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Hepatic peroxisome proliferator-activated receptor alpha mediates the major metabolic effects of Wy-14643

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Abstract

Background and Aim: Peroxisome proliferator-activated receptor alpha (PPAR α) is a molecular target of various fibrate drugs clinically used to lower serum lipids. However, the tissue-specific functions of PPAR α remain to be elucidated. This study aimed to explore the tissue-specific functions of PPAR α in response to Wy-14643.

Methods: A hepatocyte-specific *Ppara* knockout mouse line was used to explore the impact of hepatic PPAR α activity on the systemic response to treatment with the potent PPAR α agonist Wy-14643.

Results: Wy-14643 mainly activated hepatic PPAR α and regulated the expression of PPAR α target genes in liver. Hepatic *Ppara* disruption abolished the triglyceride lowering effects of Wy-14643, prevented agonist-induced hypophagia, and ablated PPAR α target gene response in the liver.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article.

Conclusions: These findings indicate that Wy-14643 treatment mainly activates hepatic PPAR α , and the hypolipidemic and hypophagic effects of Wy-14643 are dependent on PPAR α activation within hepatocytes.

Keywords

fibrate drugs; hypolipidemia; hypophagia; PPAR α ; Wy-14643

Introduction

The peroxisome proliferator-activated receptors (PPARs) are a family of ligand-activated nuclear transcription factors, consisting of three members conserved in humans and mice, PPAR α , PPAR δ , and PPAR γ .¹ PPAR α was the first PPAR cloned during an effort to identify the molecular targets of various hypolipidemic agents and peroxisome proliferator chemicals, notably the fibrate drugs, that caused pronounced peroxisome proliferation, hepatomegaly, and liver cancer in rats and mice.² Since then, numerous fatty acid metabolites were identified as endogenous PPAR α ligands, and PPAR α has been described as a principal metabolic “lipid sensor” *in vivo*. Upon agonist binding, PPAR α forms a heterodimer with retinoid X receptor alpha and regulates target gene expression by binding to specific peroxisome proliferator response elements (PPREs) within promoter regions. As many target genes encode key enzymes involved in lipid metabolism, PPAR α is considered a major transcriptional regulator of both local and systemic lipid homeostasis.³ It is now clear that PPAR α is a *bona fide* therapeutic target for fibrates and other synthetic hypolipidemic agents used in the treatment of hyperlipidemia.⁴ Therefore, a comprehensive understanding of the physiological characteristics of PPAR α activation has become essential for establishing the mechanism of regulation of dyslipidemia and related metabolic disorders, as well as determining any risks associated with off-target effects.

Full body PPAR α knockout (*Ppara*^{-/-}) mice have significantly helped in elucidating the functions of PPAR α *in vivo*. Present evidence clearly supports a critical role for PPAR α in governing fatty acid homeostasis,⁵ and PPAR α deficiency abolishes the normal physiological response to fasting and fibrate-induced peroxisome proliferation, hepatomegaly, and liver cancer in susceptible mammalian species.^{3,6-8} Recently, PPAR α was reported to regulate bile acid homeostasis,⁹ endothelial progenitor cell maturation and differentiation,¹⁰ and even neurological functions through stimulating the expression of neurotrophin¹¹ or the proteolysis of amyloid precursor protein.¹² Although many studies have identified PPAR α expression in non-hepatic tissues,^{13,14} there are few published studies that directly addressed the tissue-specific functions of PPAR α , let alone its possible role in facilitating crosstalk between different tissues. A major reason that this has not been explored is the lack of tissue-specific *Ppara* knockout models.

Given that PPAR α plays an important role in regulating metabolic regulation and that the liver is an essential metabolic organ¹⁵ and a major site of PPAR α expression,¹⁴ a hepatocyte-specific PPAR α knockout (*Ppara*^{Hep}) mouse line was developed.¹⁶ With this mouse line, the role of hepatic PPAR α in the response to Wy-14643, a potent and highly

specific agonist, was examined. Evidence suggests that hepatocyte-specific PPAR α activation principally facilitates the systemic lipid-lowering effects of Wy-14643.

Methods

Animals.

All procedures involving mice were performed in accordance with and the approval of the National Cancer Institute Animal Care and Use Committee. C57BL/6N mice were purchased from Charles River Laboratories (Wilmington, MA, USA). *Ppara* wild-type (*Ppara*^{+/+}) or *Ppara*-null (*Ppara*^{-/-}) mice on the C57BL/6N background were described previously.^{17,18} *Ppara* floxed (*Ppara*^{f/f}) or *Ppara* liver-specific knockout (*Ppara*^{Hep}) mice on the C57BL/6N background have been described.¹⁶ repTOP PPRE-luc reporter mice were purchased from Charles River Laboratories. All mice were housed in a temperature (22 °C) and light-controlled vivarium with free access to water and standard rodent chow, a standard control grain diet (vehicle grain diet), or a grain diet containing 0.1% Wy-14643 (Bio-Serv, Flemington, NJ, USA). All experiments were started on 7 to 8-week-old mice.

Wy-14643 treatment.

Mice were randomly grouped to Wy-14643 (WY) grain diet or matching control vehicle grain diet (Veh). All mice were allowed unrestricted access to a diet containing 0.1% Wy-14643 (100 mg/kg/day), or control diet, respectively, for 2 days. After treatment, all mice were killed by CO₂ asphyxiation and tissues collected for analysis.

Histological staining.

Fresh liver was placed into 10% phosphate-buffered formalin for 24 h to fix tissue and then processed into paraffin blocks. Sections were stained with hematoxylin and eosin. Histological sectioning was performed by HistoServ, Inc (Germantown, MD, USA). Slide imaging was performed using a Keyence BZ-X700 series all-in-one microscope with both 20 $\times$ and 40 $\times$ objectives, 200 $\times$ and 400 $\times$ magnification, respectively.

Cumulative food intake assay.

Cumulative food intake was measured by indirect calorimetry in mice after being placed on a vehicle grain diet for 11 days using a 12-chamber Environment Controlled CLAMS (Columbus Instruments, Columbus, OH, USA) with one mouse/chamber as previously described.¹⁹ After 48-h chamber acclimatization, mice were monitored for 24 h under vehicle grain diet followed by 20 h under Wy-14643 grain diet. During testing, water was provided *ad libitum*.

In vivo luciferase assays.

repTOP PPRE-luc reporter mice were orally gavaged with vehicle (dimethylsulphoxide in 4:1 of PEG-400 and Tween 80) or Wy-14643 (50 mg/kg). Twenty-four hours following treatment, mice were dosed with 200 μ L of D-luciferin (15 mg/mL DPBS, intraperitoneal). Ten minutes after D-luciferin injection, the mice were anesthetized using isoflurane and bioluminescence imaging was assessed using an IVIS Imaging System 200 Series (Xenogen,

Hopkinton, MA, USA). All imaging was performed between 9 and 10 AM. The image shown is representative of each treatment. The luciferase signal was quantified using IVIS Living Imaging software (version 4.5.5) and represented as average counts after subtracting background. Four mice were imaged per treatment group.

Serum triglyceride assay.

Serum triglyceride (TG) was assessed in a 96-well microplate using a commercial TG assay kit (Wako USA, Richmond, VA, USA) and monitored with a microplate reader (BioAssay Systems, Harvard, CA, USA).

Total RNA extraction, cDNA synthesis, and real-time PCR.

Total RNA was extracted from frozen tissues using TRIzol reagent according to the manufacturer's instructions. The purity and concentration of total RNA samples were determined by a NanoDrop ND-1000 spectrophotometer (Thermo-Fisher, Waltham, MA, USA). A total of 50 ng of total RNA was reverse transcribed using cDNA Synthesis SuperMix (Biotool, Houston, TX, USA). Real-time polymerase chain reaction (PCR) was carried out in an ABI 7900HT Fast Real-Time PCR System (AB Applied Biosystems, Warrington, UK) with SYBR Green PCR master mix (AB Applied Biosystems, Warrington, UK) and gene-specific primers. The sequences for the forward and reverse primers used to quantify mRNA are available listed in Table S1. The following conditions were used for real-time PCR: 95 °C for 5 min, then 95 °C for 15 s and 60 °C for 1 min and 40 cycles. The 2^{-CT} method²⁰ was used to analyze the relative changes in gene expression normalized against 18S rRNA expression.

Statistical analysis.

All results are expressed as means $\pm$ SEM. Significance was determined by (multiple) two-tailed unpaired *t*-test using Prism 7.0 software (GraphPad Software, San Diego, CA, USA). A *P* value of < 0.05 was considered significant.

Results

Wy-14643 treatment principally activates hepatic PPAR α .

Peroxisome proliferator-activated receptor alpha activation was assessed in live animals using mice expressing a firefly luciferase transgene under control of a PPAR response element Actionrepeat (repTOP PPRE-Luc mice).²¹ Pronounced hepatic PPAR α activation in response to Wy-14643 was observed (Fig. 1a). Average luciferase counts of the treatment groups revealed a 10-fold increase in signal after Wy-14643 treatment (Fig. 1b). This observation is consistent with the effect of Wy-14643 treatment on *Ppara* mRNA expression and selected PPAR α target genes in brown adipose tissue (BAT), liver, kidney, and heart, the four tissues with the highest PPAR α expression in mice.¹⁴ *Ppara* mRNA was slightly but significantly upregulated in the liver of wild-type mice but not in heart or kidney (Fig. 1c). *Fgf21*, *Acadm*, and *Acox1* mRNAs, encoded by PPAR α target genes, were upregulated in the liver with no significant induction in BAT, heart, or kidney (Fig. 1d,f,h). *Irfap* and *Irf5* mRNA were sharply suppressed in the liver (Fig. 1e,g). Employing the present study

conditions, it is reasonable to infer that hepatic PPAR α predominately mediates the hypolipidemic effects of Wy-14643.

Agonist-induced hepatomegaly is dependent on hepatocyte-specific Ppara activation.

Prolonged activation of PPAR α in rodents causes pronounced hepatomegaly. To determine the requirement for hepatocyte PPAR α activation on hepatomegaly in response to short-term agonist treatment, *Ppara* wild-type (*Ppara*^{+/+}), *Ppara*-null (*Ppara*^{-/-}), *Ppara*-floxed (*Ppara*^{f/f}), and hepatocyte-specific *Ppara* knockout (*Ppara*^{Hep}) mice were provided either a grain diet containing 0.1% Wy-14643 or matching control diet for 2 days. Following treatment, body and liver weights were measured and liver indices calculated (mg liver/g body mass) to assess the extent of hepatomegaly (Fig. 2a). Wy-14643 treated *Ppara*^{+/+} and *Ppara*^{f/f} mice exhibited significantly higher liver indices than *Ppara*^{-/-} or *Ppara*^{Hep} mice, which were similar to controls. Liver sections were stained with hematoxylin and eosin revealing pronounced hepatocyte swelling in *Ppara*^{f/f} mice treated with Wy-14643 (Fig. 2b,c). *Ppara*^{Hep} mice were protected from these effects indicating that PPAR α activation in hepatocytes is required for hepatocyte swelling and liver enlargement.

Hepatic PPAR α deficiency ablates the hypolipidemic effects of Wy-14643.

To assess the contribution of hepatocyte-specific PPAR α activation to systemic lipid homeostasis, Wy-14643 was administered to *Ppara*^{+/+}, *Ppara*^{-/-}, *Ppara*^{f/f}, *Ppara*^{Hep} mice. Consistent with current evidence,^{5,22} the lipid-lowering effects of Wy-14643 were PPAR α -dependent and only observed in *Ppara*^{+/+} but not *Ppara*^{-/-} mice (Fig. 3a). Serum TG levels in *Ppara*^{Hep} mice were completely unaffected by Wy-14643 treatment and resembled that of conventional full body *Ppara*^{-/-} knockout mice indicating that these effects are liver-dependent (Fig. 3a). As expected, the response of the *Ppara*^{f/f} mice paralleled the *Ppara*^{+/+} mice (Fig. 3a). In agreement with previous evidence showing that *Ppara* disruption prevents the upregulation of fatty acid oxidation and lipid metabolism pathways,⁵ *Ppara*^{Hep} mice exhibited undetectable levels of *Ppara* mRNA, lower basal expression levels of many fatty acid-metabolizing enzymes (Fig. 3b-l), and gene upregulation in response to agonist was abolished (Fig. 3c-l). These results indicate that hepatic PPAR α activation is necessary for the hypolipidemic actions of PPAR α agonists.

Hepatic PPAR α deficiency abolishes agonist-induced appetite-suppression.

Another common consequence of PPAR α agonist treatment is the suppression of food intake, also known as hypophagia.²³⁻²⁶ In agreement with these studies, Wy-14643 significantly reduced feeding in *Ppara*^{+/+} mice (Fig. 4a), but not *Ppara*^{-/-} mice (Fig. 4b). These results support the view that appetite suppression in this context is PPAR α -dependent. Feeding behavior in animals is at least partially governed by the central nervous system, particularly the hypothalamus,²⁷ and oleylethanolamide, an endogenous PPAR α ligand, was reported to suppress food intake mediated by the lateral hypothalamus.²⁸ Therefore, an emerging question has focused on whether PPAR α activation in the liver, or possibly other PPAR α expressing tissues, is responsible for agonist-induced appetite suppression. Considering the previous evidence, the effect of Wy-14643 on food intake was tested in *Ppara*^{f/f} and *Ppara*^{Hep} mice. Intriguingly, a decrease in food intake was only observed in *Ppara*^{f/f} mice but not hepatocyte-specific *Ppara*^{Hep} knockout mice by Wy-14643 treatment

(Fig. 4c,d). These data indicate that hepatic PPAR α deficiency is sufficient to abolish the appetitesuppressing effect of Wy-14643. The underlying molecular mechanisms responsible for the crosstalk between the liver and hypothalamus after hepatic PPAR α activation remains to be elucidated.

Discussion

The present studies revealed that hepatic PPAR α mediates the major physiological effects of Wy-14643. PPAR α agonists, namely, the fibrate class of hypolipidemic drugs, have been clinically used as for decades. However, elucidating the underlying mechanisms by which fibrates influence systemic lipid homeostasis has been an ongoing effort. Here, hepatocyte-specific PPAR α activation was found to be essential for the lipid-lowering actions of Wy-14643 and likely other therapeutic drugs that target PPAR α . Additionally, hepatic PPAR α disruption abolished agonistinduced hepatomegaly and hypophagia. The deleterious impact of loss of hepatic PPAR α on systemic lipid metabolism is logical, as the liver plays a principle role in modulating lipid catabolism and fatty acid homeostasis.²⁹ Hepatomegaly that results from longterm agonist treatment was recently shown to be dependent on hepatic PPAR α activation, and these short-term studies strengthen support for the view that hepatocyte-specific PPAR α activation plays a key role in this process.¹⁶ Hepatic gene expression data clearly indicate that the response to Wy-14643 is abolished in *Ppara*^{Hep} mice and that the agonist-induced metabolic pathway remodeling to promote lipid catabolism would be hindered. However, it is somewhat unexpected that the hypolipidemic effects would be solely dependent on hepatic PPAR α without any apparent contribution from extrahepatic tissues. In this study, the upregulation of PPAR α target genes in response to Wy-14643 was predominantly hepatic and not observed in BAT or heart. There was a slight but significant increase of *Acox1* expression in the kidney. Previous studies using Wy-14643 revealed varying degrees of PPAR α target gene response in extrahepatic tissues, namely, BAT, skeletal muscle, adipose tissue, and heart.^{30–32} Agonist dose, route of administration, mouse age, and strain background are known to influence PPAR α activity and most likely contribute to the observed inconsistencies between studies. It was also surprising that hepatic PPAR α disruption would abolish hypophagia in these mice. Although many factors might affect feeding behavior, in general, peripheral signals are integrated and often compensated for by the central nervous system then transformed into a resulting physiological or behavioral change that influences food intake.²⁷ In this context, the current study found that (i) the hypophagic effects of Wy-14643 are liver dependent, (ii) food consumption is mediated directly by activation of PPAR α within hepatocytes, and (iii) hepatic PPAR α activation forms a direct or indirect signaling axis with the central nervous system. In fact, current evidence supports crosstalk between peripheral PPAR α and the central nervous system in regards to feeding behavior.²⁴ Interestingly, a recent study found that PPAR γ influenced feeding behavior through a mechanism involving signaling through the hepatic afferent vagal nerve, which can directly deliver signals to center nervous system.³³ It is therefore interesting to speculate that PPAR α could use a similar mechanism to direct feeding behavior. Although the present data provide no direct evidence for a PPAR α -dependent liver-brain communication axis, it provides correlative support for crosstalk between these two organ systems. Future studies will be required to clarify the mechanisms

associated with hepatic PPAR α -dependent control of feeding behavior and liver-neuronal satiety pathways.

In summary, the major physiological effects of Wy-14643 appear to act primarily through hepatic PPAR α , and the loss of PPAR α expression in the liver abolishes both local and systemic response to agonist, namely, reducing circulating lipid levels and promoting hypophagia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Wy-14643 predominately activates hepatic PPAR α . (a) Representative images showing repTOP PPRE-luc mouse luciferase imaging in untreated mice and mice gavaged with vehicle or Wy-14643. (b) Quantified luciferase signal from untreated (Un), vehicle (Veh), and Wy-14643 (WY) treated PPRE-luc reporter mice ($n = 4$). (c–h) Fold change of *Ppara* (c), *Fgf21* (d), *Il1rap* (e), *Acadm* (f), *Il15* (g), and *Acox1* (h) mRNA expression in the liver, BAT, heart, and kidney of mice after treated with a vehicle grain diet (■, Veh) or 0.1% Wy-14643 grain diet (■, WY) for 2 days ($n = 5–7$ each). Data are presented as mean $\pm$ SEM.

Different uppercase letters indicate statistical significance (A, $P < 0.05$; B, $P < 0.01$; and D, $P < 0.001$ vs Veh). [Color figure can be viewed at wileyonlinelibrary.com]

Figure 2.

Hepatocyte-specific deletion of *Ppara* prevents Wy-14643 induced hepatomegaly. (a) After treatment with Wy-14643 for 2 days, total and liver mass were used to calculate liver indices (mg liver/g body mass) to assess hepatomegaly (D, $P < 0.001$). Data are presented as mean $\pm$ SEM. (b) Liver sections were stained with hematoxylin and eosin to assess hepatocyte size. Cell size and volume increased significantly in agonist-treated *Ppara* floxed (*Ppara*^{f/f}) but not hepatocyte-specific *Ppara* knockout (*Ppara*^{Hep}) mice. Representative images ($n = 5$ mice

per group) are shown at (b) 200× and (c) 400× magnification. , Veh; , WY. [Color figure can be viewed at wileyonlinelibrary.com]

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Figure 3.

Hepatic *PPARα* deficiency abolishes the hypolipidemic effect of Wy-14643. (a) Comparison of serum triglyceride (TG) concentrations in wild-type (*para*^{+/+}), *Ppara*-null (*Ppara*^{-/-}), *Ppara* floxed (*Ppara*^{f/f}), and hepatocyte-specific *Ppara* knockout (*Ppara*^{Hep}) mice fed with vehicle grain diet (Veh) or 0.1% Wy-14643 grain diet (WY) for 2 days (*n* = 5–6 each). (b–l) Fold change of *Ppara* (b), *Cd36* (c), *Cpt2* (d), *Acadl* (e), *Acadv1* (f), *Hmgcs2* (g), *Fgf21* (h), *Acox1* (i), *Aldh3a2* (j), *Cyp4a10* (k), and *Cyp4a14* (l) mRNA expression in livers of *Ppara*^{f/f} and *Ppara*^{Hep} mice fed with a vehicle grain diet (Veh) or 0.1% Wy-14643 supplemented grain diet (WY) for 2 days (*n* = 5–6 each). Data are presented as mean ± SEM. Different uppercase letters indicate statistical significance (A, *P* < 0.05; B, *P* < 0.01; C, *P* < 0.005; and D, *P* < 0.001). Black letters show the effects of WY (WY vs Veh within the same mouse strain), and red letters show the effects of *Ppara*^{Hep} versus *Ppara*^{f/f} within the same diet. [color figure can be viewed at wileyonlinelibrary.com]

Figure 4.

Hepatic PPAR α deficiency abolishes the hypophagic effect of Wy-14643. Comparison of cumulative food intake in *Ppara* wild-type (*Ppara*^{+/+}) (a), *Ppara*-null (*Ppara*^{-/-}) (b), *Ppara* floxed (*Ppara*^{f/f}) (c), and *Ppara* liver-specific knockout (*Ppara*^{Hep}) (d) mice fed with a vehicle grain diet (Veh) or 0.1% Wy-14643 grain diet (WY) ($n = 5-6$ each). Data are presented as mean $\pm$ SEM. —, Veh; —, WY. [Color figure can be viewed at wileyonlinelibrary.com]