European Journal of Pharmacology 628 (2010) 1–5

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Review

The role of peroxisome proliferator-activated receptor α in transcriptional regulation of novel organic cation transporters

Klaus Eder *, Robert Ringseis

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

ARTICLE INFO

Article history:
Received 22 August 2009
Received in revised form 8 November 2009
Accepted 17 November 2009
Available online 24 November 2009

Keywords:
Novel organic cation transporter
Carnitine
Peroxisome proliferator-activated receptor-α

ABSTRACT

Former studies in rats demonstrated that starvation or treatment with the hypolipidemic drug clofibrate causes a marked increase in the concentration of carnitine in the liver. The molecular mechanisms underlying these phenomena in rats, however, have been largely unknown. Since both, fasting and clofibrate treatment lead to an activation of peroxisome proliferator-activated receptor α (PPARα), the hypothesis has been raised that activation of this nuclear receptor could lead to an up-regulation of novel organic cation transporters (OCTN) which facilitate transport of carnitine and several other organic cations through membranes. Studies in rodents and pigs have indeed shown that treatment with PPARα agonists causes an up-regulation of OCTN2 in liver and other tissues such as muscle and small intestine. Additional experiments with PPARα-null and corresponding wild-type mice, which were either fasted or treated with the high-affinity PPARα agonist WY-14,643, revealed that transcriptional up-regulation of OCTN2 and OCTN3 is dependent on PPARα. An up-regulation of OCTN by PPARα activation could be regarded as a means to supply cells with sufficient carnitine required for transport of excessive amounts of fatty acids into the mitochondrion during fasting, and therefore plays an important role in the adaptive response of the metabolism to fasting. Due to the strong similarities in the gene response to PPARα agonists and the similar metabolic features and anatomic conditions between pigs and humans, it is likely that pharmacological PPARα agonists exert similar effects in humans.

Contents

1. Introduction

Peroxisome proliferator-activated receptor α (PPARα) is a ligand-activated transcription factor that acts as an important regulator of lipid metabolism and energy homeostasis (Desvergne and Wahli, 1999). PPARα is abundantly expressed in tissues with high rates of fatty acid oxidation such as liver, heart muscle, skeletal muscle, and kidney (Mandard et al., 2004). Transcriptional regulation of genes by PPARα is mediated by binding of activated PPAR/retinoid X receptor heterodimers to specific DNA sequences, called peroxisome proliferator response elements (PPREs) present in and around the promoter of target genes (Qi et al., 2000; Schoonjans et al., 1997; Tan et al., 2005), thereby stimulating the expression of those genes. Proteins encoded by these genes are involved in all aspects of fatty acid catabolism including cellular fatty acid uptake, intracellular fatty acid transport, fatty acid transport through the mitochondrial membrane, mitochondrial and peroxisomal fatty acid oxidation, ketogenesis as well as gluconeogenesis (Kersten et al., 1999; Mandard et al., 2004).

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

0014-2999/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2009.11.042

PPARα can be activated by both endogenous and synthetic ligands. Endogenous ligands of PPARα are fatty acids and their derivatives (eicosanoids). Endogenous ligand-activation of PPARα is observed during fasting (Kersten et al., 1999; Leone et al., 1999), since free nonesterified fatty acids are released from adipose tissue and taken up into tissues at increased levels during this state. Consequently, in the liver, where PPARα is most abundant, β-oxidation, ketogenesis as well as gluconeogenesis are dramatically elevated as a consequence of the increased expression of PPARα target genes (Kersten et al., 1999; Mandard et al., 2004). The crucial role of PPARα during fasting is evidenced by the fact that PPARα-null mice cannot sustain long-term fasting (Leone et al., 1999), because these animals are unable to adapt to food deprivation by stimulating β-oxidation, ketogenesis, and gluconeogenesis. Thus, PPARα-null mice develop hepatic steatosis and become hypoketonemic and hypoglycemic in response to a fasting challenge, despite marked elevations in circulating free fatty acids (Finck and Kelly, 2002; Hashimoto et al., 2000; Lee and Gonzalez, 1996; Leone et al., 1999). In addition to endogenous ligands, PPARα is also activated by a heterogenous group of synthetic compounds including the fibrate class of lipid lowering drugs (clofibrate, fenofibrate, bezafibrate, and gemfibrozil) (Forman et al., 1997; Krey et al., 1997).

1.1. Expression of PPARα in proliferating and non-proliferating species

Regarding the expression of PPARα in tissues and the effects of PPARα activation on transcription of its target genes, there are great differences between various species. In rodents, PPARα is highly expressed in tissues, and activation of PPARα not only induces many genes involved in various metabolic pathways but also causes severe peroxisome proliferation, hypertrophy, hyperplasia, and even hepatocarcinogenesis in the liver (Peters et al., 2005). In contrast to rodents, PPARα agonists like fibrates do not induce peroxisome proliferation in several other species such as guinea pigs, pigs, monkeys and humans. These species have a lower expression of PPARα in the liver and the response of many genes to PPARα activation is much weaker than in rodents (Holden and Tugwood, 1999). Due to the great differences in the response to PPARα activation with respect to hepatic proliferation, rodents are commonly designated as *proliferating species* while those which do not respond with hepatic peroxisome proliferation are often called *non-proliferating species*. According to differences in PPARα expression and the response of target genes, PPARα activation observed in rodents cannot be directly applied for *non-proliferating species* such as humans. In contrast, mRNA concentration of PPARα in the liver in pigs is similar to that in humans (Luci et al., 2007a), which is approximately ten-fold lower than in rats. This suggests that the pig is a useful model to study the biochemical effects of PPARα agonists. Moreover, the pig is generally considered as a suitable model object for humans because it has similar metabolic features and anatomic conditions (Schwartz et al., 1996; Sigel et al., 1994; Spurlock and Gabler, 2008).

1.2. Role of starvation and PPARα agonists on carnitine homeostasis

Many years ago it has been shown that starvation or treatment of rats with clofibrate increases the hepatic concentration of carnitine (Brass and Hoppel, 1978; McGarry et al., 1975; Paul and Adibi, 1979; Paul et al., 1986), an essential metabolite that is required for the β-oxidation of long-chain fatty acids in the mitochondrial matrix (Brass, 2004; McGarry and Brown, 1997; Steiber et al., 2004). Carnitine is derived from both dietary sources and endogenous biosynthesis which occurs in humans in liver, kidney and brain exclusively (Rebouche and Seim, 1998; Vaz and Wanders, 2002). Tissues which are incapable of producing carnitine are highly dependent on active carnitine uptake from blood. Since both, fasting and clofibrate treatment lead to an activation of PPARα, the hypothesis has been raised that activation of this nuclear receptor either stimulates carnitine biosynthesis and/or uptake of carnitine from the blood into tissues (Luci et al., 2006). Recent studies have shown that activation of PPARα indeed stimulates carnitine synthesis and leads to an up-regulation of novel organic cation transporters (OCTN) which facilitate the transport of carnitine through cell membranes. An overview about studies dealing with the effects of PPARα activation on the expression of OCTN is presented in chapter 2 of this review.

1.3. Occurrence and function of novel organic cation transporters (OCTN)

OCTN belong to the solute carrier (SLC) 22A family (Lahjouji et al., 2001; Tein, 2003). Three OCTN have been identified so far, OCTN1, OCTN2 and OCTN3 (Tamai et al., 1997, 1998, 2000), localised in the plasma and the mitochondrial membrane, respectively, of cells. OCTN1 and OCTN2 are expressed in several tissues such as kidney, intestine, skeletal muscle, heart, liver and brain (Ohashi et al., 2001; Tamai et al., 2000; Wu et al., 1999). OCTN3 has been found only in the mouse and is expressed exclusively in testes, kidney and small intestine (Durán et al., 2005; Tamai et al., 2000). OCTN are polyspecific. All the three OCTN are able to transport carnitine, and the involvement in carnitine homeostasis might be their most important physiologic function. The fact that inborn or acquired defects of OCTN lead to primary or secondary systemic carnitine deficiency demonstrates their essential role in carnitine homeostasis (Lahjouji et al., 2004). Among the three OCTN, OCTN3 has the highest specificity for carnitine, OCTN1 has the lowest one (Tamai et al., 2000). OCTN operate on the intestinal absorption and renal reabsorption of carnitine and play a major role in tissue distribution by catalysing the uptake of carnitine into body cells. Due to its high binding affinity for carnitine and its wide expression, OCTN2 is the physiologically most important carnitine transporter, operating for the reabsorption of carnitine from the urine as well as playing a major role in tissue distribution. OCTN1 contributes less to carnitine transport than OCTN2 due to its low carnitine transport activity. In mice, OCTN3 may be important for carnitine uptake into testis, and may contribute to reabsorption of carnitine in kidney (Tamai et al., 2000). OCTN1 transports, besides carnitine, also the zwitterions ergothioneine and stachydrine and several monovalent organic cations such as tetraethylammonium, quinidine, pyrilamine and verapamil. OCTN2 is a Na^+/carnitine cotransporter but can function alternatively as a polyspecific and Na^+-independent organic cation uniporter. In the presence of Na^+, OCTN2 transports also the zwitterionic β-lactam antibiotic cephaloridine, L-lysine and L-methionine. Tetraethylammonium, choline, verapamil and pyrilamine are cationic substrates which are Na^+ independently transported by OCTN2 (Koepsell and Endou, 2004).

2. Evidence for the involvement of PPARα in the transcriptional regulation of OCTN in *proliferating* and *non-proliferating species*

Recently, it has been observed that treatment of rats, representing a *proliferating species*, with clofibrate leads to a strong increase of the hepatic mRNA content of OCTN2 (Luci et al., 2006). In accordance with these findings in rats, treatment of rat hepatoma cells also caused an up-regulation of OCTN2 (Luci et al., 2006). These findings suggested for the first time that PPARα activation leads to an up-regulation of OCTN2. Subsequent studies confirmed that treatment of rats with clofibrate increases mRNA concentrations of OCTN2 in liver and small intestine (Ringseis et al., 2007a, 2008a). The increased expression of OCTN2 in small intestine was associated with an increased absorption rate of carnitine in small intestine (Ringseis et al., 2008a). Interestingly, clofibrate treatment did not increase OCTN2 mRNA concentrations in other tissues such as skeletal muscle, heart, kidney or brain while fasting or energy restriction caused an up-regulation of OCTN2 in nearly all these tissues (Koch et al., 2007; Ringseis et al., 2008a). Clofibrate also caused an up-regulation of OCTN1 in the liver of rats, which was however less pronounced than the up-regulation of