Regulation of liver carnitine palmitoyltransferase I gene expression by hormones and fatty acids

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Abstract

This brief review focuses on the transcriptional regulation of liver carnitine palmitoyltransferase I (L-CPT I) by pancreatic and thyroid hormones and by long-chain fatty acids (LCFA). Both glucagon and 3,3',5-tri-iodothyronine (T₃) enhanced the transcription of the gene encoding L-CPT I, whereas insulin had the opposite effect. Interestingly, the transcriptional effect of T₃ required, in addition to the thyroid-responsive element, the co-operation of a sequence located in the first intron of L-CPT I gene. Non-esterified fatty acids rather than acyl-CoA ester or intra-mitochondrial metabolite were responsible for the transcriptional effect on the gene encoding L-CPT I. It was shown that LCFA and peroxisome proliferators stimulated L-CPT I gene transcription by distinct mechanisms. Peroxisome proliferator stimulated L-CPT I gene transcription through a peroxisome-proliferator-responsive element (PPRE) located at -2846 bp, whereas LCFA induced L-CPT I gene transcription through a peroxisome-proliferator-activated receptor α (PPARα)-independent mechanism owing to a sequence located in the first intron of the gene.

Introduction

Long-chain fatty acids (LCFA) have an essential role as an energy source for many tissues of the organism, especially the heart and skeletal muscles. In these tissues, LCFA are oxidized into the mitochondria by β-oxidation to provide ATP. In the liver, LCFA have an additional role. The acetyl-CoA generated by β-oxidation can be used for the production of ketone bodies (acetoacetate and β-hydroxybutyrate), which are released into the circulation for further use as alternative fuels for extrahepatic organs, especially the brain. In adult mammals, ketogenesis occurs mainly in the liver, in response to fasting, diabetes and prolonged exercise. In suckling rats, ketogenesis also occurs in the intestine and to a smaller extent in the kidney (reviewed in [1]). In mammalian liver, fatty acid oxidation occurs in both mitochondria and peroxisomes. During the past decade the role of peroxisomes received considerable attention since the discovery that hypolipidaemic drugs induce peroxisomal proliferation in rodents and after the characterization of related human diseases [2]. However, the contribution of peroxisomal β-oxidation represents only 10–15% of total β-oxidation in the neonatal rat liver [3,4].

Hepatic fatty acid oxidation involves four specific steps (Scheme 1): (1) uptake and activation of fatty acids into fatty acyl-CoA, (2) translocation of fatty acyl-CoA into the mitochondria, (3) β-oxidation of fatty acyl-CoA and (4) ketone body production. The capacity for long-chain fatty acid oxidation is very low in fetal tissues and it increases markedly during the neonatal period (reviewed in [1]). The postnatal development of liver mitochondrial LCFA oxidation and ketogenesis is closely related to the appearance of the carnitine palmitoyltransferase (CPT) system. The CPT system allows the transfer of LCFA inside the mitochondria and is composed of three distinct entities: CPT I, localized in the outer mitochondrial membrane; carnitine-acylcarnitine translocase; and CPT II, localized on the inner mitochondrial membrane (reviewed in [5]). It is generally accepted that the oxidation of LCFA is regulated at the level of CPT I through different mechanisms: (1) changes in CPT I activity, (2) changes in the concentration of malonyl-CoA (a physiological inhibitor of this enzyme), and (3) changes in the sensitivity of CPT I to malonyl-CoA inhibition.

In the rat, profound nutritional and hormonal modifications occur immediately after birth (reviewed in [1]). The high-carbohydrate/low-fat diet of the fetus is abruptly replaced by a high-
fat/low-carbohydrate diet (milk). This dramatic change in nutrition increases plasma glucagon and decreases plasma insulin, and there is a gradual increase in the concentration of plasma thyroid hormones (reviewed in [1]). Under these conditions, there is a marked increase in CPT I gene transcription, an accumulation of CPT I mRNA and the synthesis of CPT I protein (reviewed in [1]). These results suggest that the regulation of CPT I gene expression is dependent on hormonal and/or nutritional factors.

The aim of the present review is to discuss recent advances in the understanding of L-CPT I gene expression, with emphasis on the possible mechanisms by which LCFA and hormones mediate their effects on gene transcription.

### Scheme I

Schematic representation of the mitochondrial metabolism of fatty acids in the liver

Abbreviation: MCFA, medium-chain fatty acids.

#### Regulation of L-CPT I gene expression by hormones

The regulation of specific gene expression in response to changes in the hormonal environment has become a major aspect of modern biology. Here we briefly review the role of insulin, glucagon and thyroid hormones in the regulation of L-CPT I gene expression.

Very few studies have been performed to address the question of direct effects of hormones on the regulation of L-CPT I gene expression in vitro with cultured hepatocytes or hepatoma cells. Recent studies in our laboratory have shown that the addition of glucagon or dibutyryl cAMP to cultured fetal hepatocytes increased the concentration of L-CPT I mRNAs in a dose-dependent manner.
manner [6]. This resulted from a stimulation of gene transcription without modification of CPT I mRNA half-life [6]. In cultured fetal rat hepatocytes, insulin antagonized the cAMP-induced accumulation of CPT I mRNA [6]. Insulin also decreased L-CPT I mRNA in H4IIE rat hepatoma cells without affecting the half-life of its mRNA [7]. By contrast, neither cAMP nor insulin affects CPT II gene expression [6]. This marked difference in gene regulation by pancreatic hormones was probably due to differences in the promoter structure of these two genes. There was no CCAAT-enhancer-binding protein (CREB)-binding site in the proximal promoter region of the CPT II gene [8], whereas four CREB-binding sites were present in the promoter region of the gene encoding L-CPT I (J.-F. Louet, J.-F. Decaux, J.-P. Pegorier and J. Girard, unpublished work).

Thyroid hormones [3,3',5-tri-iodothyronine (T3)] also have profound effects on β-oxidation in the liver (reviewed in [9]). In the rat, the hypothyroid state increases the oxidation of long-chain fatty acids. Under these conditions, a 5-fold increase in L-CPT I mRNA concentration was observed in comparison with control animals. By contrast, a marked decrease in CPT I mRNA level was observed in hypothyroid rats. The effects of T3 are mediated through the binding of the thyroid hormone receptor (TR) to T3 response elements (TREs) in the promoter of several genes (reviewed in [10]). The consensus sequence of TRE contains a direct repeat of two AGGTCA motifs separated by four nucleotides (DR4). TR binds to DNA as a heterodimer with the retinoid X receptor (RXR). Two isoforms of TR are known: TRα and TRβ [11]. In the liver, the TRβ isoform seems to mediate many of the effects of T3. The induction of several hepatic genes (S14 and malic enzyme) in response to T3 is suppressed in TRβ knock-out mice [12]. Recently, it has been shown that L-CPT I promoter contains a functional TRE located at approx. −3 kb from the cap site of the gene [13]. Studies of DNA–protein binding reveal that TRβ binds this element as a heterodimer with RXRα. This interaction is not sufficient to obtain maximal induction of the gene encoding CPT I in response to T3. Co-operation with a sequence located in the first intron of the gene encoding CPT I is required [13]. Similar results have been reported for other hepatic genes such as those for glutamine synthase [14] and the very-low-density apolipoprotein II (apoVLDLII) [15].

**Regulation of L-CPT I gene expression by LCFA**

LCFA are important nutrients for growth and development. Apart from their role in membrane structure, metabolism and signal transduction, it has become increasingly clear that they have also a role in the expression of a number of genes encoding enzymes involved in carbohydrate metabolism and lipid metabolism. Here we focus our discussion on the most recent reports on the effects of LCFA on L-CPT I gene expression.

In the liver, the expression of a number of genes is stimulated by LCFA in a physiologically relevant manner. LCFA caused a marked increase in the specific CPT I mRNA level in primary culture of fetal rat hepatocytes [6]. This accumulation, which is time- and dose-dependent, results from two mechanisms: (1) stimulation of gene transcription (run-on assays), (2) stabilization of the mRNA level. Many other genes are regulated at both the transcriptional and the post-transcriptional levels, such as stearoyl-CoA desaturase [16] and the insulin-regulatable glucose transporter Glut-4 in adipocytes [17]. In contrast, other genes are regulated only at the post-transcriptional level, such as the adipocyte lipid-binding protein (aP2) [18].

The inhibitory effect of LCFA on hepatic lipogenic gene expression seems to be restricted to polyunsaturated fatty acids (PUFA) (reviewed in [19]). In contrast, the stimulatory effect of LCFA on L-CPT I gene expression is independent of the degree of saturation of LCFA [6]. A similar phenomena has been reported for the genes encoding liver fatty-acid-binding protein [20], medium-chain acyl-CoA dehydrogenase [21] and mitochondrial 3-hydroxymethylglutaryl-CoA synthase [22]. Interestingly, the gene encoding L-CPT I is also induced by LCFA in a pancreatic β-cell line INS 1 [23].

**Are the effects of LCFA on gene transcription mediated by a metabolite?**

The nature of the metabolite(s) triggering the transcriptional effect of LCFA in mammals is still controversial. For example, recent studies suggested that mitochondrial oxidation of LCFA is required to exert their inhibitory effect on gene expression in rat pancreatic β-cells [24]. In contrast, several studies have shown that when the oxidation of LCFA is blocked by specific inhibitors (tetradecylglycidic acid) or by a non-metabolizable analogue of fatty acids (2-
bromopalmitate), the stimulation of LCFA on gene expression is still present. This was clearly demonstrated for aP2 [25], liver CPT I [6], long-chain fatty acyl-CoA synthase (ACS) [26] and liver-type fatty-acid-binding protein in distal ileum [27]. This suggested that the effects could be mediated by LCFA itself or by their CoA derivatives. The role of long-chain fatty acyl-CoA in the regulation of gene transcription has been clearly established in bacteria (reviewed in [28]). Acyl-CoAs are also active in modulating gene transcription in yeast (reviewed in [28]). However, there is no clear demonstration of the role of LCFA or their corresponding CoA esters as modulators of gene expression in higher eukaryotes. Indirect evidence suggests that LCFA rather than their acyl-CoA esters could be active components in gene expression: (1) in COS-7 cells transfected with the peroxisomal acyl-CoA oxidase promoter linked to the chloramphenicol acetyltransferase reporter gene, the transcription was increased more efficiently by free arachidonic acid than by arachidonyl-CoA [29]; (2) in an adipocyte cell line, the induction of aP2 gene transcription by LCFA occurred before the expression of the gene encoding ACS [30]; (3) in the FaO hepatoma cell line, the stimulation of CPT I gene expression by LCFA was maintained even in the presence of ACS inhibitors [31,32]. These experiments, with chimaeric gene transfection, metabolic inhibitors and assays in vitro, provide only indirect evidence and it is clear that additional experiments will be required before it can be concluded that LCFA, and not their acyl-CoA derivatives or other metabolites, are the active modulators of gene transcription.

**Contribution of nuclear receptors to LCFA-induced gene expression**

The molecular mechanisms by which LCFA or their metabolites regulate gene transcription are still unclear in mammalian cells. On the basis of the comparison between the effects of LCFA and the peroxisome proliferator, it was suggested that LCFA could mediate gene transcription through the activation of nuclear receptors of the steroid-thyroid superfamily, the peroxisome-proliferator-activated receptor (PPAR) (reviewed in [33]). Several mechanisms have been proposed for PPAR activation by fatty acids: a ligand-mediated process, activation through binding to a cytosolic heat shock protein (HSP72) or activation secondary to a phosphorylation-mediated MAP (mitogen-activated protein) kinase (reviewed in [31,34]). The modulation of gene transcription is due to the binding of the heterodimer PPAR/RXR to a specific DNA sequence, the peroxisome-proliferator-responsive element (PPRE) (reviewed in [33]). PPRE was initially defined as a direct repeat of two core recognition motifs AGGTCA spaced by one nucleotide (DR1) [35]. Further analyses have completed this first definition by adding the following properties: an imperfect core DR1, an adenine as the spacing nucleotide between the two hexamers, and an extended 5′-AAct sequence, which seems to be crucial for the polarity and for the selectivity of recognition (reviewed in [33,36]). This modulation is accomplished with the help of co-activator complexes that bind to the AF2 sequence located in helix 12 of the ligand-binding domain of the nuclear receptor (reviewed in [37,38]). In keeping with this, it has been shown that LCFA stimulated the transcription of a DNA construct consisting of the gene promoter for human muscle CPT I (−1025 bp to −12 bp from the cap site) linked to a luciferase reporter gene in transfected HepG2 hepatoma cells [39]. However, this stimulation by LCFA required the co-transfection of a PPARα construct, so it is questionable whether the overexpression of nuclear receptors reflect a physiologically relevant process. In fact, if the transcriptional effect of LCFA was due to the synthesis of a putative ligand of PPARα, it is difficult to understand why the induction of the muscle CPT I–luciferase chimaeric gene in response to LCFA did not occur with endogenous PPARα. The role of PPAR in mediating transcriptional effects of LCFA is still a matter of controversy. For instance, it was shown that the repression of hepatic Spot 14 gene transcription (a gene encoding a lipogenic-like enzyme) by PUFA was not mediated though the activation of PPARα [40]. This was confirmed recently with a PPARα-null mouse in which the induction of peroxisomal β-oxidation genes by PUFA was blunted, whereas inhibition of the expression of the gene encoding spot 14, fatty acid synthase or L-pyruvate kinase normally occurred [41,42]. Several experimental results obtained in cultured fetal rat hepatocytes suggest that the effects of LCFA on L-CPT I gene transcription are mediated by a pathway different from that of peroxisome proliferators (Figure 1). First, LCFA induce the gene expression of CPT I but not CPT II, whereas clofibrate (a peroxisome proliferator) enhances the concentration of both CPT I and
CPT II mRNA [6]. Secondly, the LCFA-induced accumulation of CPT I mRNA is inhibited by insulin, whereas the clofibrate-induced expression of the CPT I and II genes is not [6] (Figure 1). Thirdly, the starvation-induced expression of the gene encoding CPT I is maintained in the liver of PPARα-null mice [43–45]. As the expression of this gene is regulated both by LCFA and by the changes in pancreatic hormones [6], its induction during starvation is probably due to the decrease

**Figure 1**

Effects of fatty acids, peroxisome proliferator and insulin on L-CPT I gene expression in cultured hepatocytes from fetal rats

Abbreviations: Lino, linoleate; Clo, clofibrate (a peroxisome proliferator), Palm, palmitate, 2-Br-Palm, 2 bromopalmitate, TDGA, tetradecylglycidic acid.

**Figure 2**

Description of cis-elements in the promoter and first intron of L-CPT I gene

Abbreviations: PP, peroxisome proliferator; TRβ, thyroid receptor β; RXRα, retinoid X receptor; X, unknown protein factor; CRE, cAMP-response element; FA-RE, fatty-acid-responsive element.
in plasma insulin levels and the rise in plasma glucagon levels. Thus the stimulation of the gene encoding L-CPT I by LCFA seems to be a PPARα-independent mechanism. This was confirmed in cultured hepatocytes from PPARα-null mice in which the absence of LCFA-induced L-CPT I gene expression was not due to the absence of PPARα receptor but to an intrinsic defect of response to LCFA because they also failed to stimulate L-CPT I gene expression in wild-type mice [32]. Such a dissociation between the transcriptional effects of LCFA or peroxisome proliferators has already been observed for other genes. ApoA-II [15] and FAT-CD36 genes contain a PPRE but do not respond to LCFA (reviewed in [36]). Moreover, PUFA inhibit the transcription of the Δ5 and Δ6 desaturases [46,47], whereas the same genes are stimulated by peroxisome proliferators.

To investigate further the molecular mechanisms involved in the regulation of L-CPT I gene expression by LCFA and peroxisome proliferators, we performed transient transfection of plasmids containing different sequences of the L-CPT I promoter into cultured hepatocytes and hepatoma cells. The results confirm that clofibrate stimulates L-CPT I through a classical DR1 motif (PPRE), whereas LCFA induce L-CPT I via elements localized in the first intron of the gene [32] (Figure 2). With the use of gel-shift experiments we have shown that the nuclear receptor complex PPARα-RXRα binds to the L-CPT I-PPRE sequence [32]. PPARα alone was unable to bind to this sequence, indicating that heterodimerization with RXRα is necessary for DNA binding [48]. Previous experiments have shown that distinct sequences were responsible for the effects of peroxisome proliferators and PUFA on the hepatic promoter of the Spot 14 gene [40,41]. Similarly, the PUFA-induced suppression of the transcription of the gene encoding L-type pyruvate kinase did not require the presence of PPARα. The heterodimer PPARα–RXRα did not bind the PUFA-responsive element sequence of the L-type pyruvate kinase promoter [42]. Finally, our results demonstrate that LCFA can up-regulate gene expression through PPARα-independent pathways and suggest that the regulation of hepatic gene expression by dietary lipids is more complex than previously suggested. Further studies will be necessary to identify the LCFA response element (FARE) and the protein and co-activators involved in the activation of genes in response to LCFA.

Conclusions
This brief review has underlined the notion that peroxisome proliferators and LCFA can mediate gene transcription (at least for L-CPT I) by distinct mechanisms. This indicates that, for a given gene, its potential for control by LCFA does not necessarily result from the presence of a putative PPRE consensus sequence in the regulatory region of the gene. Moreover, this review has also underlined the importance of the first intron of L-CPT I gene for effects mediated by LCFA and by thyroid hormone.

References
Post-transcriptional regulation of rat carnitine octanoyltransferase

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Abstract

Carnitine octanoyltransferase (COT) produces three different transcripts in rat through cis- and trans-splicing reactions, which can lead to the synthesis of two proteins. The occurrence of the three COT transcripts in rat has been found in all tissues examined and does not depend on sex, fat feeding, peroxisome proliferators or hyperinsulinemia. Rat COT exon 2 contains a putative exonic splicing enhancer (ESE) sequence. Mutation of this ESE (GAAGAAG) to AAAAAAA decreased trans-splicing in vitro, from which it is deduced that this ESE sequence is partly responsible for the formation of the three transcripts. The protein encoded by cis-spliced mRNA of rat COT is inhibited by malonyl-CoA and etomoxir. CDNA species encoding full-length wild-type COT and one double mutant COT were expressed in Saccharomyces cerevisiae. The recombinant enzymes showed full activity towards both substrates, carnitine and decanoyl-CoA. The activity of the doubly mutated H131A/H340A enzyme was similar to that of the rat peroxisomal enzyme but was completely insensitive to malonyl-CoA and etomoxir. These results indicate that the histidine residues His-131 and His-340 are the sites responsible for the interaction of these two inhibitors, which inhibit COT by interacting with the same sites.

Introduction

Carnitine octanoyltransferase (COT) facilitates the transport of medium-chain fatty acids from peroxisomes to mitochondria through the con-

Key words: etomoxir, exonic splicing enhancers, malonyl-CoA, trans-splicing.

Abbreviations used: COT, carnitine octanoyltransferase; CPT, carnitine palmitoyltransferase; ESE, exonic splicing enhancer; etomoxir, 2-[6-(4-chlorophenoxo)hexyl]oxirane carboxylic acid.

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