Control of Human Muscle-type Carnitine Palmitoyltransferase I Gene Transcription by Peroxisome Proliferator-activated Receptor*

Cristina Mascaró, Elena Acosta, José A. Ortiz, Pedro F. Marrero, Fausto G. Hegardt, and Diego Haro§

From the Unit of Biochemistry, School of Pharmacy, University of Barcelona, 08028 Barcelona, Spain

The expression of several genes involved in intra- and extracellular lipid metabolism, notably those involved in peroxisomal and mitochondrial β-oxidation, is mediated by ligand-activated receptors, collectively referred to as peroxisome proliferator-activated receptors (PPARs). To gain more insight into the control of expression of carnitine palmitoyltransferase (CPT) genes, which are regulated by fatty acids, we have examined the transcriptional regulation of the human MCPT I gene. We have cloned by polymerase chain reaction the 5'-flanking region of this gene and demonstrated its transcriptional activity by transfection experiments with the CAT gene as a reporter. We have also shown that this is a target gene for the action of PPARs, and we have localized a PPAR responsive element upstream of the first exon. These results show that PPAR regulates the entry of fatty acids into the mitochondria, which is a crucial step in their metabolism, especially in tissues like heart, skeletal muscle and brown adipose tissue in which fatty acids are a major source of energy.

The incorporation of activated long-chain fatty acids into the mitochondria to be catabolized through β-oxidation is produced by the mitochondrial carnitine palmitoyltransferase (CPT)1 enzyme system. CPT I, the outer membrane component of this system, is the main control point in the β-oxidation pathway. CPT I is thus a suitable site for pharmacological control of fatty acid oxidation in conditions such as diabetes or heart diseases.

Two isoforms of CPT I have been described, which have been designated LCPT I and MCPT I since these isoforms are mainly expressed in liver and muscle respectively. The MCPT I gene is expressed not only in skeletal muscle but also in heart and brown and white adipose tissue (1–4). This expression pattern may be of great significance since fatty acids are a major source of energy for heart, skeletal muscle, and brown adipose tissue.

The CPT I gene expression is regulated by fatty acids and peroxisome proliferators (5, 6). To gain more insight into the control of CPT I gene expression by fatty acids, we have examined the transcriptional regulation of CPT I genes. The expression of several genes involved in intra- and extracellular lipid metabolism, notably those involved in peroxisomal and mitochondrial β-oxidation, is mediated by ligand-activated receptors collectively referred to as peroxisome proliferator-activated receptors (PPARs); these receptors are members of the nuclear receptor superfamily. PPARs are activated by a wide array of peroxisome proliferators and also by natural and synthetic fatty acids (7, 8), antidiabetic drugs (9, 10), prostaglandin F2α (10), and leukotriene B4 (11).

We have amplified by polymerase chain reaction (PCR) the 5’ region of the human heart and brown adipose tissue CPT I gene and demonstrate, first, the transcriptional activity of this fragment and, second, the presence of a PPRE in the 5’-flanking region of this gene. In CV1 cells, the activation of the CPT I gene by PPAR was dependent on the addition of exogenous ligands.

**EXPERIMENTAL PROCEDURES**

Plasmids—pCPTCAT, containing an 882-base pair fragment of the human MCPT I gene, was constructed by the application of the PCR using a pair of oligonucleotide primers, CPTF (5'-CCTGGTCTGAGCAGTTAGAATAA) and CPTR (5'-GGAGTGTATCCAGACGCAGATTAG), corresponding to coordinates −909 to −889 and +126 to +92, respectively, of the human MCPT I gene (12) and human genomic DNA as a template. The PstI-AvrII-digested PCR product was cloned into the PstI-BamHI sites of chloramphenicol acetyltransferase (CAT) vector pCAT-BASIC reporter gene (Promega). To confirm the sequence, the PCR-amplified fragment was automatically sequenced using the fluorescent terminator kit (Perkin-Elmer).

Heterologous promoter plasmids were constructed in the herpesvirus thymidine kinase gene promoter upstream of the CAT reporter gene pBLCAT2 (13). pTRCATpCPT contains a fragment corresponding to coordinates −774 to −775 of the mitochondrial HMG-CoA synthase gene. It was constructed by cloning the oligonucleotide 5’-agetTGCAGCTTTCTCCATACGTGT annealed to 5’-gcACAAATGTAGAAAAGGCTA into pBLCAT2 (nucleotides designated in lowercase were added to provide cohesive HindIII-SalI ends at the 5’ and 3’ termini, respectively). The insert in this plasmid had the same 5’→3’ orientation as found in the human MCPT I gene promoter. DNA sequence analysis, by the fluorescent terminator kit was performed to confirm insert orientation.

Cell Culture and Transfections—CV1 cells were cultured in minimal essential media supplemented with 10% fetal calf serum. Cells were typically cotransfected with 10 μg of the reporter MCPT I-CAT gene construct and, when indicated, with 1 μg of effector plasmids expressing full-length cDNAs for mouse PPARα, PPARγ2, or PPARδ. 4 μg of plasmid pRSVβGal (Rous sarcoma virus promoter β-galactosidase) was included as internal control in cotransfections. Transfection experiments were carried out by the calcium-phosphate method as described (14, 15). After removal of the calcium-phosphate-DNA precipitate, cells were re-fed with medium supplemented with 10% delipidated calf serum. Experiments with ligand included either vehicle (dimethyl sulfoxide or ethanol) or ligand (10 μM PGJ2 (15-deoxy-Δ12,13-prostaglandin J2), 30 μM LY-171883, or 30 μM linoleic acid). All ligands used were from Sigma. Cells were harvested 48 h after re-feeding.

β-Galactosidase and CAT Assays—Extracts of harvested cells were prepared by liquid nitrogen freeze/thaw disruption (three times) after

* This research was supported by Grant PB84–0840 from Dirección General de Investigación Científica y Técnica. 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.

§ To whom correspondence should be addressed: Unitat de Bioquimica, Facultat de Farmàcia, Avda. Diagonal, 643, 08028 Barcelona, Spain.

† Present address: IGIBM, CNRS INSERM, Université Louis Pasteur, C.U. de Strasbourg 67404, France.

© 1998 by The American Society for Biochemistry and Molecular Biology, Inc.

Printed in U.S.A.
Human MCPT I Is a Target for PPAR

**RESULTS AND DISCUSSION**

The Human MCPT I Gene 5'-Flanking Region Contains a Consensus PPRE—PPAR α, γ, and δ bind to the MCPT I PPRE as heterodimers with RXR. To elucidate the control of CPT I gene expression by fatty acids, we have examined the transcriptional regulation of CPT I genes. A BLAST search performed using the NCBI BLAST WWW Server revealed that the sequence for the human muscle type CPT I gene was included in the sequence of a BAC clone containing a part of the q arm of chromosome 22 (GenBank™ accession number U62317). The analysis of the 5'-flanking region of this gene was linked to a promoter-less bacterial CAT gene. These plasmids were introduced into cultured CV1 cells by the calcium-phosphate method, with or without an expression vector for PPARs, together with a plasmid that contains the β-galactosidase coding region driven by the SV40 promoter as a control of the effi-

---

**Fig. 1.** A, scheme of the 5'-flanking region of the human MCPT I. Indicated (open rectangles) are the positions of exons 1A, 1B, and 2 containing the translation initiation codon and the presence of the PPRE (screened box). B, comparison of the sequence of the proposed PPRE with the consensus (CONS).

---

Electrophoretic Mobility Shift Analysis—2 µl of mPPARα, mPPARγ, and mPPARδ with or without hRXRα (2 µl) synthesized in vitro were preincubated on ice for 10 min in 10 mM Tris-HCl, pH 8.0, 40 mM KCl, 0.05% (v/v) Nonidet P-40, 6% glycerol, 1 mM dithiothreitol, and 2 µg of poly(dI-dC). The total amount of reticulocyte lysate was kept constant in each reaction (4 µl) by the addition of unprogrammed lysate. For competition experiments, a 25–100-fold molar excess of MCPT I PPRE or MCPT I MPPRE double-stranded probes, relative to the labeled probe, was included during preincubation. MCPT I PPRE is the fragment corresponding to coordinates −774 to −755 of the MCPT I gene, which was used to prepare pTKCATCPT. MCPT I MPPRE is the fragment corresponding to coordinates −782 to −748 of the MCPT I gene, but the nucleotides corresponding to the PPAR binding sequence have been mutated (CACATGGTGACCctcgagggatccTTGGCTATTT, nucleotides described in lowercase correspond to those that have been changed from the wild type sequence). Next, 2 ng of MCPT I PPRE, 32P-labeled by fill-in with Klenow polymerase, was added, and the incubation was continued for 15 min at room temperature. The final volume for all reactions was 20 µl. Samples were electrophoresed at 40°C on a 4.5% polyacrylamide gel in 0.5× TBE buffer (45 mM Tris, 45 mM boric acid, 1 mM EDTA, pH 8.0).

---

**Fig. 2.** Electrophoretic mobility shift assay of the muscle CPT I PPRE with PPAR-RXR heterodimers. PPAR α, γ, and δ and RXRα were translated in vitro, incubated with the proposed CPT I PPRE labeled probe, and analyzed by electrophoretic mobility shift assay. Additions were as indicated on the top of the figure. Shown in panel B is a competition of the complex PPARα-RXR-PPRE with a 25–100-fold molar excess of two different unlabeled oligonucleotides: MCPT I PPRE, containing the proposed PPRE, or MCPT I MPPRE, with the proposed PPRE mutated. All isoforms of PPAR are identically competed (data not shown).

---

not able to compete with the wild-type probe for the formation of the complex. The binding of the three subtypes of PPAR to the MCPT I PPRE is as strong as the binding to the mitochondrial HMG-CoA synthase PPRE, which allows the formation of the strongest complexes for all PPAR subtypes (17) (data not shown).
The expression vector for mPPAR thymidine kinase gene promoter. Average values of β-galactosidase-normalized CAT activity (means ± S.D.), from three independent transfections with two plates each, are expressed as -fold induction relative to the activity in the absence of both PPARs and activators.

Fig. 3. PPARα- and γ-dependent activation of the human MCPT I. pCPTCAT was cotransfected with expression vectors for PPARα, γ, and δ into CV1 cells either in the absence or presence of LY, PGJ2, or linoleic acid, respectively as activators of the different isoforms of PPAR. Average values of β-galactosidase-normalized CAT activity (means ± S.D.), from three independent transfections with two plates each, are expressed as -fold induction relative to the activity in the absence of expression vectors and activators.

ciency of the transfection. Following transfection, cells were incubated in the presence or absence of a PPAR activator, and after 48 h, the cells were harvested and CAT activity measured. As can be seen in Fig. 3, cotransfection of PPAR expression vectors lead to a marked increase in CAT activity in the presence of the PPAR activators. Surprisingly, even though PPARδ is able to bind the MCPT I PPRE in vitro, it does not activate the expression of the chimeric gene even in the presence of linoleic acid as activator.

The Human MCPT I PPRE Confers PPAR Responsiveness to Thymidine Kinase Gene Promoter—Next a pair of oligonucleotides containing the human MCPT I PPRE were inserted into pBLCAT2, a plasmid containing the CAT gene under the control of the thymidine kinase gene promoter. As can be seen in Fig. 4, this sequence conferred PPAR responsiveness to the otherwise unresponsive thymidine kinase gene promoter. The results demonstrate that this human MCPT I element is able to confer PPARα and γ responsiveness both on its natural context and on a normally unresponsive promoter.

Our data provide evidence that extends the influence of PPARs in the regulation of mitochondrial fatty acid metabolism. They influence not only activation, through the control of acyl-CoA synthetase (18), β-oxidation, through medium-chain acyl-CoA dehydrogenase (19), and ketogenesis, through mitochondrial HMG-CoA synthase (20), but also, mitochondrial import through CPT I (Fig. 5). These results also support the suggestion that in higher organisms, as well as in bacteria and yeast, there is metabolic control of gene expression.

Non-insulin-dependent diabetes mellitus (NIDDM) affects between 5 and 20% of the population in Western industrialized societies (21), but despite decades of research, the pathogenesis of NIDDM remains incompletely understood. It has recently been suggested that NIDDM may have more to do with abnormalities in fat than in carbohydrate metabolism (22). There is evidence that free fatty acids are an important link between obesity and insulin resistance and NIDDM (reviewed in Ref. 23). There is also evidence that the antidiabetic action of the thiazolidinediones (insulin sensitizers that significantly reduce glucose, lipid, and insulin levels in animal models of NIDDM and obesity) are directly mediated through binding to PPARγ and the resulting active conformation of the receptor (24), whose expression is high in the skeletal muscle of obese and type II diabetic subjects (25). Our hypothesis is that the transcriptional control of the muscle type CPT I gene produced by thiazolidinedione-activated PPARγ may contribute to the antidiabetic effect of these agents by controlling glucose utilization in skeletal muscle through modulation of fatty acids metabolism in such cells, and studies to examine this hypothesis are now under way.

4. Esser, V., Brown, N. F., Cowan, A. T., Foster, D. W., and McGarry, J. D. (1996) J. Biol. Chem. 271, 6972–6977
5. Asino, G., Serra, D., and Hegardt, F. G. (1994) Biochim. Pharmacol. 47, 1373–1379
6. Chatelain, F., Kohl, C., Esser, V., McGarry, J. D., Girard, J., and Pogier, J. P. (1996) Eur. J. Biochem. 235, 789–796
7. Forman, B. M., Chen, J., and Evans, R. M. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 4312–4317
8. Kliever, S. A., Sundested, S. S., Jones, S. A., Broen, P. J., Wisely, G. B., Koble, C. S., Devchand, P., Wahl, W., Willson, T. M., Lenhard, J. M., and Lehmann, J. M. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 4318–4323
9. Lehmann, J. M., Moore, L. B., Smith-Oliver, T. A., Wilkinson, W. O., Willson, T. M., and Kliever, S. A. (1995) J. Biol. Chem. 270, 12956–12958
10. Forman, B. M., Tomonoz, P., Chen, J., Brun, R. P., Spiegelman, B. M., and Evans, R. (1995) Cell 83, 803–812
11. Devchand, P. R., Keller, H., Peters, J. M., Vázquez, M., Gonzalez, F. J., and Wahl, W. (1996) Nature 384, 39–43
Human MCPT I Is a Target for PPAR

12. Yamazaki, N., Yamanka, Y., Hashimoto, Y., Shinohara, Y., Shima, A., and Terada, H. (1997) FEBS Lett. 409, 401–406
13. Luckow, B., and Schütz, G. (1987) Nucleic Acids Res. 15, 5490
14. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. and Struhl, K. (1987) Current Protocols in Molecular Biology, pp. 9.14–9.1.6, Green Publishing Associates/Wiley-Interscience, New York
15. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., pp. 16.66–16.67, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
16. Palmer, C. N. A., Hou, M-H., Griffin, K. J., and Johnson, E. F. (1995) J. Biol. Chem. 270, 16114–16121
17. Juge-Aubry, C., Pernin, A., Favez, T., Burger, A. G., Wahl, W., Meier, C. A., and Desvergne, B. (1997) J. Biol. Chem. 272, 25252–25259
18. Schoonjans, K., Watanabe, M., Suzuki, H., Mahfoudi, A., Key, G., Wahl, W., Grimaldi, P., Staels, B., Yamamoto, T., and Auwerx, J. (1995) J. Biol. Chem. 270, 19269–19276
19. Gulick, T., Cresce, S., Caira, T., Moore, D. D., and Kelly, D. P. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 11012–11016
20. Rodriguez, J. C., Gil-Gómez, G., Hegardt, F. G., and Haro, D. (1994) J. Biol. Chem. 269, 18767–18772
21. Harris, M. I. (1999) Diabetes Care 22, 464–474
22. McGarry, J. D. (1992) Science 258, 766–770
23. Boden, G. (1997) Diabetes 46, 3–10
24. Berger, J., Bailey, P., Biswas, C., Cullinan, C. A., Doolber, T. W., Hayes, N. S., Saperstein, R., Smith, R. G., and Leibowitz, M. D. (1996) Endocrinology 137, 4189–4195
25. Park, K. S., Ciaraldi, T. P., Abrams-Carter, L., Mudaliar, S., Nikouлина, S. E., and Henry, R. R. (1997) Diabetes 46, 1230–1234