression by PPARα.

REFERENCES

1. Neely, J. R., Rovetto, M. J., and Oram, J. F. (1972) Prog. Cardiovasc. Dis. 15, 289–329
2. Bing, R. J. (1955) Harvey Lect. 50, 27–70
3. Taegtmeyer, H. (1994) Curr. Protoc. Cardiovasc. Med. 47, 11–57
4. Bishop, S. P., and Altschuld, R. A. (1970) Am. J. Physiol. 218, 153–159
5. Taegtmeyer, H., and Overturf, M. L. (1988) Hypertension 11, 416–426
6. Scheuer, J. (1993) Circulation 87, V154–V157
7. Moalic, J.-M., Charlemagne, D., Mansier, P., Chevalier, B., and Swyngedauw, B. (1983) Circulation 77, II21–II26
8. Takeyama, Y., Kogai, Y., Yamaoka, S., Shiba, N., Chida, M., Takahashi, T., Ido, T., Ishida, N., and Takishima, T. (1995) Cardiovasc. Res. 29, 763–767
9. Massie, B. M., Schaefer, S., Garcia, J., McKirnan, D., Schwartz, G. G., Wisse, J., A., Weiner, M. W., and White, F. C. (1995) Circulation 91, 1814–1823
10. Alpern, P., and Muller, L. A. (1982) Circ. Res. 50, 491–500
11. Christe, M. E., and Rodgers, R. L. (1984) J. Mol. Cell. Cardiol. 16, 1371–1375
12. Kelly, M. N., Gorden, T. A., Park, S., McCune, S. A., and Kelly, D. P. (1996) Circulation 94, 2837–2842
13. Disch, D. L., Rader, T. A., Cresci, S., Leone, T. C., Barger, P. M., Vega, R., Wood, P. A., and Kelly, D. P. (1990) Mol. Cell. Biol. 16, 4043–4051
14. Sack, M. N., Dieth, D., Rockman, H., and Kelly, D. P. (1997) Proc. Natl. Acad. Sci. U.S.A. 94, 6438–6443
15. Luesing, T. C., Cresci, S., Carson, M. E., Zhang, Z., Lala, D. S., Strauss, A. W., Kelly, D. P. (1995) J. Biol. Chem. 270, 16308–16314
16. Carter, M. E., Gulick, T., Moore, D. D., and Kelly, D. P. (1994) Mol. Cell. Biol. 14, 4360–4372
17. McGarry, J. D., Wohlet, K. F., Kawajima, M., and Foster, D. W. (1989) Diabetes Metab. Rev. 5, 271–284
18. McGarry, J. D. (1995) Biochem. Soc. Trans. 23, 321–324
19. Esser, V., Britton, C. H., Weiss, B. C., Foster, D. W., and McGarry, J. D. (1993) J. Biol. Chem. 268, 5817–5822
20. Britton, C. H., Schultz, R. A., Zhang, B., Esser, V., Foster, D. W., and McGarry, J. D. (1995) Proc. Natl. Acad. Sci. U.S.A. 92, 1984–1988
21. Yamazaki, N., Shinohara, Y., Shima, A., and Terada, H. (1995) FEBS Lett. 363, 41–45
22. Yamazaki, N., Shinohara, Y., Shima, A., Yamana, T., and Terada, H. (1996) Biochim. Biophys. Acta 1275, 157–164
23. Esser, V., Brown, N. F., Cowan, A. T., Foster, D. W., and McGarry, J. D. (1996) J. Biol. Chem. 271, 6972–6977
24. McGarry, J. D., Mills, S. E., Long, C. S., and Foster, D. W. (1983) Biochem. J. 214, 21–28
25. Singh, B., Stakkestad, J. A., Bremer, J., and Borrebaek, B. (1983) Anal. Biochem. 138, 107–111
26. Kelly, D. P., Gorden, T. A., Alpers, D. J., and A., Strauss, A. W. (1989) J. Biol. Chem. 264, 18921–18925
27. Levy, F. H., and Kelly, D. P. (1997) Am. J. Physiol. 41, C457–C465
28. Lee, S. B. T., Pineau, T., Drago, J., Lee, E. J., Owens, J. W., Kroetz, D. L.,
Fernandez-Salguero, P. M., Westphal, H., and Gonzalez, F. J. (1995) Mol. Cell. Biol. 15, 3012–3022
29. Gorman, C. (1985) in DNA Cloning: A Practical Approach (Glover, D. M., ed) Vol. II, pp. 143–190, IRL Press, Oxford
30. Gulick, T., Cresci, S., Cairn, T., Moore, D. D., and Kelly, D. P. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 11012–11016
31. van der Leij, F. R., Takeu, J., van der Veen, A. Y., Terpstra, P., and Kuipers, J. R. G. (1997) Biochim. Biophys. Acta 1352, 123–128
32. Yamazaki, N., Yamanaka, Y., Hashimoto, Y., Shinozuka, Y., Shima, A., and Terada, H. (1997) FEBS Lett. 409, 401–406
33. Lemberger, T., Desvergne, B., and Wahli, W. (1996) Annu. Rev. Cell Dev. Biol. 12, 335–363
34. Klierwer, S. A., Sundseth, S. S., Jones, S. A., Brown, P. J., Wisely, G. B., Kohle, C. S., Devchand, P., Wahl, W., Willen, T. M., Lenhard, J. M., and Lehmann, J. M. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 4318–4323
35. Forman, B. M., Chen, J., and Evans, R. M. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 4312–4317
36. Klierwer, S. A., Umesono, K., Noonan, D. J., Heyman, R. A., and Evans, R. M. (1993) Nature 358, 771–774
37. Gebel, T., Arund, M., and Oesch, F. (1992) FEBS Lett. 309, 37–40
38. Aoyama, T., Peters, J. M., Iritani, N., Nakajima, T., Furihata, K., Hashimoto, T., and Gonzalez, F. J. (1998) J. Biol. Chem. 273, 5678–5684
39. Nagao, M., Parimoo, B., and Tanaka, K. (1993) J. Biol. Chem. 268, 24114–24124
40. Assimacopoulos-Jeannet, F., Thumelin, S., Roche, E., Esser, V., McGarry, J. D., and Prentki, M. (1997) J. Biol. Chem. 272, 1659–1664
41. Chatelain, P., Kohl, C., Esser, V., McGarry, J. D., and Prentki, M. (1996) Biochem. 235, 789–798
42. Isserman, I., and Green, S. (1990) Nature 347, 645–650
43. Mascaro, C., Acosta, E., Ortiz, J., Marrero, P., Hegardt, F., and Haro, D. (1998) J. Biol. Chem. 273, 8560–8563