Biochimica et Biophysica Acta 1819 (2012) 357–365

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagrm

The mouse gene encoding the carnitine biosynthetic enzyme 4-N-trimethylaminobutyraldehyde dehydrogenase is regulated by peroxisome proliferator-activated receptor α

Gaiping Wen, Robert Ringseis, Christine Rauer, Klaus Eder *

Institute of Animal Nutrition and Nutritional Physiology, Justus-Liebig-Universität, Heinrich-Buff-Ring 26–32, 35392 Giessen, Germany

ARTICLE INFO

Article history:
Received 14 September 2011
Received in revised form 9 January 2012
Accepted 10 January 2012
Available online 21 January 2012

Keywords:
Carnitine synthesis
PPARα
Functional PPRE
Target gene

ABSTRACT

Genes involved in carnitine uptake and synthesis, such as organic cation transporter-2 (OCTN2) and γ-butyrobetaine dioxygenase (BBD), have been shown to be regulated by peroxisome proliferator-activated receptor (PPAR)α directly. Whether other genes encoding enzymes involved in the carnitine synthesis pathway, such as 4-N-trimethylaminobutyraldehyde dehydrogenase (TMABA-DH) and trimethyllysine dioxygenase (TMLD), are also direct PPARα target genes is less clear. In silico-analysis of the mouse TMLD promoter and first intron and the TMABA-DH promoter revealed several putative peroxisome proliferator response elements (PPRE) with high similarity to the consensus PPRE. Luciferase reporter gene assays using either a 2 kb TMLD promoter or a 4 kb TMLD first intron reporter constructs revealed no functional PPRE. In contrast, reporter gene assays using wild-type and mutated 5′-truncation TMABA-DH promoter reporter constructs showed that one PPRE located at position −132 in the proximal promoter is probably functional. Using gel shift assays we observed in vitro-binding of PPARα to this PPRE. Moreover, using chromatin immunoprecipitation assays we found that PPARα also binds in vivo to a nucleotide sequence spanning the PPRE at −132, which confirms that this PPRE is functional. In conclusion, the present study shows that the mouse TMABA-DH gene is a direct PPARα target gene. Together with the recent identification of the mouse BBD and the mouse OCTN2 genes as PPARα target genes this finding confirm that PPARα plays a key role in the regulation of carnitine homeostasis by controlling genes involved in carnitine synthesis and carnitine uptake.

1. Introduction

Carnitine is a water soluble quaternary amine which is essential for normal function of all tissues. Carnitine is derived from both, dietary sources and endogenous biosynthesis [1]. Carnitine is synthesized endogenously from trimethyllysine, a product from lysosomal protein breakdown, by a series of enzymatic reactions involving trimethyllysine dioxygenase (TMLD), 3-hydroxy-N-trimethyllysine aldolase and 4-N-trimethylaminobutyraldehyde dehydrogenase (TMABA-DH). The enzymatic reaction catalyzed by TMABA-DH provides γ-butyrobetaine which is the ultimate precursor of carnitine. In the final biosynthetic step, γ-butyrobetaine is hydroxylated by γ-butyrobetaine dioxygenase (BBD) to form carnitine [2]. In humans, the primary organs responsible for carnitine synthesis are the liver and the kidneys because these are the only tissues with a considerable activity of BBD [2]. However, other tissues than liver and kidney also contribute to carnitine synthesis through formation of γ-butyrobetaine because activities of TMLD and TMABA-DH are found in most tissues. The γ-butyrobetaine is excreted from these tissues and transported via the circulation to the liver and the kidneys, where it is converted into carnitine [2]. All tissues which are incapable of producing carnitine are highly dependent on active carnitine uptake from blood. Delivery of carnitine from plasma into cells is catalyzed by novel organic cation transporters (OCTNs), from which the OCTN2 isoform has the highest binding affinity for carnitine and is therefore the physiologically most important carnitine transporter [3,4].

Recent studies provided clear evidence that both, carnitine synthesis and carnitine uptake are regulated by peroxisome proliferator-activated receptor α (PPARα) (reviewed by [5]). PPARα is a ligand-activated transcription factor that acts as an important regulator of lipid metabolism and energy homeostasis that is abundantly expressed in tissues with high rates of fatty acid oxidation such as liver and kidney [6,7]. Regulation of gene expression by PPARα is mediated by complex formation of PPARα with the retinoid X receptor (RXR) and subsequent binding of this complex to a specific DNA consensus sequence, called peroxisome proliferator response element (PPRE), present in the regulatory region of target genes [7–11], thereby stimulating the expression of those genes. Typical proteins encoded by these genes are involved in all aspects of fatty acid catabolism,

* Corresponding author. Tel.: +49 641 9939230; fax: +49 641 9939239.
E-mail address: klaus.eder@ernaehrung.uni-giessen.de (K. Eder).

1874-9399/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbagrm.2012.01.004

ketogenesis as well as gluconeogenesis [7]. PPARα can be activated by either endogenous ligands such as fatty acids, which are released from white adipose tissue during fasting and taken up into tissues during this state, or exogenous ligands such as fibrates (WY-14,643, clofibrate, fenofibrate, bezafibrate, and gemfibrozil) [12,13]. The regulation of carnitine synthesis and carnitine uptake by PPARα is evidenced by the fact that activation of PPARα by fibrates or fasting increases mRNA abundance of OCTN2 and BBD and carnitine concentrations in the liver [14–16]. In contrast, genetic disruption of PPARα as in PPARα-knockout mice or down-regulation of hepatic PPARα as observed in mice during lactation causes a reduction of hepatic mRNA levels of OCTN2 and BBD and hepatic carnitine concentrations [17–19]. In addition, the mouse genes encoding OCTN2 and BBD have been recently identified as direct PPARα target genes containing a functional PPRE in the first intron and the promoter region, respectively [11,20]. Whether other genes encoding enzymes involved in the carnitine synthesis pathway, such as TMABA-DH and TMLD, are also direct PPARα target genes is less clear. TMABA-DH was shown to be up-regulated by WY-14,643 in the liver of wild-type mice but not PPARα-knockout mice and to be expressed at lower levels in PPARα-knockout mice than in wild-type mice [18] indicating that TMABA-DH is a direct PPARα target gene. However, administration of the PPARα activator clofibrate in rats failed to increase hepatic mRNA levels of TMABA-DH in two other studies [14,15]. With respect to TMLD it was reported that administration of WY-14,643 in wild-type mice does not increase its mRNA abundance in the liver and that hepatic mRNA abundance of TMLD does not differ between wild-type and PPARα-knockout mice [18]. In contrast, hepatic mRNA level of TMLD was found to be increased in one study with rats fed clofibrate [15]. These findings suggest that TMLD is not a direct PPARα target gene but may be regulated by PPARα indirectly. Therefore, the present study aimed to clarify whether TMABA-DH and TMLD are direct PPARα target genes by identifying functional PPRE using reporter gene experiments, gel shift assays and chromatin immunoprecipitation assays. Our data show that at least the mouse TMABA-DH gene is regulated by PPARα directly.

2. Materials and methods

2.1. Animal experiment

For the chromatin immunoprecipitation (ChIP) assay, we used livers from a recent study with mice [18]. The experimental procedures described followed established guidelines for the care and handling of laboratory animals and were approved by the council of Saxony-Anhalt. The mice experiment was carried out with male, 11–12 wk old PPARα$^{-/-}$ mice (129S4/SvJae-*Ppara*^{tm1Gonz}/J) and corresponding wild-type control mice (129S1/SvImJ). The mice had an average initial body weight (±SD) of 24.3±3.2 g. Mice of each genotype were randomly assigned to two groups and kept individually in Macrolon cages in a room with controlled temperature (22±2 °C), relative humidity (50–60%), and light (12:12-h light:dark cycle). Mice in the treatment groups (wild-type mice, $n=8$; PPAR$_\alpha^{-/-}$ mice, $n=8$) received 40 mg/kg WY-14,643 once daily 2 h after the beginning of the light cycle for 4 days. WY-14,643 was dissolved in DMSO and sunflower oil (50:50, v/v) at a final concentration of 8 mg/ml as described. The daily dose of WY-14,643 (in 0.12 ml) was given by gavage. Control animals (wild-type mice, $n=8$; PPAR$_\alpha^{-/-}$ mice, $n=8$) were given the appropriate volume of the vehicle (DMSO and sunflower oil). All mice were fed a commercial, standard basal diet ("altromin 1324," Altromin GmbH, Lage, Germany) providing 11.9 MJ metabolizable energy per kg diet. To standardize food intake, the mice were fed restricted amounts (4 g daily). Water was available *ad libitum* from nipple drinkers during the entire experiment. On day 4 of treatment, mice received the last dose of WY-14,643 or vehicle alone and 1 g of the diet and were killed 4 h later by decapitation under light anesthesia with diethyl ether. Livers were immediately excised for ChIP experiment.

2.2. Cell culture

HepG2 cells, a human hepatoma cell line (CLS Germany) that is commonly used for transient transfection assays [11], were cultured in RPMI1640 GlutaMax-1 medium (Invitrogen, Karlsruhe, Germany) supplemented with 10% fetal calf serum (Invitrogen) and 0.05 mg/mL gentamycin (Invitrogen). Cells were maintained at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. Medium was changed every 2 days. After reaching a confluence of 70–80%, the cells were either subcultivated or used for reporter gene experiments.

2.3. In silico-analysis of mouse TMLD and TMABA-DH promoter

Using cDNA (accession number BC10495), genomic DNA (accession number AC134612) from NCBI Genbank and genomic sequences from http://genome.ucsc.edu/ an approximately 2 kb fragment of mouse TMLD promoter from −2036 to +128 relative to transcription start site, and a 4024 bp fragment of mouse TMLD first intron before the translation start site were scanned and analyzed for putative PPRE using the NUBIScan (nuclear receptor binding site scanner; [21]). The same was performed for an approximately 3 kb fragment of mouse TMABA-DH promoter from −2752 to +245 relative to transcription start site using cDNA and genomic sequences from NCBI Genbank (Accession numbers NM_019993 and AL113970).

2.4. Generation of mouse TMLD and TMABA-DH promoter reporter gene constructs

A 1867 bp promoter fragment from −1739 to +128 relative to transcription start site containing eight predicted PPRE was PCR amplified from mouse BAC clone RP23-120L11 (imaGene, Berlin, Germany) by using the primer pair [TMLDpro_down(HindIII) and TMLDpro-PPRE1-8(XhoI)] shown in Table 1. The generated PCR fragment with HindIII and XhoI restriction sites introduced at the 5′ and 3′ ends was subcloned into the HindIII and XhoI digested pGL4.10 [luc2] vector (Promega, Mannheim, Germany) upstream of the luciferase reporter representing the full-length construct pGL4.10-TMLD-1867. In addition, four mouse TMABA-DH promoter-truncation constructs were designed. The 891 bp promoter fragment from −645 to +246 relative to transcription start site containing four predicted PPRE was PCR amplified from mouse BAC clone RP23-125E16 (imaGene). The generated PCR fragment with XhoI and KpnI restriction sites introduced at the 5′ and 3′ ends was subcloned into the XhoI and KpnI digested pGL4.10 [luc2] vector (Promega, Mannheim, Germany) upstream of the luciferase reporter gene, thereby, providing the full-length construct pGL4.10-TMABA-DH-891 (TMABA-DH construct 1). The TMABA-DH promoter-truncation constructs pGL4.10-TMABA-DH-698 (TMABA-DH construct 2) from −452 to +246 containing three putative PPRE, pGL4.10-TMABA-DH-469 (TMABA-DH construct 3) from −223 to +246 containing two putative PPRE, pGL4.10-TMABA-DH-319 (TMABA-DH construct 4) from −73 to +245 containing one putative PPRE were PCR amplified from parental clone pGL4.10-TMABA-DH-891 by using different 5′-primers flanking the putative PPRE and a common 3′-primer. The primer sequences are shown in Table 1. The generated PCR products containing two adapters of XhoI and KpnI site at the end were subcloned into the XhoI and KpnI digested pGL4.10 [luc2] vector upstream of the luciferase reporter gene. To confirm the integrity of the constructs the cloned DNA fragments were sequenced.