PPAR Research

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Editorial

Peroxisome Proliferator-Activated Receptor δ: A Target with a Broad Therapeutic Potential for Drug Discovery

Francine M. Gregoire

Department of Biology, Metabolex Inc., Hayward, CA 94545, USA

Correspondence should be addressed to Francine M. Gregoire, fmgregoire@metabolex.com

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The biology of Peroxisome proliferator-activated receptor (PPAR) alpha (α) and gamma (γ) has been intensely scrutinized for the last 20 years and the clinical use of both PPAR-α (fibrates) and PPAR-γ (thiazolididiones) agonists has led to the understanding of their key role in the treatment of hypertriglyceridemia and type 2 diabetes mellitus [1, 2]. In contrast, the understanding of PPAR delta (δ) biology still lags behind. The identification of small molecule agonists for PPAR-δ has shed some light on the function of this ubiquitously expressed receptor in preclinical models and early clinical studies [3]. They have revealed the multiple benefits of PPAR-δ activation on lipid disorders, diabetes, and inflammation [3, 4]. However, synthetic PPAR-δ agonists have yet to be marketed for clinical use in humans, partly due to the burden associated with their clinical development [3].

In this special issue of PPAR Research, the broad potential of PPAR-δ agonists for the treatment of metabolic disease is highlighted by 3 key articles. They include a review from de Lange et al. on the regulation of the oxidative capacity of muscle by PPAR-δ, an article by Perreault et al. which tackles opportunities and issues with the development of PPAR-δ agonist for the treatment of obesity, and finally a review from Wang that addresses the effect of PPAR-δ activation on vascular pathophysiological processes. A key question regarding the result of PPAR-δ activation, either via natural or via synthetic ligands, is its effect on cell proliferation and the risk of inducing cancer. This has been an area of intense debate as both pro- and antitumorigenic effects have been reported. This topic is concisely reviewed in this issue by Muller et al. Last but not least, two interesting and not well-characterized portions of PPAR-δ biology are presented. First, as PPAR-δ is expressed at high level in the brain, Hall et al. investigate the potential neuroprotective role of PPAR-δ activation in this organ. Second, although the role of PPAR-δ in embryo implantation was recognized early on with studies in knockout mice [5], the reproductive functions of PPAR-δ are still unclear. This topic and the projected potential applications of PPAR-δ ligands in assisted reproductive technology are addressed in Huang's review.

Taken together, it is obvious that there is an urgent need for additional basic research to better characterize PPAR-δ function. The current availability of synthetic ligands should help to further dissect PPAR-δ-mediated responses in the brain as well as in other functions not addressed in this issue, including gut and skin homesotasis. Although challenges for the development of PPAR-δ agonists remains, they clearly hold great therapeutic promise, as highlighted by recent clinical findings indicating that MBX-8025, one of the most advanced PPAR-δ agonists currently in phase II clinical trial for dyslipidemia, displays hypolipidemic features not observed with the currently available dyslipidemia therapies [6, 7].

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Review Article

Peroxisome Proliferator-Activated Receptor Delta: A Conserved Director of Lipid Homeostasis through Regulation of the Oxidative Capacity of Muscle

Pieter de Lange,[1] Assunta Lombardi,[2] Elena Silvestri,[3] Fernando Goglia,[3] Antonia Lanni,[1] and Maria Moreno[3]

[1] *Dipartimento di Scienze della Vita, Seconda Università degli Studi di Napoli, Via Vivaldi 43, 81100 Caserta, Italy*
[2] *Dipartimento delle Scienze Biologiche, Sez. Fisiologia ed Igiene, Università degli Studi di Napoli "Federico II", Via Mezzocannone 8, 80134 Napoli, Italy*
[3] *Dipartimento di Scienze Biologiche ed Ambientali, Università degli Studi del Sannio, Via Port'Arsa 11, 82100 Benevento, Italy*

Correspondence should be addressed to Pieter de Lange, pieter.delange@unina2.it

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The peroxisome proliferator-activated receptors (PPARs), which are ligand-inducible transcription factors expressed in a variety of tissues, have been shown to perform key roles in lipid homeostasis. In physiological situations such as fasting and physical exercise, one PPAR subtype, PPARδ, triggers a transcriptional program in skeletal muscle leading to a switch in fuel usage from glucose/fatty acids to solely fatty acids, thereby drastically increasing its oxidative capacity. The metabolic action of PPARδ has also been verified in humans. In addition, it has become clear that the action of PPARδ is not restricted to skeletal muscle. Indeed, PPARδ has been shown to play a crucial role in whole-body lipid homeostasis as well as in insulin sensitivity, and it is active not only in skeletal muscle (as an activator of fat burning) but also in the liver (where it can activate glycolysis/lipogenesis, with the produced fat being oxidized in muscle) and in the adipose tissue (by incrementing lipolysis). The main aim of this review is to highlight the central role for activated PPARδ in the reversal of any tendency toward the development of insulin resistance.

1. INTRODUCTION

The modern Western lifestyle is characterized by excessive food intake and lack of physical exercise. This has led to obesity caused by a disturbance of lipid homeostasis, becoming one of the most prevalent and serious global chronic disorders. In lean organisms, fatty acids derived either from food or from hepatic lipogenesis are utilized as energy substrates by the heart and skeletal muscles. A strict physiological equilibrium between lipid availability and lipid consumption needs to be maintained to prevent development of both an impairment of insulin responsiveness and a metabolic dysfunction. In adult obesity and obesity-associated metabolic disorders (which have strict associations with type 2 diabetes, hypertension, and hyperlipidemia, and are often referred to as metabolic syndrome [1]), a disturbance of lipid homeostasis causes excess fat accumulation in various tissues (predominantly in adipose tissues, but also in other insulin-responsive organs, such as skeletal muscle and liver).

Skeletal muscle is quantitatively the largest organ in the body, and it contributes 30–40% of the resting metabolic rate in adults. It is a major site for the oxidation of fatty acids and glucose (accounting for approximately 80% of insulin-stimulated glucose uptake), and it exhibits a remarkable flexibility in its usage of fuel. One notable aspect of skeletal muscle plasticity is the specificity of its structural, biochemical, and functional adaptations to a given stimulus [2]. In view of the above features, it is perhaps not surprising that the predominant feature of type 2 diabetes is insulin resistance in skeletal muscle [3, 4].

The peroxisome proliferator-activated receptors (PPARs), which are ligand-inducible transcription factors expressed in a variety of tissues, have been shown to perform key roles in lipid homeostasis. In physiological situations such as fasting [5] and physical exercise [6],

one PPAR subtype, PPARδ, triggers a transcriptional program in skeletal muscle leading to a switch in fuel usage from glucose/fatty acids to solely fatty acids, and thereby drastically increasing this tissue's oxidative capacity. In addition, recent evidence has highlighted the possibility that activating PPARδ in human subjects could increase skeletal muscle's oxidative capacity and so reverse metabolic abnormalities [7]. In mouse models, PPARδ has been shown to play a crucial role in whole-body lipid homeostasis as well as in insulin sensitivity, and to be predominantly active in skeletal muscle (as an activator of fat burning [8, 9]) but also in the liver (where it can activate glycolysis/lipogenesis, with the produced fat being oxidized in muscle [10]) and in the adipose tissue (by incrementing lipolysis [11]). Thus PPARδ activation provides a multiorgan "energy substrate-switching" phenotype that triggers tissue-specific transcriptional programs, and in which skeletal muscle plays a crucial role by reducing fat content.

From the current data presented in the literature, a central role for activated PPARδ can be deduced in skeletal muscle, liver, and white adipose tissue (increased fat oxidation in muscle, increased carbohydrate catabolism and fat synthesis in the liver, and increased lipolysis in white adipose tissue) in the reversal of any tendency toward the development of insulin resistance. Following a brief description of the structure, the mode of activation and the action of the PPARs, this review aims to highlight the multiorgan energy switching role of PPARδ and its ultimate impact on insulin resistance. Figure 1 represents an overview of the central role played by PPARδ in the use of fatty acids as fuel by enhancing: (a) their oxidation in skeletal muscle (described in Section 3), (b) their synthesis from glucose in the liver and their subsequent release (described in Section 4), and (c) their release from the white adipose tissue (described in Section 5).

2. PPARS, NUCLEAR RECEPTORS ACTIVATED BY FATTY ACIDS

PPARs are nuclear receptors that act as ligand-inducible transcription factors. The three known isoforms PPAR α, β (also termed δ) , and γ display tissue-specific expressions and possess different gene-regulatory profiles. PPARγ is a key regulator of adipose development and adipose insulin sensitivity [12], whereas PPARα-regulated genes are involved in hepatic lipid oxidation [13]. The PPARδ isoform, the function of which has only recently been elucidated and will be discussed in detail in this review, is predominantly expressed in skeletal muscle (where it induces fatty acid oxidation), but it is also expressed in brain, heart, liver, adipose tissue, and small intestine [14, 15].

It is generally known that PPARs heterodimerize with the retinoid X-receptor (RXR) and bind to a specific DNA sequence, (a sequence termed peroxisome proliferators response element (PPRE), that is found in a variety of genes involved in lipid and carbohydrate metabolism, inflammation, and cell proliferation and differentiation) [16, 17]. However, alternative mechanisms exist. Recently, an interplay has been reported between PPARδ and thyroid hormone receptor β (TRβ) in the activation of the gene encoding uncoupling protein 3 (UCP3) [18] in rat skeletal muscle as well as in cotransfection experiments in rat L6 myoblasts containing a reporter construct driven by the rat UCP3 promoter. Activation of UCP3 gene transcription in vivo by thyroid hormone (T3) requires the presence of fatty acids, while in the absence of fatty acids this transcription can be restored by the PPARδ agonist L165,041 [18]. The UCP3 gene promoter has been shown to contain a noncanonical thyroid hormone response element (TRE) termed TRE1 that is conserved from rodents to humans [18, 19], and this response element is also recognized by PPARs [19]. Interestingly, fatty acid responsiveness after T3 treatment was only observed in cells transfected with the rat UCP3 promoter, which suggests a species-specific regulation [18].

Dietary fatty acids and fatty acid derivatives are the natural ligands of PPARs, which display the greatest preference for monounsaturated and polyunsaturated fatty acids (MUFAs and PUFAs, resp.), as demonstrated by means of various ligand-binding assays [20, 21]. The fact that each PPAR activates a different gene program, despite their overlapping expressions, would seem to suggest ligand-specificity for each PPAR. Indeed, the structure of the ligand-binding pocket differs considerably among the various PPARs as revealed by X-ray crystal-structure analysis [21, 22]. Nevertheless, natural fatty acids can be ligands of all three PPAR isoforms. It is well known that binding of the ligand promotes a conformational change that is permissive for interactions with tissue-specific coactivator proteins, allowing nucleosome remodelling and activation of the transcription of cell type-specific target genes [21, 23]. It is, therefore, conceivable that a given fatty acid induces different conformational changes when binding to the ligand-binding pockets of the various PPAR subtypes. Given that the transcriptional activity induced by each PPAR subtype is cell type-specific [24], the different conformations induced following ligand binding may determine the cell-specificity of the different PPARs (through heterodimerization with different receptors and binding to cell-type specific cofactors). However, further research is needed to establish which natural ligands might activate each PPAR in a given cellular context.

3. ROLE OF PPARδ IN SKELETAL MUSCLE: A SWITCH TO FAT OXIDATION

3.1. *PPARδ-induced fuel switching evidenced by its overexpression or ablation and by the use of synthetic ligands in rodents*

In skeletal muscle, the relatively high expression of PPARδ (at 10- and 50-fold higher levels than PPARα and PPARγ, resp.; [15, 25]) as well as its preferential expression in oxidative rather than glycolytic myofibers [9], has led to the suggestion that this receptor isoform may be involved in promoting the utilization of fatty acids as fuel. Indeed, treatment of rat L6 cells with the highly specific PPARδ agonist GW0742 has been shown to increase fatty acid oxidation and induce expression of several lipid regulatory genes [26]. In addition,

treatment of C2C12 cells with GW0742 [27] or with another the PPARδ-specific agonist (GW1516 [28]) induced expressions of genes involved in lipid catabolism and energy uncoupling in skeletal muscle cells [29]. However, although an in vivo magnetic resonance spectroscopy (MRS) study of rats treated with the latter agonist revealed increased lipid metabolism, no change in mitochondrial energy coupling was detected efficiency despite an increased UCP3 expression [29]. Skeletal muscle has the capacity to adapt its structure to metabolic changes, as indicated by changes in the myosin type and a subsequent shift in the number and type of muscle fibers. An increased demand for fat metabolism is reflected by a shift in fiber-type away from the fast, glycolytic form (type II) toward the slow-oxidative form (type I) [2]. PPARδ has emerged as an important stimulus in the induction of this fiber shift. Indeed, studies on transgenic mice harboring a constitutively activated form of PPARδ (VP16-PPARδ) have clearly shown an increase in skeletal muscle lipid metabolism as well as the formation of type I fibers [9]. Mice overexpressing peroxisome proliferator-activated receptor γ coactivator 1 α (PGC-1α), which activates PPARδ via a direct protein-protein interaction [30], have been shown to possess a high content of type I fibers [31]. However, experiments on mice overexpressing VP16-PPARδ have revealed that type I fiber formation can be directly stimulated by PPARδ without an induction of PGC-1α [9]. Nevertheless, in physiological situations, an upregulation of PGC-1α is likely to be involved in the early events leading to muscle-fiber shifts, since PGC-1α is a coactivator of the PPARs. Indeed, PGC-1α-null mice show clear muscle dysfunction [32], and evidence that PPARδ induces an expression of PGC-1α has come from treatment of mice with GW1516 [8] as well as from a study in which skeletal muscle C2C12 myocytes transfected with a PGC-1α promoter-driven reporter construct were treated with the same ligand [33]. A wild-type PPARδ transgene (specifically overexpressed in mouse skeletal muscle, but not constitutively activated) has been shown to promote a net increase in the number of fibers with an oxidative metabolic capability through an increase in fiber numbers in soleus and tibialis anterior muscle, while in plantaris muscle the increase was more closely related to a shift from glycolytic to more oxidative fibers [34]. Interestingly, this nonactivated PPARδ transgene failed to induce the formation of type I fibers in skeletal muscle [34]. In this case, despite its high expression, actual activation of the transgene would depend on the presence of natural ligands, such as fatty acids. As the fatty acid concentrations fluctuate, fatty regulation of PPARδ transcription is likely to be highly regulated.

This raises the question as to whether, instead of overexpression, increasing the availability of ligand (natural or artificial) would induce fuel switching as well as fiber switching toward type I fibers. Indeed, a fuel-switching role of PPARδ activation has been demonstrated by incubating rat isolated skeletal muscle strips with the agonist GW1516 for 24 hours [35]. This led to an increased use of fatty acids over glucose, as reflected by increased fatty acid oxidation and reductions in glucose oxidation, glycogen synthesis, lactate release, and glucose transport. Interestingly, this switch

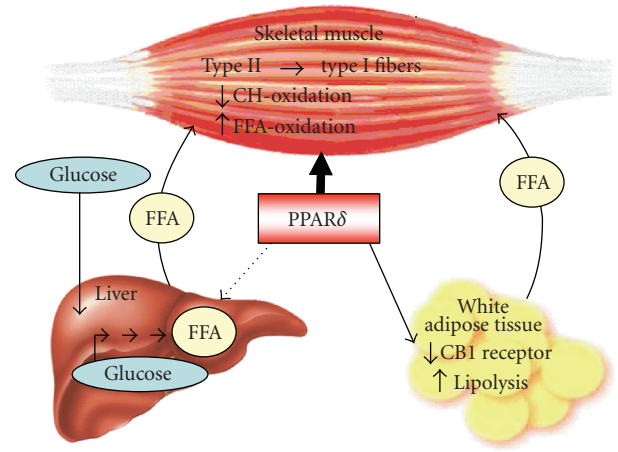

Figure 1: Central, fuel-switching mechanisms by which PPARδ increases the use of fatty acids in skeletal muscle without provoking insulin resistance. Dotted arrow: indirect effect. For abbreviations, the reader is referred to the text.

was independent of insulin stimulation or fiber type [35]. The PPARδ agonist GW610742 also induced this fuel switch and in addition triggered a genetic program toward initiation of muscular atrophy without compromising mitochondrial activity in rat skeletal muscle [36]. Furthermore, a somatic gene transfer of PPARδ into adult rat fibers has recently been shown to lead to activation of fuel switching as well as to a shift in the existing fiber profile toward the "slow," oxidative phenotype, an effect that caused the number of type I fibers in the extensor digitorum longus (EDL) muscle to be tripled after 14 days [37]. From these experiments, it is clear that PPARδ rapidly programs all fiber types in skeletal muscle to oxidize fatty acids.

3.2. *Evidence supporting a role for activated PPARδ in mediating fuel switching in human skeletal muscle: data from in vitro and clinical studies*

Recent studies have revealed clear roles for PPARδ in the regulation of lipid and glucose metabolism in human skeletal muscle [7, 38]. PPARδ stimulates the expression of genes involved in (a) increasing of lipid oxidation (fatty acid binding protein 3 (FABP3) and CPT1) and (b) reducing carbohydrate oxidation (pyruvate dehydrogenase kinase 4 (PDK4)) in human skeletal muscle [38], as it does in rodents [5, 8–10]. These in vitro human skeletal muscle data with GW1516 support the long-known observation that an increase in fatty acid oxidation reduces the glucose utilization of isolated muscle reflecting the mutual inhibition in the metabolism of substrates involved in the glucose-fatty acid cycle [35, 36, 39]. Recent data from a clinical study indicate that this situation holds true in human subjects. A statistically significant reduction in fasting insulin levels was observed in a study performed on moderately obese men given a dose of 10 mg o.d. of GW1516 was given for 2 weeks

[7]. These subjects also showed decrease fasting plasma nonesterified fatty acid (NEFA) concentrations and increased expression of CPT1b in muscle. Interestingly, GW1516 treatment slightly reduced the expression of PPARδ, showing that overexpression of the receptor itself is not necessary for the induction of PPARδ-mediated effects, the resident levels of the receptor protein being sufficient. In contrast, similar treatment with a PPARα agonist (GW590735) did not result in any change in plasma insulin levels, nor did it cause a significant increase in CPT1b mRNA [7].

3.3. PPARδ: a regulator of fuel use in skeletal muscle under physiological conditions

Since fasting promotes increased utilization of fatty acids, fasting might also regulate PPARδ expression. However, the initial reports appearing in the literature did not seem to confirm this. For instance, a 24-hour fasting period was found not to upregulate PPARδ in rat skeletal muscle [40], and our group even reported reduced levels of PPARδ (and PPARα) in gastrocnemius muscle from 48-hour-fasted rats [25]. Decreased expressions of PPARδ and PPARα upon 48 hours of fasting have recently been reported in humans, too [41]. In contrast, in mice, skeletal muscle PPARδ levels were found to be upregulated after a 24-hour fast [42]. One explanation is that studying the responses to physiological stimuli by making single time point measurements may give misleading results, due to possible transient modifications. Indeed, time course studies have shown that PPARδ actually is upregulated in fasted rat skeletal muscle, but within the first 6 hours [43]. This upregulation of PPARδ (and of PGC-1α) during fasting is, however, transient [43], downregulation (after the initial upregulation at 6 hours to around or below the control levels being evident at 48 hours). Data showing that rapid nuclear accumulations of both PGC-1α and PPARδ upon food deprivation [43], and their physical interaction within the nucleus [30], occur concomitantly with increases in fatty acid levels and an increase in the expression of myosin heavy chain Ib (MHC Ib), underline the role of PPARδ as a key regulator of fatty acid metabolism and muscle fiber switching (in concert with its coactivator, PGC-1α).

Following the nuclear accumulation of PPARδ, its target genes (such as carnitine palmitoyl transferase 1b (CPT1b), mitochondrial thioesterase I (MTE I), and UCP3) are upregulated simultaneously, and so is the rate of mitochondrial fatty acid oxidation [43]. In starved mice, the mRNA for a member of the FOXO family, FKHR (forkhead homolog in rhabdomyosarcoma), is upregulated rapidly and transiently (starting within 6 hours, peaking at 12 hours, and decreasing at 24 hours), and this is followed by an upregulation of FKHR protein and a nuclear accumulation of nonphosphorylated FKHR levels with a consequent upregulation of its target gene pyruvate dehydrogenase kinase 4 (PDK4) [44]. In mouse skeletal muscle, PDK4 has been shown to be a target of PPARδ, since it is activated in vivo by GW1516 [8]. This kinase plays an important role in the switching from glucose usage to fat usage since it phosphorylates the E1 component of the pyruvate dehydrogenase (PDH) complex, thereby downregulating carbohydrate (CH) oxidation [45]. Similarly, in human skeletal muscle, PDK4 mRNA and protein levels have been found to be elevated at both 24 hours and 48 hours of fasting, whereas at these time points the FOXO1 mRNA/protein levels were unchanged [41]. However, it is possible that regulation of the mRNA/protein levels of FOXO1 (a target of PGC-1α [46], which are transiently regulated during fasting in rats [40]) is a transient event preceding upregulation of PDK4 mRNA and protein in humans, too. If so, such transient regulatory changes may have been missed by measuring only at 24 hours and 48 hours of starvation in humans. At the 48-hour time point of starvation, PPARδ mRNA levels in the human vastus lateralis muscle were decreased [41], a finding in line with the decreased levels of PPARδ mRNA [25] and nuclear protein [43] at this time point in fasting rat gastrocnemius muscle. In the rat, this decline was preceded by a rapid rise in the mRNA and protein levels of this transcription factor, elevated levels being detected at 6 hours and 12 hours of food deprivation [43]. Unfortunately, these time points were not investigated in the human study [41]. Thus kinetic studies in the fasting situation, in rodents as well as in humans, have made it clear that the expression of PPARδ, and that of its target genes, is tightly regulated. These results offer an explanation for the rapid structural and metabolic changes favoring an increased use of lipids as fuel that occur in this condition.

The reported kinetics of the PPARδ upregulation occurring during the recovery period after exercise-imposed energy stress in humans are in line with those seen in the fasting rat; a single exhaustive bout of cycling increasing PPARδ mRNA and protein expression within 3 hours after completion of the exercise [47, 48]. Regular physical training, which elevates the levels of PPARδ and PGC-1α, leads to increases in both mitochondrial capacity and insulin sensitivity [34, 49–51], effects similar to those seen after PPARδ activation. The observations made in exercise and fasting experiments showing that PPARδ-expression kinetics are rapid, and the changes almost immediate, are supported by PPARδ acutely bringing about the fuel switching effects inpre-existing adult rat muscle fibers (as demonstrated by short-term agonist treatment [35, 36] and somatic PPARδ gene transfer [36]). Recently, a direct role for PPARδ in the suppression of glucose oxidation in fasting skeletal muscle has been shown by Nahlé et al. [52]. First, the authors demonstrated that deficiency of the fatty acid translocase CD36 (mediating muscle fatty acid uptake during fasting, [5]) blunts fasting induction of FOXO1 and PDK4 and the associated suppression of glucose oxidation in mouse skeletal muscle [52]. Next, they demonstrated that loss of PPARδ abolishes the fasting induction of muscle FOXO1 and PDK4 in vivo [52]. As the authors identified several PPRE sites in the FOXO1 promoter, these results suggest that CD36-dependent activation of PPARδ results in the trascriptional regulation of FOXO1 as well as PDK4 in fasted mice.

Taken together, the control of fuel use by activated PPARδ has emerged to be crucial for the rapid metabolic adaptation of skeletal muscle to energy stress.

4. ANTIGLYCEMIC ACTION OF PPARδ IN LIVER: CONVERTING GLUCOSE INTO FAT

At first glance, the above described PPARδ-mediated fuel switching in skeletal muscle would be expected to give rise to insulin resistance, since a preferential uptake and oxidation of fatty acids (FFAs) over glucose (mediated by PPARδ stimulation in skeletal muscle) would lead to an accumulation of blood glucose, muscle being a major player in blood-glucose homeostasis [1, 2]. However, as well as stimulating skeletal muscle fatty acid oxidation, PPARδ plays a surprising role in ameliorating hyperglycemia. It does this by increasing hepatic glucose flux through the pentose phosphate pathway and by enhancing fatty acid synthesis, the fatty acids being destined for oxidization in muscle [10]. Whereas PPARδ-deprived mice are metabolically less active and glucose intolerant, db/db mice treated with GW1516 show increased levels of CPT in muscle [10] (similar effects of this agonist being reported in [8, 9]). This effect on CPT is indicative of increased fatty acid oxidation, so it was a surprising finding that gene array analysis revealed increased expression of gene clusters involved in fatty acid synthesis and the pentose phosphate cycle in the liver [7], both pathways using glucose or its metabolites as substrates. The authors [10] suggested that PPARδ may increase glucose catabolism through these processes, with the result that peripheral insulin sensitivity may be improved [10]. Indeed, GW1516-treated db/db mice showed an improved performance in the glucose tolerance test, and also showed a tendency toward lowered fasting serum insulin levels [10].

5. ROLE FOR PPARδ IN WHITE ADIPOSE TISSUE: INCREASING LIPOLYSIS

Interestingly, adipocyte hypertrophy induced by high-fat diet was accompanied by increased cannabinoid receptor type 1 (CB1R) expression and by a decrease in PPARδ expression in adipose tissue [11]. Exercise attenuates adipocyte hypertrophy and normalizes expression of CB1R and PPARδ. Functional cross-talk between CB1R and PPARδ is established by RNA interference experiments in 3T3-L1 preadipocytes. For example, selective silencing of PPARδ by RNA interference significantly increased CB1R and increased adipocyte differentiation and adenovirus-mediated overexpression of PPARδ reduced CB1R expression [11]. The role of CB1R in obesity is well established [53]. Depletion of CB1R in knockout mice is known (in animals fed a high-fat diet) to reduce obesity through increased lipolysis [53].

The simultaneous regulation of skeletal muscle fatty acid oxidation and adipose proliferation by PPARδ underlines the powerful role this receptor may play in preventing weight gain in physiological situations.

6. CONCLUSIONS

Recent evidence shows that the fuel-switching role of PPARδ in skeletal muscle is conserved from rodents to humans. This receptor is crucial for a modulation of skeletal muscle flexibility that directs this tissue toward the use of fatty acids as fuel, an effect with rapid kinetics. It is becoming increasingly clear that even though the fat oxidation-inducing effect of PPARδ is exerted in skeletal muscle, this receptor also activates transcriptional programs in other tissues (such as adipose tissue, in which PPARδ directly inhibits proliferation and induces lipolysis), thereby channelling the fat consumed in the diet directly to muscle for oxidation. In addition, in an action that serves to prevent insulin resistance developing as a consequence of a reduction of carbohydrate oxidation in muscle, the liver is activated to form fat from glucose, thereby both allowing skeletal muscle to oxidize more fat and concomitantly lowering blood glucose levels. PPARδ is emerging as a crucial nuclear receptor for the multiorgan regulation of whole body fuel turnover, with skeletal muscle as the central organ in the burning of fat and the consequent prevention or reduction of obesity (see Figure 1). These features make PPARδ a good candidate as a central target for the future treatment of metabolic disturbances linked to obesity and insulin resistance.

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Review Article

PPARδ Agonism for the Treatment of Obesity and Associated Disorders: Challenges and Opportunities

Mylène Perreault, David V. Erbe, and James F. Tobin

Department of Cardiovascular and Metabolic Diseases, Wyeth Research, 200 Cambridge Park Drive, Cambridge, MA 02140, USA

Correspondence should be addressed to Mylène Perreault, mperreault@wyeth.com

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The prevalence of obesity in the USA and worldwide has reached epidemic proportions during the last two decades. Drugs currently available for the treatment of obesity provide no more than 5% placebo-adjusted weight loss and are associated with undesirable side effects. Peroxisome proliferator-activated receptor (PPAR) modulators offer potential benefits for the treatment of obesity and its associated complications but their development has been complicated by biological, technical, and regulatory challenges. Despite significant challenges, PPAR modulators are attractive targets for the treatment of obesity and could offer a viable alternative to the millions of patients who fail to lose weight following rigorous dieting and exercise protocols. In addition, PPAR modulators have the potential-added benefit of ameliorating the associated comorbidities.

1. INTRODUCTION

During the last two decades, the incidence of obesity has tripled in developing countries, with more than 1.1 billion adults overweight worldwide and 312 million of them obese [1]. Obesity has been causally linked to the development of certain forms of cancer, cardiovascular disease, sleep apnea, psychiatric disorder, and type 2 diabetes (T2D) [2–6]. In parallel to the growing rate of obesity, the incidence of T2D has also dramatically increased worldwide, and it is believed that more than 366 million people will become diabetic by 2030 [1]. Recent findings point toward visceral adiposity as a causal link for the development of obesity-induced T2D. Visceral adipose tissue (VAT) is metabolically more active than subcutaneous adipose tissue and secretes a number of adipokines [7, 8]. The surgical removal of as little as 0.6 kg (0.8% total fat) of VAT significantly ameliorated insulin sensitivity in obese patients [9], whereas removal of large amounts of subcutaneous adipose tissue did not [10]. These findings suggested that adipose tissue can no longer be considered an inert tissue strictly involved in lipid storage but is rather an endocrine organ capable of regulating several aspects of metabolism.

Currently, a few drugs are approved for the long-term treatment of obesity but their efficacy is limited. Both sibutramine (Meridia) and orlistat (Xenical) have been available for approximately 10 years for the treatment of obesity but these agents only induce modest one-year weight loss of 4.2% and 2.9%, respectively [11]. More recently, the cannabinoid receptor-1(CB1) inverse agonist, rimonabant, has been associated with significant weight loss in several global clinical trials, including RIO-Europe, RIO-Lipids, RIO-Diabetes, and RIO-North America [11]. Although rimonabant induced an approximately 5% placebo-adjusted weight reduction in those studies, it was not approved in the USA principally due to neurological and psychiatric safety concerns [12]. Recently, the antidiabetic GLP-1 agonist exenatide (Byetta) was shown to significantly reduce mean body weight (−4.7%) from baseline after 2 years of treatment [13]. This drug offers benefits over existing therapies as it combines weight loss and improvements in glycosylated hemoglobin (HbA1c), liver function, and blood pressure [13]. Side effects including nausea and vomiting have been noted [14] and rare cases of acute pancreatitis have been reported [15]. However, exenatide is currently not approved for the treatment of obesity.

With no more than 5% placebo-adjusted weight loss observed with currently available treatments, more drugs are thus needed to provide patients with safe and efficacious therapeutic options. Recent findings have suggested

that peroxisome proliferator-activated receptor (PPAR) delta agonists could induce body weight loss by increasing energy expenditure and fatty acid oxidation [16]. In fact, PPARδ agonism either alone or in combination with agonism of PPARα and/or PPARγ could not only induce body weight loss but could also potentially improve other metabolic parameters including insulin sensitivity and lipid profiles, thus producing benefits associated with the comorbidities of the obese state.

2. MECHANISM OF ACTION OF PPARs

PPARs belong to the nuclear receptor family of transcription factors and are master regulators of genes involved in glucose and lipid metabolism. The PPARs are modular in nature, containing an amino-terminal ligand-independent AF1 transactivation domain, a DNA binding domain containing two zinc finger motifs, and a carboxy-terminal domain that contains a dimerization domain and a ligand-dependent AF-2 transactivation domain [17]. There are three members of the PPAR family, PPARα, PPARγ, and PPARδ [18]. They are 60–80% conserved in their DNA and ligand-binding domains and are activated by a number of natural and synthetic ligands, including eicosanoids, free fatty acids (FFA), lipid lowering drugs (fibrates), and the insulin sensitizers (thiazolidinediones). PPARs heterodimerize with the retinoic X receptor (RXR) and bindspecific promoter sequences termed PPAR responsive elements (PPREs) in their target genes. In the absence of a ligand, PPARs form complexes with corepressors such as NcoR, RIP140, and SMRT [19–21], which repress transcription through the recruitment of histone deacetylases. Ligand binding induces a conformational change that results in the dissociation of corepressors and the recruitment of coactivators, such as PPARγ coactivator-1 (PGC-1), mediator complex, P300/CBP [22–24]. The specificity of complexes formed between the receptor and corepressors or coactivators appears to be determined by cell type, conformational change induced by the ligand, and sequence of the DNA binding element. This level of complexity allows fine-tuning of the physiological response and explains the variability of gene expression changes when a receptor is activated by different ligands.

2.1. PPARγ

PPARγ is the molecular target of thiazolidinediones (TZDs) and has been well characterized in obese diabetic rodent models and in diabetic patients. It is primarily expressed in adipocytes, where it plays a pivotal role in the regulation of adipocyte differentiation and lipid storage. Treatment of diabetic animals or patients with TZDs increases subcutaneous fat mass area by inducing the number of small insulin sensitive adipocytes in subcutaneous fat [25–28], while decreasing visceral fat mass [29, 30]. This effect of TZDs most likely contributes to the improvement of insulin sensitivity, although a direct effect of PPARγ on skeletal muscle, liver, and macrophages has also been demonstrated. For example, it has been reported that the insulin sensitizing effect of rosiglitazone was attenuated in macrophage-specific PPARγ knockout mice, suggesting that macrophages are important for TZD-induced insulin sensitivity [31]. TZD treatment has also been associated with significant improvement of skeletal muscle, liver, and adipose tissue insulin signaling in both rodents and humans [30, 32, 33]. Additionally, PPARγ agonists increase the expression of adiponectin, which decreases hepatic glucose production through activation of AMP-activated protein kinase (AMPK) [34] and could thus contribute to TZD-induced improvement in insulin sensitivity.

2.2. PPARα

PPARα is predominantly expressed in highly oxidative tissues, including liver, skeletal muscle, brown adipose tissue, and heart [35, 36] and is the target of the fibrate class of drugs used for the treatment of dyslipidemia. Fibrates decrease triglyceride levels in both rodents and humans by depleting the pool of free fatty acids (FFAs) through peroxisomal and mitochondrial fatty acid oxidation. PPARα activation has been associated with increased expression of genes involved in all steps of fatty acid utilization, including the breakdown of triglyceride particles to free fatty acids by lipoprotein lipase (LPL), the transport of free fatty acids inside the cells by fatty acid transport proteins (FATPs), and the peroxisomal and mitochondrial β-oxydation by activation of acyl CoA oxydase (ACO) and medium chain acyl CoA decarboxylase (MCAD). In addition, PPARα is involved in the reverse cholesterol transport pathway and contributes to HDL production in humans by increasing the expression of the major constituent of high-density lipoprotein (HDL) cholesterol, apolipoprotein A1 (ApoA1) [37], as well as increasing the ATP-binding cassette transporter A1 (ABCA1) [37].

Recently, PPARα has also been implicated in the regulation of body weight through appetite suppression. In rodents, PPARα agonists, including WY-14643, fenofibrate, and oleoylethanolamide (OEA), have been reported to induce body weight loss in a PPARα-specific manner [38, 39]. One of the proposed mechanisms involves stimulation of specific regions of the brain controlling satiety through [40] vagal nerve activation. Interestingly, PPARα agonists can no longer induce appetite suppression in rats in which the vagal nerve has been severed [38]. It is also possible that PPARα induces appetite suppression through other mechanisms, including FGF-21 secretion [41–43] or increased fatty acid oxidation and production of ketone bodies [44, 45].

2.3. PPARδ

PPARδ has recently been characterized in rodents and humans and is more widely distributed than its two counterparts with high level of expression in skeletal muscle, heart, kidney, adipose tissue, liver, and macrophages [46]. The physiological role of PPARδ has been elucidated not only through genetic approaches, but also by pharmacological activation of the receptor with specific agonists, including GW501516. A major role for PPARδ in body

weight regulation has been reported in mice overexpressing a constitutively active form of PPARδ in adipose tissue. These mice are resistant to diet-induced obesity (DIO) and are protected against the development of genetically induced obesity through induction of energy expenditure via mitochondrial fatty acid oxidation and energy uncoupling [16]. It is also possible that PPARδ induces body weight loss by centrally regulating appetite suppression since PPARδ was shown to mediate the hyperphagic response following focal cerebral ischemia [47].

In addition to inducing body weight loss, PPARδ activation has been shown to improve lipid profile by depleting the pool of fatty acids through mitochondrial fatty acid oxidation [16, 48] and by increasing reverse cholesterol transport [49]. Two-week treatment of healthy volunteers with GW501516 significantly increased HDLc and decreased triglycerides as compared to placebo-treated subjects [50]. PPARδ expression is upregulated in skeletal muscle of T2D subjects and high-fat fed rats after bouts of exercise [46, 51–54] and may play a role in providing a continuous source of energy to support the increasing energy demand. Consistent with this hypothesis, skeletal muscle overexpression of PPARδ was associated with significant muscle-type switching from glycolytic to oxidative fibers, further supporting its role in lipid oxidation [55]. Interestingly, skeletal muscle PPARδ transgenic mice were more resistant to fatigue after intense exercise [55], suggesting a PPARδ-induced switch in substrate utilization that helped maintain a higher level of energy demand.

In addition to inducing weight loss and improving lipid profiles, PPARδ agonism has the potential to improve insulin sensitivity and glucose metabolism [46, 56]. Recently, this has been shown to include a crucial role for PPARδ expressed in tissue macrophages [57, 58]. Activation of resident macrophages in metabolic tissues toward the inflammatory (M1) phenotype appears to play a role in insulin resistance as a result of obesity. However, through production of Th2 cytokines, both adipocytes and hepatocytes are able to induce PPARδ expression in resident macrophages leading to their alternative activation toward the anti-inflammatory M2 phenotype. This then results in increased insulin sensitivity in these tissues, and could underlie some of the improvements in glucose metabolism seen in PPARδ-treated animals.

3. CHALLENGES TO THE DEVELOPMENT OF PPARδ MODULATORS

Following the publication of several high-profile studies implicating PPARδ as a potential target for obesity and associated metabolic disorders, multiple pharmaceutical companies have initiated drug discovery efforts to identify specific PPARδ, PPARα/δ, PPARγ/δ, and pan PPAR modulators. However, development of these PPAR agonists faces significant challenges including major differences in PPAR biology between rodents and humans, difficulties in predicting in vivo efficacy from simple in vitro models of activity, toxicological findings arising from weight reduction through targeting β-oxidation of fatty acids, and safety requirements unique to PPAR biology and drug development.

3.1. Differences in PPAR pharmacology between rodents and humans

Despite very high homology between human and rodent PPAR receptors, major biological and physiological differences exist between these species. One clear and well-understood example relates to peroxisome proliferation and liver enlargement with PPARα agonists. In rodents, but not humans, PPARα activation leads to hepatic peroxisome proliferation associated with liver enlargement and cell necrosis. One potential biological explanation for these differences is that hepatic PPARα expression is approximately 10 times higher in rodents versus humans [59].

Similarly, it has been reported that PPARδ pharmacology differs between higher species and rodents. Contradicting reports suggest that pharmacological PPARδ activation inconsistently regulates body weight [55, 60, 61] and cholesterol or triglyceride levels in rodents [55, 60–62]. However, significant elevation of HDL cholesterol and/or reduction of LDL cholesterol and triglyceride have been reported in obese rhesus monkeys and humans after chronic PPARδ agonist administration [50, 63]. One potential explanation may reside in the observation that the rodent ApoA-1 promoter differs from the human promoter by 3 nucleotides, resulting in a nonfunctional PPRE site [64, 65]. Interestingly, PPARδ agonists have been shown to decrease triglyceride and increase HDL cholesterol in humanized ApoA-1 transgenic mice [66] suggesting that mice can positively respond to PPARδ activation once provided with a functional ApoA1 gene. Overall, these species differences could make the observation of PPARδ driven outcomes more difficult in rodents, and thus complicate the development of PPARδ modulators.

3.2. Complexity induced by ligand-specific effects

Traditionally, the potency and affinity of PPAR modulators have been determined using multiple assays, including displacement of radiolabeled compound, displacement and recruitment of corepressors and coactivators, and cell-based transcriptional activation assays. Often, the in vitro potency in binding and transcriptional activation assays correlates with in vivo efficacy, a notable example is the hypoglycemic effects of TZDs. TZDs with more potent in vitro activities are often more active in rodent models [67, 68]. In some cases, these correlations do not hold, reflecting a more complex relationship between simple in vitro measures and in vivo pharmacology that is best understood in the context of the selective PPAR modulator (SPPARM) hypothesis. The SPPARM concept emerged to describe the complete spectrum of PPAR conformation/activation states leading to each specific biological response. This model stipulates that PPAR modulators turn on and off specific genes by recruiting or releasing a complex assortment of coactivators and corepressors. The coactivators and corepressors are likely tissue specific and different modulators are believed to recruit different sets of proteins leading to various degree of gene activation/repression. This level of complexity provides a potential strategy to eliminate the known liabilities

associated with PPAR activation by selecting modulators that are recruiting/releasing specific sets of coactivators and corepressors. However, this complexity also complicates the development of PPAR agonists because species differences can exist concerning coactivator expression and recruitment, tissue specificity and availability.

Consistent with the SPPARM hypothesis, Berger et al. reported that PPARγ/δ agonists L-165461 and L-783483 had similar binding affinities and transcriptional activities against PPARγ and PPARδ while the glucose lowering effect was more pronounced with L-783483 in db/db mice [67]. We have observed that PPAR pan modulators with similar profiles in transcriptional cell-based assays can yield significantly different in vivo activities (Table 1) despite similar pharmacokinetic properties. These differences likely stem from differential interactions with the ligand-binding pocket of each PPAR resulting in modulator-specific differential recruitment of coactivators/corepressors.

3.3. Challenges associated with the development of weight-reducing agents targeting oxidation of fatty acids

It is well understood that body weight is physiologically regulated through two major mechanisms: food intake and energy expenditure. Energy expenditure can be positively or negatively modulated by regulating metabolic rate, body temperature, or level of physical activity. Targeting metabolic rate through oxidation of free fatty acids and/or energy uncoupling are effective ways to induce body weight loss. For example, it has been shown that targeted deletion of acetyl-CoA carboxylase-2 (ACC2), an enzyme responsible for the synthesis of malonyl-CoA, is associated with approximately 10–20% body weight loss caused by significant increase in total energy expenditure, with no change [71] or increased food consumption [72]. The increased energy expenditure was mostly explained by a significant increase in fatty acid oxidation and not an elevation of the level of physical activity [71, 72]. Similarly, transgenic mice with muscle-specific activation of PPARα are resistant to diet-induced obesity despite consuming similar amounts of food compared to nontransgenic mice [73]. As expected, the rate of palmitate oxidation and the level of expression of genes regulating mitochondrial and peroxisomal β-oxidation were increased in muscle-specific PPARα transgenic mice [73]. Overall, these studies provided convincing evidence that specifically targeting oxidation of fatty acids is a relevant mechanism to induce body weight loss.

PPARδ is known to influence all of the parameters regulating energy expenditure by increasing the transport and oxidation of free fatty acids in adipose tissue and skeletal muscle, increasing thermogenesis and energy uncoupling in adipose tissue and inducing muscle fiber-type switching to promote endurance and resistance to fatigue. Recently, PPARδ has been suggested as a potential target for body weight loss because of its role in fatty acid oxidation. In mouse and human skeletal muscle, PPARδ activation increases the expression of several genes involved in mitochondrial oxidation of free fatty acids, including carnitine palmitoyltransferase-1 (CPT-1), medium chain acyl-CoA dehydrogenase (MCAD), long chain acyl-CoA dehydrogenase (LCAD), and PGC-1α [16, 48, 55, 74] and its pharmacological activation has been associated with body weight loss in rodents [48, 55].

Currently, controversies exist as to whether an increased rate of fatty acid oxidation is beneficial. Numerous studies have been published demonstrating that an elevated rate of β-oxidation is beneficial because it depletes the pool of fatty acids required to synthesize triglyceride particles, prevents accumulation of fatty acid metabolites in skeletal muscle and other nonfat tissues, and induces body weight loss. However, it has been shown that sustained level of fatty acid oxidation, through long-term fibrate treatment or PPARα overexpression, is associated with skeletal and heart muscle degeneration [75], cardiomyopathy [76, 77], and insulin resistance [73] in mice. Consistent with these reports, we have previously observed that pan PPAR activation induced significant levels of skeletal muscle degeneration and liver vacuolation and necrosis in mice, but these observations were completely absent in PPARα knockout animals (M. Perreault et al., unpublished observations). Collectively, these results suggest that sustained levels of fatty acid oxidation through PPARα and potentially PPARδ could significantly improve some metabolic parameters but with potential deleterious effects on muscle and liver.

It has been proposed that the detrimental effects of sustained fatty acid oxidation result from the production of reactive oxygen species produced during peroxisomal and mitochondrial oxidation of fatty acids. The first and rate-limiting step of peroxisomal β-oxidation, ACO, generates hydrogen peroxide during oxidation of acyl-CoAs. It has been reported that hydrogen peroxide levels are significantly increased in the heart of cardiac-specific PPARα transgenic mice and this effect is exacerbated on a high-fat diet, where the substrates for fatty acid oxidation are more abundant [76]. Similarly, oxidation of fatty acids through mitochondrial β-oxidation has been associated with an increased production of reactive oxygen species (ROSs) as long-chain fatty acids provide reducing equivalents that fuel the electron transport chain.

While the role of PPARα in skeletal and cardiac muscle degeneration has been established in rodents, the effect of PPARδ activation on these parameters is less clear. It has been reported that PPARδ activates distinct metabolic programs in the mouse heart as compared to PPARα, leading to cardioprotection in the setting of myocardial ischemia/reperfusion injury [77]. The exact mechanism for the cardioprotection is currently unknown, but in contrast to PPARα, PPARδ overexpression induced cardiac glucose oxidation as opposed to fatty acid oxidation [77]. Normal hearts exhibit substrate flexibility by switching between lipid and glucose to match the metabolic state. However, diabetic hearts mostly rely on fatty acids, leading to excessive rates of myocardial fatty acid oxidation concomitant with reduced glucose oxidation. In the heart of cardiac-specific PPARα transgenic mice, genes involved in fatty acid uptake, lipogenesis, and triglyceride synthesis (fatty acid transport protein (FATP), CD36, glycerol-3-phosphate

Table 1: Compounds with similar in vitro profiles induced different in vivo efficacy.

Compound	In vitro potentcy (μM)			Weight loss	Insulin (% reduction)	Adiponectin (fold increase)
	α	γ	δ			
1 [69, 70]	6.0	0.1	5.0	Weight neutral	30%	2.1
2 [69, 70]	3.0	0.1	1.0	14%	80%	3.2

acyltransferase (GPAT), acyl-CoA synthetase (ACS), fatty acid synthase (FAS), microsomal triglyceride transfer protein (MTP)) were significantly elevated while genes involved in glucose metabolism (glucose transporter 4 (GLUT4), phosphofructokinase (PFK)) were not. In contrast, the expression of fatty acid transport and esterification genes was unchanged in cardiac-specific PPARδ transgenic mice while genes regulating glucose metabolism were significantly elevated. These results further contribute to the hypothesis suggesting that increased and sustained rate of fatty acid oxidation could be detrimental for cardiac functions and suggest that this mechanism is PPARα-specific. Furthermore, it has been reported that PPARδ activation is associated with elevated expression of catalase [78], an enzyme responsible for hydrogen peroxide degradation potentially providing an additional protective effect.

Recently, the concept of incomplete fatty acid oxidation has emerged as another potential explanation for fatty acid oxidation-induced metabolic disturbances. It was proposed that obesity results in an excessive fatty acid load on mitochondria causing accumulation of incompletely oxidized intermediates, including acylcarnitine esters, while decreasing levels of metabolites of the tricarboxylic acid (TCA) cycle [79]. The exact mechanism leading to metabolic disturbances is still unknown and whether acylcarnitine esters directly induce insulin resistance and metabolic disorders remains to be determined.

3.4. Safety study requirements unique to PPAR biology and drug development

PPAR gamma (TZDs) and alpha (fibrates) agonists have been used for many years to treat type 2 diabetic and dyslipidemic patients, respectively. TZDs (Avandia and Actos) are very well characterized in rodents and humans and result in significant improvements in insulin sensitivity and glycemic control but are associated with increases in body weight and fluid retention that can exacerbate congestive heart failure. The liabilities associated with PPARγ agonists are observed in a small but significant number of patients and are very closely related to their efficacy, as reduction in HbA1c is directly correlated to body weight gain [80]. PPARγ-associated weight gain and fluid retention liabilities are well understood and can be reversed by discontinuing drug administration or by treating with diuretics.

Recently, the PPARγ activator rosiglitazone has been suggested to directly affect cardiac function independent from its effects on fluid retention. A number of meta-analyses concluded that rosiglitazone significantly increased the rates of myocardial infarction and death by cardiovascular causes [81, 82]. However, controversies exist as several recent studies refuting those results have been published [83, 84]. Interestingly, a significant lower risk of death, myocardial infarction and stroke was observed with pioglitazone (Actos) [85], suggesting a difference between rosiglitazone and pioglitazone in relation to cardiovascular effects. Pioglitazone has been previously shown to improve lipid profile (triglyceride, LDL and HDL cholesterol) by weakly activating PPARα, while rosiglitazone seems to worsen these parameters which could contribute to the deleterious effects on cardiac functions [86–88]. The findings from these meta-analyses will have to be confirmed with cardiovascular outcome studies, as the clinical trials included in these analyses were not originally designed to evaluate cardiovascular endpoints. The RECORD study is a clinical trial evaluating the effects of rosiglitazone in approximately 2000 patients to specifically address cardiovascular effects as primary outcomes. The interim analysis indicated no significant increased myocardial infarction and death with rosiglitazone, however, the authors admitted that their study might be underpowered due to lower cardiovascular events in this patient population and that the analysis was performed before completion of the study [89–92].

Whether the cardiovascular effects of rosiglitazone and pioglitazone are confirmed or not with clinical outcome studies, these recent findings have changed the way regulatory agencies review and approve drugs and protein therapeutics for the treatment of T2D. Earlier this year, the FDA published their draft guidance for the development of new diabetes therapeutics. The guidance affirms that in the absence of cardiovascular signal, long-term cardiovascular studies could be conducted postapproval in a reasonable timeframe [93]. However, large outcome trials should be conducted prior to submission of regulatory dossiers for drugs that show nonclinical or clinical evidence of increasing cardiovascular risk [93].

In addition to the cardiovascular effects, multiple PPAR modulators have been discontinued over the last several years due to carcinogenicity findings in rodents. Because of the prevalence of positive carcinogenicity findings with PPAR agonists and the lack of complete understanding of PPAR-induced tumor development, 2-year carcinogenicity studies in mice and rats are now required before clinical trials longer than 6 months in duration can be initiated [93, 94]. Moreover, PPARγ activation has recently been linked to an increased risk of fracture in humans [95–97]. It has been proposed that PPARγ activators could have a direct effect on osteoblastogenesis [98, 99] and osteoclastogenesis [100] in rodents, but further clinical studies will be necessary to understand if these mechanisms are also involved in TZDs-induced bone loss in humans. Despite an increased risk of fracture with PPARγ activators in humans, the role of

other PPAR isoforms on bone formation and resorption is less clear. The presence of PPARδ both at protein and mRNA levels has been reported in rat bone tissue sections, preosteoblasts, rodent and human osteoblastic cell lines as well as rabbit osteoclasts [101–104], while PPARα has been found in preosteoblasts, chondrocytes, and human peripheral blood mononuclear cell (PBMC)-derived osteoclasts [101]. The role of PPARα and PPARδ in bone formation and resorption needs to be further determined.

4. SUMMARY AND PERSPECTIVES

Obesity and associated disorders are serious diseases affecting millions of people worldwide. The therapeutic options currently offered are providing limited efficacy and are coupled with several serious side effects. Despite significant challenges associated with their development, PPAR modulators are potential new obesity therapies that could offer not only weight management opportunities but also amelioration of the associated disorders by correcting the causes of insulin resistance and dyslipidemia. Molecules targeting PPARδ, alone or in combination with other PPARs, offer significant advantages as PPARδ seems to have beneficial cardioprotective effects in rodents by modulating fuel utilization in addition to its anti-inflammatory and lipid effects. Whether or not such weight-modulating therapies will be relevant to humans and ultimately approvable by regulatory agencies remain to be determined.

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Review Article

PPAR-δ in Vascular Pathophysiology

Nanping Wang

Laboratory of Vascular Molecular Biology and Bioengineering, Institute of Cardiovascular Science and Diabetes Center, Peking University Health Science Center, Beijing 100191, China

Correspondence should be addressed to Nanping Wang, npwang@bjmu.edu.cn

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Peroxisome proliferator-activated receptors belong to the superfamily of ligand-dependent nuclear receptor transcription factors, which include three subtypes: PPAR-α, β/δ, and γ. PPAR-δ, play important roles in the regulation of cell growth and differentiation as well as tissue wound and repair. Emerging evidence has also demonstrated that PPAR-δ is implicated in lipids and glucose metabolism. Most recently, the direct effects of PPAR-δ on cardiovascular processes such as endothelial function and angiogenesis have also been investigated. Therefore, it is suggested that PPAR-δ may have critical roles in cardiovascular pathophysiology and is a potential target for therapeutic intervention of cardiovascular disorders such as atherosclerosis.

1. INTRODUCTION

Peroxisome proliferator-activated receptors (PPARs) are members of nuclear receptor/ligand-activated transcription factors superfamily. PPAR subfamily consists of 3 subtypes: PPAR-α, -β/δ, and -γ. PPARs form heterodimers with a retinoid X receptor (RXR) and bind to specific PPAR-responsive elements (PPREs) to regulate target gene expression. In the absence of specific ligands, the PPAR-RXR heterodimer forms repressive complex with corepressors and histone deacetylases. Upon ligand binding, the receptor undergoes conformational changes that cause the dissociation of repressors, the recruitment of coactivators, and the activation of gene transcription [1]. Recently, extensive studies have been performed to characterize the biological and pathophysiological roles of PPAR-α and -γ, which are pharmacological targets of the clinical interventions for dyslipidemia and type 2 diabetes, respectively. Fibrates class of lipid-lowering drugs such as fenofibrate and gemfibrozil are agonists for PPAR-α. Thiazolidinedione class of insulin sensitizers including troglitazone (Rezulin), rosiglitazone (Avandia), and pioglitazone (Actos) are specific ligands for PPAR-γ [2]. PPAR-α, primarily expressed in liver, muscles, heart, and kidney, plays a key role in fatty acid catabolism such as β-oxidation. PPAR-γ is highly expressed in fat, controls adipogenesis, and regulates insulin action. However, relatively little has been known with regards to the function of PPAR-δ, the only subtype of PPARs that is not a target of current drug. Newly developed synthetic ligands and genetically modified mouse models for PPAR-δ have rapidly advanced our understanding of the important roles of PPAR-δ in tissue development, repair, inflammation, and metabolism [3–7]. Most recently, the direct effects of PPAR-δ on cardiovascular processes such as endothelial function and angiogenesis have also been investigated. In this review, we will focus on the recent advancements regarding the roles of PPAR-δ in vascular pathophysiological processes.

2. MATERIALS AND METHODS

2.1. Gene and protein

PPAR-δ, also known as nuclear hormone receptor 1 (NUC1), PPAR-β, or NR1C2, was first cloned in 1992 [8, 9]. The PPAR-δ gene is mapped to human chromosome 6p21.2-p21.1 and has 11 exons, spanning 35 kilobase-pair [10]. Like other PPARs, PPAR-δ protein has a modular structure consisted of 5 regions: an N-terminal region (A/B), a DNA-binding domain (C), a flexible hinge region (D), ligand-binding domain (E), and a C-terminal region (F). X-ray crystallographic study revealed that PPAR-δ has an exceptionally large ligand binding pocket, which maybe related to the promiscuous accommodation of a large range of mostly amphipathic ligands [11, 12].

2.2. Endogenous and synthetic ligands

Several 14- to 18-carbon saturated fatty acids and 16- to 20-carbon polyunsaturated fatty acids can bind PPAR-δ [13–15]. Naturally occurring or synthetic eicosanoids such as prostaglandin A1 and carbaprostacyclin have been shown to bind and activate PPAR-δ [16]. Very low-density lipoprotein (VLDL) derived has also been demonstrated to activate the PPAR-δ target genes in a receptor-dependent manner [17]. Since these agonists activate PPAR-δ all with affinities at molar range, it raises a question as to whether these are bona fide physiological ligands for PPAR-δ. However, many of the above-mentioned agonists under the physiological or pathological conditions are either released by the vessels, such as PGI2, or being exposed to vascular endothelium, such as VLDL. It would be intriguing to examine whether PPAR-δ in the vessel wall is activated in vivo. In addition, it was recently shown that retinoic acid, a ligand for retinoic acid receptor, also can activate the PPAR-δ with nanomolar affinity without affecting the other two subtypes of PPARs [18]. This finding expanded our understanding of the mechanisms of PPAR-δ activation.

Recently, several synthetic ligands have been reported to selectively activate PPAR-δ. The PPAR-δ agonists reported to date were discovered using several strategies: GW501516 and GW-0742 (GlaxoSmithKline) were optimized from a library of hydrophobic carboxylates [19]; L165461 (Merck) was derived from an in silico approach [20]. These derivatives of phenoxyacetic acid are the highly selective PPAR-δ ligands with a nanomolar affinity and 1000-fold selectivity over PPAR-α and -γ. Other PPAR-δ agonists including KD3010 (Kalypsys) and MBX-8025 (Metabolex) are currently in clinical development. On the other hand, the development of these specific agonists has greatly aided the investigation in the biological functions of PPAR-δ. A selective antagonist for PPAR-δ, GSK0660, has also been recently demonstrated as it by itself exhibits inverse agonist activity and competes with agonist in a cellular context [21] (Table 1).

2.3. Effects on the vessel wall

Although nearly ubiquitously expressed with highest levels in placenta, skeletal muscles, and adipose tissue, PPARδ is also expressed in the vascular cells including endothelial cells [22], smooth muscle cells, and macrophages. Particularly, a number of studies during the past 2 years have demonstrated that PPAR-δ plays direct roles in various basic vascular processes such as apoptosis, survival, angiogenesis, and inflammation.

2.4. Endothelial apoptosis

Vascular endothelium, when unperturbed, is considered to provide a relatively nonadhesive and nonthrombotic interface. This characteristic is likely essential to physiological homeostasis. However, endothelial cells (ECs) can undergo apoptosis in vitro in response to a variety of pathophysiological conditions including hypoxia, proinflammatory cytokines, bacterial endotoxins, and atherogenic risk factors such as homocysteine and lipoproteins [23, 24]. EC apoptosis has been implicated in numerous pathophysiological processes, such as angiogenesis, thrombosis, and atherosclerosis. On the other hand, ECs produce a plethora of bioactive molecules to maintain vascular homeostasis. Among those, prostacyclin (PGI2) protects ECs from apoptosis. Although PPAR-δ has been previously documented to protect against the hypertonicity-induced apoptosis in renal cells [25] and the growth factor deprivation- or anoikis-induced apoptosis in keratinocytes [6], its role in vascular cells has been recently demonstrated. Liou et al. showed that PGI2 protects ECs from H_2O_2-induced apoptosis via the action of PPAR-δ. By inducing the expression of its target gene 14-3-3alpha, PPAR-δ prevents Bad-triggered apoptosis [26]. Treatment with L165041 or overexpression of PPAR-δ also has a similar effect. In addition, small interfering RNA-mediated knockdown of PPAR-δ abrogated the antiapoptotic effect, suggesting that the antiapoptotic role of PPAR-δ appeared to be specific for and dependent on the endogenous PPAR-δ receptor. Interestingly, gene expression of 14-3-3epsilon was also induced by PPAR-δ through a PPRE-independent mechanism and an interaction between PPAR-δ and CCAAT/enhancer binding protein (C/EBP) [27].

2.5. Endothelial activation

When exposed to proinflammatory stimuli such as tumor necrosis factor (TNF) or lipopolysaccharide (LPS), normally quiescent endothelium undergoes a phenotypic change, which is characterized by induction of proinflammatory and procoagulant factors such as adhesion molecules and tissue factor. Such a phenotypic conversion, referred to as EC activation, is implicated in a number of proinflammatory diseases including atherosclerosis and thrombosis [28]. PPAR-α and -γ have been previously shown to suppress EC expression of proinflammatory adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VCAM-1), E-selectin, monocyte chemoattactant protein-1 (MCP-1), and ensuing recruitment of leukocytes [22, 29–32]. However, there has also been evidence that suggests a proinflammatory role of PPAR-α or -γ [33, 34]. Recent studies suggested that PPAR-δ also plays a role in inflammatory processes and atherosclerosis. In macrophages, the PPAR-δ agonist GW0742 inhibited lipopolysaccharide (LPS)-induced expression of proinflammatory genes, such as cyclooxygenase (COX)-2 and inducible nitric oxide synthase (iNOS) [35]. GW0742 reduced atherosclerotic lesions and decreased the expression of MCP-1 and ICAM-1 in the aorta of LDLR$^{-/-}$ mice [36, 37]. Given the beneficial effects of PPAR-δ agonists on lipid profiles, it is likely that PPAR-δ agonists may inhibit endothelial activation by improving dyslipidemia. A direct anti-inflammatory effect was also demonstrated. In EAhy926 cells, Rival et al. found that L-165041, at high a concentration up to 100 μM, inhibited TNF-α-induced VCAM-1 and MCP-1 expressions [38]. In primary culture of human umbilical vein ECs (HUVECs), specific agonists GW0742 and GW501516 inhibited the TNF-α- or interleukin-1β-induced expression of adhesion

Table 1: Ligands for PPAR-δ.

Ligands	Nature	Affinity	Clinical status
Natural agonists			
Linoleic acid	Dietary fatty acid	μM	
Oleic acid	Dietary fatty acid	μM	
Arachidonic acid	Dietary fatty acid	μM	
Eicosapentaenoic acid	Dietary fatty acid	μM	
Docosahexaenoic acid	Dietary fatty acid	μM	
Prostaglandin A1	Endogenous prostaglandin	μM	
Synthetic agonists			
Carbaprostacyclin	Synthetic stable PGI2 analogue	μM	
Iliprost	Prostacyclin analogue	μM	
Compound F	Phenoxyacetic acid derivative	nM	
L165,041	Phenoxyacetic acid derivative	nM	
GW501516	Phenoxyacetic acid derivative	nM	
GW0742	Phenoxyacetic acid derivative	nM	
KD2010	not disclosed	nM	Phase I
MBX-8025	not disclosed	nM	Phase II
Synthetic dual agonists			
Compound 23	Dual agonist for γ and δ	nM	
Synthetic antagonist			
GSK0660	Antagonist for δ	nM	

molecules and the monocyte adhesion to ECs. PPAR-δ agonist induced the gene expression of antioxidative enzymes, such as superoxide dismutase-1, catalase, and thioredoxin, and it decreased reactive oxygen species production in ECs. Unexpectedly, the anti-inflammatory effect not only persisted but it also was further enhanced after the decrease of PPAR-δ expression by siRNA knockdown [39]. Given the evidence that the ligand binding caused the dissociation of the transcription repressor BCL-6 from PPAR-δ and the subsequent association of BCL-6 with the VCAM-1 promoter region, this seemingly paradoxical result could be plausibly interpreted with the previously proposed PPAR-δ/Bcl-6 interaction action mode: the synthetic ligand binds to PPAR-δ and recruits the coactivators to replace the corepressors such as Bcl-6. The released corepressors relocate to repress the transcription of proinflammatory genes such as VCAM-1 and E-selectin and thus contribute to the vascular protection.

Ghosh et al. recently showed that the metabolism of endocannabinoids by the endothelial COX-2 coupled to the prostacyclin synthase activates PPAR-δ, which negatively regulates the expression of tissue factor (TF), the primary initiator of blood coagulation [40]. As COX-2 inhibitors suppressed PPAR-δ activity and induced TF expression, these results may help explaining the prothrombotic adverse effects of the cox-2 inhibitors rofecoxib and valdecoxib [41].

2.6. Angiogenesis

Angiogenesis is referred to the formation of new capillaries from the existing blood vessels. Physiological angiogenesis is involved in wound healing and aerobic exercise, whereas pathological or therapeutic angiogenesis is implicated in cardiovascular diseases, diabetic complications, inflammatory diseases, and cancers [42]. Recent studies have also linked metabolic homeostasis to angiogenesis and further interrogate the potential effects of PPARs on the angiogenic process [43, 44]. An earlier study demonstrated that the PPAR-δ agonist GW501516 dose-dependently stimulates HUVEC proliferation with increased mRNA expression of vascular endothelial growth factor α and its receptor flt-1 [45]. Later, GW501516 was shown to promote endothelial tube formation on an extracellular matrigel, EC outgrowth in a murine aortic ring model, and increased angiogenesis in the implanted matrigel plug assay in vivo through a PPAR-δ- and VEGF-dependent manner [22]. Most recently, Gaudel et al. found that treatment with GW0742 or muscle-specific overexpression of PPAR-δ promoted angiogenesis in mouse skeletal muscle [46]. Besides, arising from sprouts on existing vessels, vessels also arise from endothelial progenitorcells (EPCs), a process referred to as vasculogenesis [47]. Culminating evidence further suggests that circulating EPCs is capable of stimulating angiogenesis [48]. A recent study showed that the proangiogenic effects of human EPCs are in part dependent on the biosynthesis and release of PGI_2, and subsequent activation of PPAR-δ [48, 49]. Furthermore, functional genomic approach provided evidence that silencing of PPAR-δ in the tumor microenvironment impairs angiogenesis and tumor growth, identifying PPAR-δ as one of a few hub nodes in the angiogenic network [49, 50]. Up-to-date results have been largely consistent and pointed toward a proangiogenic activity of PPAR-δ. In corroboration with this, Müller-Brüsselbach et al. found that the growth of syngeneic PPAR-δ wild-type tumors was

impaired in PPAR-$\delta^{-/-}$ mice, concomitant with a reduced blood flow and hyperplastic vascular structures, suggesting that PPAR-δ maybe required in tumor ECs for the formation of functionally mature vessels [51]. Nevertheless, a full understanding of the specific roles of PPAR-δ in specific scenarios of angiogenesis will be imperative for a safe and rational therapeutic strategy.

2.7. Smooth muscle cells

PPAR-δ is expressed in SMCs and is induced in response to platelet-derived growth factor (PDGF) in SMCs, which involved the phosphatidylinositol 3-kinase/Akt signaling pathway. Initial study using overexpression showed that PPAR-δ increased SMC proliferation, indicating a proliferation-promoting effect in SMCs [52]. However, L-165041, a selective PPAR-δ agonist, inhibited SMC proliferation and migration via inhibition of the PDGF-induced expression of cyclin D1 and cyclin-dependent kinase (CDK) 4 and cell cycle progression [53]. In SMCs, GW501516 increased the expression of transforming growth factor-β1 (TGF-β1) and the effect seemed to depend on endogenous PPAR-δ. Subsequently, TGF-β1 was likely responsible for suppression of the IL1β-induced expression of MCP-1 and proliferation of SMCs [54]. In rats, administration of L-165041 decreased neointima formation in balloon-injured carotid arteries [53]. Thus, synthetic PPAR-δ agonists appear to have antiproliferative and anti-inflammatory properties in SMCs. This is consistent with previous reports that adenovirus-mediated gene transfer of prostacyclin synthase, which produces the endogenous PPAR-δ ligand PGI2, inhibited SMCs proliferation and intimal hyperplasia [55–57].

2.8. Macrophages

Macrophage infiltration in vessel wall is known to play important role in atherogenesis. PPAR-δ is expressed in macrophages. During past years, the role of PPAR-δ in macrophage biology has been extensively studied. However, existing results still remain controversial. Oliver Jr. et al. showed that GW501516 in a human monocytic cell line increased the expression of ATP-binding cassette A1 (ABCA1) and Apo AI-mediated cholesterol efflux [58]. However, Vosper et al. found that a different PPAR-δ agonist, compound F, increased lipid accumulation in both human primary macrophages and THP-1 cells. Compound F induced the expression of genes involved in lipid uptake and storage such as class A and B scavenger receptors (SRA and CD36) but repressed key genes involved in lipid metabolism and efflux such as Apo E and cholesterol 27-hydroxylase [59]. In mouse macrophages, neither genetic loss of PPAR-δ nor treatment with the PPAR-δ agonists GW501516 or GW0742 significantly influenced cholesterol efflux or accumulation [36, 60]. Beside the effects on lipid trafficking, PPARδ agonists have a potent anti-inflammatory effect in macrophages. Welch et al. first demonstrated that, in mouse peritoneal macrophages, PPAR-δ agonist GW0742 inhibited LPS-induced expression of COX-2 and iNOS [32, 35]. Recently, Barish et al. found that GW501516 in mouse macrophages suppressed the gene induction of MCP-1, -3, -5 by IL-1, interferon-γ (IFN-γ), and phorbol ester. The agonist treatment also inhibited transendothelial migration of THP-1 cells [61]. The anti-inflammatory effects of the agonist was lost in the receptor-deficient macrophages [60]. However, in other cell types such as epithelial cells, eosinophils, neutrophils, and lymphocytes, the PPAR-δ agonist was ineffective in inhibiting inflammatory processes, indicating that the effect is cell-type-specific [62].

2.9. Atherosclerosis

To date, several studies have been reported regarding the roles of PPAR-δ in atherosclerosis in different mouse models with different approaches. Lee et al. transplanted PPAR-δ-null bone marrow progenitor cells into LDL receptor-null (LDLR$^{-/-}$) mice. Unexpectedly, the adoptive transfer of PPAR-δ-null macrophages led to a less severe atherosclerosis, suggesting that endogenous PPAR-δ maybe proatherogenic. Although overexpression or deletion of PPAR-δ in macrophages suggested that PPAR-δ is proinflammatory, the agonist GW501516 decreased MCP-1, seemingly having an opposite effect [60]. To reconcile this contradiction, they postulated an unconventional ligand-dependent transcriptional mechanism, which switches PPAR-δ between a "proinflammatory" and "anti-inflammatory": in the absence of ligand, PPAR-δ sequesters a transcriptional repressor of inflammatory responses such as Bcl-6, permitting induction of proinflammatory genes; in the presence of ligand, PPAR-δ releases the repressor, which is then free to exert its anti-inflammatory effects. Following this loss-of-function approach, two independent studies examined the effect of the PPAR-δ agonist GW0742 on atherogenesis in high fat- and cholesterol-fed LDLR$^{-/-}$ mice and yielded divergent results. In the first study, Li et al. found that GW7842 decreased gene expression of proinflammatory cytokines and adhesion molecules within atherosclerotic lesions but failed to alter the progression of atherosclerosis after 14 weeks of treatment ($5\,mg^{-1}\,kg^{-1}\,day^{-1}$). In another one, Graham et al. used female LDLR$^{-/-}$ mice fed with a diet that induced moderate levels of hypercholesterolemia and observed that GW0742 reduced the lesion size at a higher dose ($60\,mg^{-1}\,kg^{-1}\,day^{-1}$) after 10 weeks of treatment [37]. Discrepancy between these two studies may be caused by differences in the levels of hypercholesterolemia and different drug doses used. However, the anti-inflammatory effect was generally consistent in both studies, regardless the different effects on the lesion sizes. It is likely that the anti-inflammatory properties of the PPAR-δ agonists on the vessel wall per se are not sufficient to attenuate the progression of atherosclerotic lesions if it is not corroborated by an efficient improvement of metabolic abnormalities. This notion is supported by the data from recently published results. Most recently, GW501516, which has a potent lipid-modifying capacity, has also been demonstrated to have a clear antiatherosclerotic property in apoE$^{-/-}$ mice. Barish et al. showed that GW501516 significantly reduced atherosclerotic lesions with an increase in HDL level and

a reduced expression of chemokines in the aorta and in macrophages [61]. Furthermore, in a model of angiotensin II-accelerated atherosclerosis ($LDLR^{-/-}$ mice), Takata et al. confirmed the atheroprotective effect of GW0742 [63]. After 4 weeks of treatment, GW0742 at both doses (1 and 10 $mg^{-1}\,kg^{-1}\,day^{-1}$) significantly inhibited the Ang II induction of atherosclerosis without altering blood pressure. This beneficial effect was likely mediated via the potent anti-inflammatory property since GW0742 increased vascular expression of Bcl-6, the regulators of G protein-coupled signaling (RGS4 and 5) in the artery and suppressed Ang II-induced activation of p38 and ERK in macrophages. However, the metabolic effect of GW0742 may also have contributed to the atheroprotective outcome because GW0742 significantly reduced plasma levels of insulin, glucose, leptin, and decreased triglycerides [63]. Overall, studies in mouse models suggest that PPAR-δ may have an attractive therapeutic target for the treatment of atherosclerosis.

2.10. Cardiovascular risk factors

In addition to the direct effects on the vessel wall, PPAR-δ also has profound effects on various metabolic parameters associated with cardiovascular diseases such as obesity, dyslipidemia, and insulin resistance.

2.11. Obesity

PPAR-δ deficiency causes embryonic lethality due to a placental defect. Some surviving PPAR-δ null mice had reduced fat mass [3], indicating a role of PPAR-δ in adipogenesis. Transgenic mice specifically expressing VP16-PPAR-δ, a constitutively active receptor, in adipose tissue had a reduced body weight, fat mass, and lower levels of circulating free fatty acids and triglycerides [64]. These animals were less susceptible to high-fat diet-induced obesity. In contrast, PPAR-δ null mice were more prone to weight gain on a high-fat diet. Similarly, GW501516 ameliorated diet-induced obesity [65]. It has been known that PPAR-δ activates genes involved in fatty acid oxidation and energy dissipation, such as carnitine palmitoyltransferase 1 (CPT1), acyl-CoA oxidase (AOX), and long chain acyl-CoA dehydrogenase (LCAD), uncoupling proteins. In addition to the direct effects on obesity, PPAR-δ agonists may also have regulatory effects on adipokine profile. For example, administration of L-165041 in rats increased the expression of visfatin and adiponectin, which are known to improve insulin sensitivity and are vasoprotective, but decreased the production of resistin in visceral adipose tissue [66].

2.12. Dyslipidemia

Increased levels of LDL and triglycerides and decreased HDL in plasma are independent risk factors for atherosclerosis and associated with metabolic syndrome as well. Recent studies have demonstrated that activation of PPAR-δ may modify lipid profile in animal models as well as in human. Oliver Jr. et al. first reported that GW501516 significantly improved dyslipidemia in obese primates with an increase in HDL and a decrease in LDL cholesterol and triglycerides [58]. The beneficial effect of GW0742 and L-165041 on HDL level was also observed in obese and nonobese mice [67, 68]. In addition to enhancing fatty acid oxidation in muscles, PPAR-δ agonists upregulated expression of ABCA1 in several types of cells, which may lead to an increase in HDL and cholesterol reverse transport. In intestinal cells, it also inhibited gene expression of Niemann-Pick C1-like 1, the key molecule for cholesterol absorption [67]. However, the precise mechanisms underlying these lipid-modifying effects still remain to be elucidated. In a small number of healthy human volunteers, GW501516 has been reported to increase HDL cholesterol level and improved the triglycerides clearance [69].

2.13. Insulin resistance and glucose homeostasis

It has been previously known that, in obese primates, GW501516 declined fasting insulin level [58]. GW501516 treated *ob/ob* mice also showed a significantly improved glucose tolerance and with a lower postprandial levels of plasma glucose and insulin [65]. In cultured myotubes, PPAR-δ agonists were found to directly stimulate glucose uptake independent of insulin action. The agonist-stimulated glucose uptake in myotubes appeared to require AMP-activated protein kinase (AMPK) but not PPAR-δ [70, 71]. However, GW501516 had no acute effect on glucose transport in rat skeletal muscles [72]. Recently, Lee et al. showed that PPAR-$\delta^{-/-}$ mice were glucose intolerant. Euglycemic hyperinsulinemic clamp experiments showed that GW501516 improved insulin sensitivity in multiple tissues including hepatic and peripheral tissues. The agonist suppresses hepatic glucose output and increases glucose disposal [73]. Gene array analysis suggested that PPAR-δ might ameliorate hyperglycemia by increasing glucose flux through the pentose phosphate pathway, which is known to enhance de novo fatty acid synthesis. Thus, it could be a concern whether PPAR-δ improves hyperglycemia at the cost of exacerbation of hepatosteatosis, a problem commonly associated with metabolic syndrome and diabetes. However, recent studies demonstrated that PPAR-δ also had a beneficial effect on hepatosteatosis. In a diet-induced mouse model of nonalcoholic steaohepatitis, GW501516 reduced triglycerides accumulation in the livers. In a most recent study, it was demonstrated that PPAR-δ suppressed hepatic lipogenesis via the induction of insulin-induced gene-1 (insig-1) and the inhibition of lipogenic sterol-regulatory element binding prortein-1 (SREBP-1) activation. In obese diabetic mice, hepatic overexpression of PPAR-δ ameliorated hepatosteatosis [74]. Since Insig-1 is a critical regulator of lipid homeostasis, identification of the insig-1 as a target gene of PPAR-δ may also facilitate our understanding of the profound effects of PPAR-δ activation on adipogenesis and lipid metabolism [75, 76]. Recently, in a double-blind and randomized study, PPAR-δ agonist (10 mg o.d. GW501516) was given to a small number of healthy overweight subjects. The results showed that treatment with GW501516 for 2 weeks significantly reduced liver fat content by 20% without increasing oxidative stress [77].

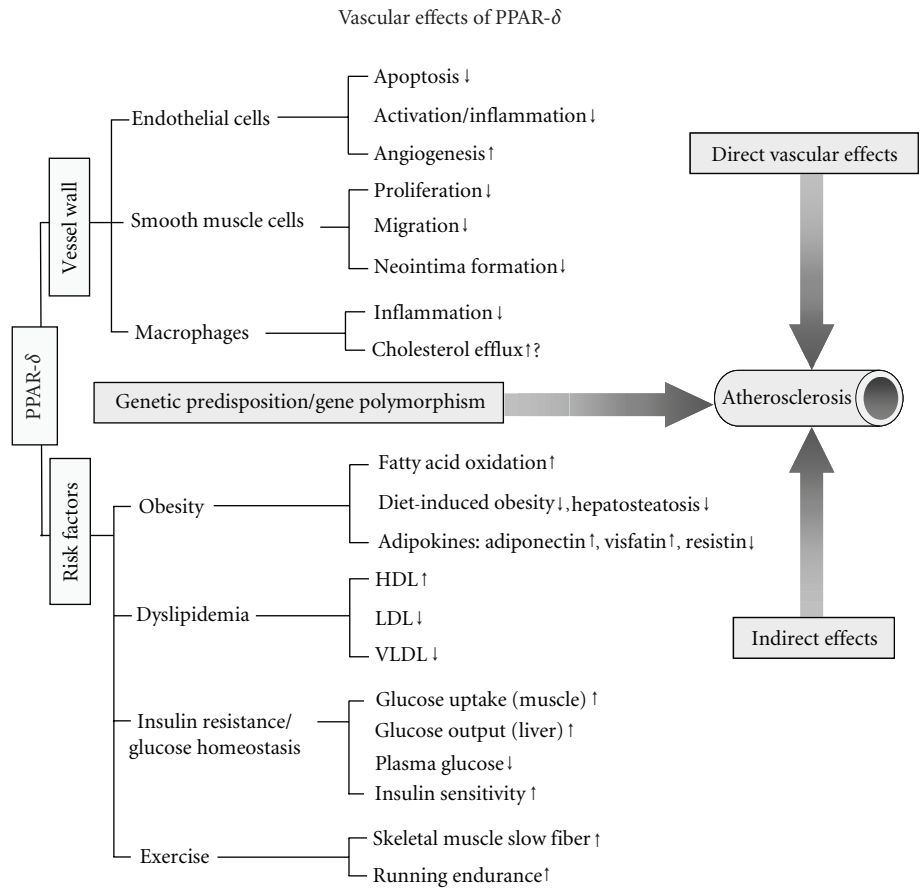

Figure 1: Activation of PPAR-δ may have profound effects on vascular homeostasis and coronary artery diseases. These include both the direct actions in the vessel wall and the indirect effects on multiple cardiovascular risk factors. PPAR-δ gene polymorphisms are also linked to cardiovascular diseases.

2.14. Gene polymorphisms

Skogsberg et al. initially described 4 polymorphisms: −409C/T (in the promoter region), +73C/T (exon 1), +255A/G (exon 3), and +294T/C (exon 4). The +294T/C polymorphism showed a significant association with a metabolic trait. The homozygotes for the C allele had a higher plasma LDL and a tendency toward higher risk of CHD compared with homozygous carriers of the T-allele [78, 79]. In addition, there is a highly significant association between the rare C allele and lower plasma HDL concentrations in the female patients with mixed hyperlipidemia. Associations were also found for the C-allele with coronary heart disease and body mass index (BMI) [80, 81]. Chen et al. also demonstrated that, in "lipoprotein and coronary atherosclerosis study" (LCAS) subjects, the PPAR-δ SNPs were strongly associated with the dyslipidemia, the responses to statin, and the atherosclerotic lesions [82]. Vänttinen et al. investigated the effects of the PPAR-δ gene SNPs on tissue glucose uptake and suggested that the SNPs regulate insulin sensitivity primarily in skeletal muscles [83]. The PPAR-δ SNPs were genotyped in type II diabetes subjects and normal control. Although no significant association was detected with the risk of type II diabetes, several SNPs were associated with fasting plasma glucose and BMI [84]. In addition, the association has been found between the PPAR-δ SNPs and metabolic syndrome, and the association was influenced by dietary fat intake [85].

3. CONCLUSIONS

During the last few years, rapid progress has been made with regards to the roles of PPAR-δ in vascular biology. Emerging evidence supports the notion that activation of PPAR-δ may have profound effects on vascular homeostasis and coronary artery diseases. These include both the direct actions in the vessel wall and the beneficial effects on central metabolic pathways (Figure 1). Importantly, PPAR-δ agonists have an unprecedented role in raising HDL level in animals. In addition to a previously reported function of PPAR-δ in increasing oxidative muscle fibers and running endurance, a most recent study revealed that AMPK and PPAR-δ pathways have synergistic effects in terms of exercise-enhancing capacity [86]. The outcome of currently ongoing clinical trial is awaited to prove its clinical efficacy in the treatment of dyslipidemia. Despite the studies in rodent models point to a vascular-protective effect for PPAR-δ agonists, their efficacies in human coronary

artery diseases remain to be clarified. With regards to the effects of PPAR-δ on tumor angiogenesis and the unsettled role in carcinogenesis, safety issues also call for attention [87, 88]. Clearly, further studies are warranted to explore the roles of PPAR-δ in cardiovascular pathophysiology and to exploit this lipid-sensing receptor as a therapeutic target for metabolic syndrome and its cardiovascular complications.

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Review Article

Regulation of Cell Proliferation and Differentiation by PPARβ/δ

Rolf Müller, Markus Rieck, and Sabine Müller-Brüsselbach

Institute of Molecular Biology and Tumor Research (IMT), Philipps-University, Emil-Mannkopff-Strasse 2, 35032 Marburg, Germany

Correspondence should be addressed to Rolf Müller, rmueller@imt.uni-marburg.de

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Peroxisome proliferator-activated receptor-β/δ (PPARβ/δ) is a ligand-activated transcription factor with essential functions in the regulation of lipid catabolism, glucose homeostasis, and inflammation, which makes it a potentially relevant drug target for the treatment of major human diseases. In addition, there is strong evidence that PPARβ/δ modulates oncogenic signaling pathways and tumor growth. Consistent with these observations, numerous reports have clearly documented a role for PPARβ/δ in cell cycle control, differentiation, and apoptosis. However, the precise role of PPARβ/δ in tumorigenesis and cell proliferation remains controversial. This review summarizes our current knowledge and proposes a model corroborating the discrepant data in this area of research.

1. INTRODUCTION

Peroxisome proliferator-activated receptor-β/δ (PPARβ/δ) is a transcription factor that is activated by endogenous fatty acid ligands and by synthetic agonists [1, 2]. Major functions of PPARβ/δ are associated with the regulation of glucose, energy, and lipid metabolism [3], and the control of inflammatory responses [4, 5]. PPARβ/δ, therefore, represents a promising drug target for the treatment of common diseases such as obesity, metabolic syndrome, chronic inflammation, and arteriosclerosis, which has led to the development of synthetic drug agonists with subtype selectivity and high-affinity binding [6]. Mice lacking PPARβ/δ show an aberrant development of the placenta and exhibit a defect in wound healing associated with alterations in cell proliferation, differentiation, and cellular survival [7–10]. Experimental evidence obtained with cultured cells has provided additional strong evidence for a role of PPARβ/δ in cell cycle regulation and differentiation in different cell types (see Table 1). Consistent with these physiological functions, there is also clear evidence for a role of PPARβ/δ in oncogenesis and tumor growth. These findings might provide a basis for the development of novel strategies for the treatment of proliferative diseases, but also demand some caution with respect to the clinical use of PPARβ/δ-directed dugs. A detailed knowledge of the role of PPARβ/δ in cell proliferation and its effects on tumor growth are therefore of paramount importance.

2. PPARβ/δ AFFECTS TUMORIGENESIS

The role of PPARβ/δ in tumorigenesis has been explored predominantly in epithelial tumors of the skin, lung, and intestine and in the tumor stroma. PPARβ/δ inhibits chemically induced skin carcinogenesis, since an enhancement of chemically induced skin tumor growth is seen in mice with a global disruption of *Pparb* [38]. However, no effect on skin carcinogenesis is observed in mice lacking PPARβ/δ specifically in basal keratinocytes [39], suggesting that the tumor suppressive effect of PPARβ/δ is due to a function in other cell types. A tumor suppressive role for PPARβ/δ has also been described for a transgenic mouse model of Raf oncogene-induced lung adenoma formation, but similar to skin carcinogenesis the precise mechanisms and cell types involved are not known [40]. Effects of PPARβ/δ have also been reported in different mouse models of intestinal carcinogenesis, that is, the Apc/Min mouse lacking functional APC protein and chemically induced intestinal carcinogenesis, but these studies differ in their conclusions [41]. Thus, PPARβ/δ has been reported to have either no effect on intestinal tumorigenesis [9] to attenuate tumor growth by promoting terminal differentiation of colonocytes

Table 1: Effects of PPARβ/δ on cell proliferation and differentiation.

Cell type	Exp. approach	Role of PPAR β/δ in		Affected pathway	References
		Prolif.	Diff.		
Epithelial cells					
Keratinocyte	Agonist, wt versus null	↘	↗	AKT	[11]
Keratinocyte	Agonist, wt versus null	↘	↗	ERK	[12–17]
Keratinocyte	Agonist, RNAi, wt versus null	↗			[18, 19]
Adipocyte	Agonist, wt versus null		↗	PPARγ	[20–22]
Trophoblast	wt versus null		↗	AKT	[10, 23]
Paneth cells (in vivo)	wt versus null		↗	Hedgehog	[24]
Hepatic stellate cell	Agonist	↗			[25]
Oligodendrocyte	Agonist		↗		[26]
Mesenchymal cells					
Fibroblast	Agonist	↘	(↘*)	*G0S2***, *PTEN*	[27]
Fibroblast	wt versus null, re-expression in null	↘		$p57^{KIP2}$	[28, 29]
Vascular smooth muscle cells	Agonist	↘		PDGF	[30]
Tumor endothelium	wt versus null	↘	↗		[29]
Endothelial cells	Agonist	↗	↗		[31]
Human tumor cell lines					
MCF-7 breast carcinoma; UACC903 melanoma	Agonist	↘			[32]
HT29, HCT116, LS-174T colon carcinoma; HepG2, HuH7 hepatoma	Agonist	↘			[33]
HCT116 colon carcinoma	RNAi	↘			[34]
SH-SY5Y neuroblastoma	Agonist		↗		[35]
NSC lung carcinoma	Agonist	↗		AKT, NFκB	[36]
A549 NSC lung ca.	Agonist	↘			[37]

* transdifferentiation into myofibroblasts.
**G0S2: *G0/G1 switch gene 2* (cell cycle inhibitor).

[33, 42–45] or to potentiate tumorigenesis [46–48]. The reason for these discrepancies remains unclear at present [49], but may be in part related to a function of PPARβ/δ in host cells recruited by the tumor, such as endothelial cells, fibroblasts, and macrophages [50]. Indeed, recent work showed that PPARβ/δ is indispensable for the formation of functional tumor microvessels [29, 51], suggesting that PPARβ/δ may have different functions in the tumor stroma and in tumor cells with opposing effects on tumor growth. The role of PPARβ/δ in tumor stroma cells is further discussed below.

3. ATTENUATION OF TUMOR STROMA CELL PROLIFERATION BY PPARβ/δ

The inhibition of syngeneic tumor growth in mice lacking PPARβ/δ strongly correlates with a lower density of functional tumor microvessels [29, 51], which is associated with a striking increase in the proliferation of tumor endothelial cells and an inhibition of their maturation [29]. The immature microvascular structures are also frequently surrounded by perivascular cells expressing vast amounts of α-smooth muscle actin, giving rise to an overall picture characteristic of tumor endothelial hyperplasia. In vivo microarray analysis led to the identification of PPARβ/δ target genes with known inhibitory functions in angiogenesis, including *Cd36* and *Cdkn1c* [29]. A crucial function of CD36 is to serve as a receptor for thrombospondins which are known to attenuate the proliferation of endothelial cells [52], and *Cdkn1c* codes for the cyclin-dependent kinase inhibitor $p57^{KIP2}$ [53]. Consistent with the existence of a PPARβ/δ – $p57^{KIP2}$ pathway in stroma cell types, it was shown that the forced expression of PPARβ/δ in *Pparb* null fibroblasts results in a *Cdkn1c*-dependend inhibition of cell proliferation [29]. Other PPARβ/δ target genes with potential functions in cell proliferation and differentiation were identified in the same study, suggesting that PPARβ/δ regulates multiple genes with functions in cell proliferation in the context of tumor stroma development and tumor angiogenesis.

An antiproliferative effect of PPARβ/δ agonists in fibroblasts and vascular smooth muscle cells has also been observed in two other studies [27, 30], while opposite effects have been described for endothelial cells [31]. At present, it is difficult to explain these apparent discrepancies, since they cannot be narrowed down to a single parameter, such as experimental strategy, cell type, expression level of PPARβ/δ, or state of the cell (e.g., metabolic activity, proliferative status, stage of differentiation, exogenous factors). This issue is discussed further in the Conclusions section below.

4. ROLE OF PPARβ/δ IN WOUND HEALING AND KERATINOCYTE PROLIFERATION

Pparb null mice exhibit a defect in wound healing by inhibiting apoptosis in keratinocytes [8]. This survival function of PPARβ/δ has been explained by an induction of AKT/protein kinase B (PKB) activity by PPARβ/δ resulting from an upregulation of the *Pdk1* and *Ilk* genes and a downregulation of *Pten* [11]. Increased AKT signaling is generally associated with enhanced proliferation, yet others have reported that PPARβ/δ inhibits cell proliferation [7, 15]. In this case, however, AKT activity was not affected by PPARβ/δ activation. Instead, a downregulation of protein kinase C and MAP kinase signaling was observed [14]. The reason for these discrepancies is not clear at present, however, in light of the relatively small effects of PPARβ/δ on the signaling pathways discussed above it is possible that subtle differences in the experimental settings account for the apparent lack of consistency.

5. ROLE OF PPARβ/δ IN DIFFERENTIATION

Mice lacking PPARβ/δ show a very high degree of embryonic lethality due to an aberrant development and malfunction of the placenta [7, 9, 10]. Consistent with this finding, the differentiation and metabolic functions of trophoblast giant cells in vitro are dependent on PPARβ/δ [10]. In the same model, stimulatory effect of PPARβ/δ on AKT signaling was observed. Another tissue where PPARβ/δ plays a role in differentiation is the digestive tract, where PPARβ/δ promotes the differentiation of Paneth cells in the intestinal crypts by down-regulating the hedgehog signaling pathway [24]. A differentiation promoting effect of PPARβ/δ has also been described for keratinocytes, adipocytes, endothelial cells, and oligodendrocytes (see Table 1 for details).

6. CONCLUSIONS

Studies addressing the role of PPARβ/δ in differentiation have yielded a consistent picture and point to a differentiation promoting in a wide spectrum of different cell types. Numerous reports have also clearly documented a role for PPARβ/δ in cell proliferation and tumorigenesis, yet different studies have produced controversial results, even though the majority of studies describe antiproliferative effects by PPARβ/δ (see Table 1).

One reason for the apparently discrepant data may be associated with the use of different experimental strategies. Since the precise mechanisms of PPARβ/δ-mediated gene regulation are often not known, the results from gain-of-function and loss-of-function are not always easy to interpret. Thus, ligand activation and genetic inactivation of PPARβ/δ may have opposite effects, as in the case of classical PPRE-driven genes, but may also give similar results in other regulatory settings. The latter has been described, for instance, for PPARβ/δ-mediated gene repression through direct interaction with the transcriptional repressor BCL-6 in macrophages [54]. This aspect has not been thoroughly analyzed to date so that it is difficult to judge its contribution to the deviant results published in different studies.

To help explain the discrepant published data, we would therefore like to put forward another hypothesis. This model postulates that PPARβ/δ is not a *bona fide* cell cycle regulator with a defined function but rather affects the expression of both inducers and inhibitors of cell proliferation (e.g., regulators of the AKT pathway and PDGF versus the cell cycle inhibitors $p57^{KIP2}$ and *G0S2*; see Table 1). This is conceivable both in view of the large number of potential PPAR target genes, estimated at several thousand for the human genome [55]. Depending on the particular cell type, the metabolic or proliferative state of the cell or other experimental conditions, positive or negative regulators of the cell cycle may prevail resulting in opposite effects. This suggests that the precise effects of PPARβ/δ on cell proliferation are highly context-dependent and not predictable on the basis of our current knowledge. Clearly, a better and detailed understanding of the effects of PPARβ/δ on cell cycle regulation and differentiation will be a prerequisite for the development of PPARβ/δ directed drugs and their clinical application.

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Review Article

Peroxisome Proliferator-Activated Receptor β/δ in the Brain: Facts and Hypothesis

M. G. Hall, Laure Quignodon, and Béatrice Desvergne

Center of Integrative Genomics, Faculty of Biology and Medicine, University of Lausanne, 1015 Lausanne, Switzerland

Correspondence should be addressed to Béatrice Desvergne, beatrice.desvergne@unil.ch

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peroxisome proliferator-activated receptors (PPARs) are nuclear receptors acting as lipid sensors. Besides its metabolic activity in peripheral organs, the PPAR beta/delta isotype is highly expressed in the brain and its deletion in mice induces a brain developmental defect. Nevertheless, exploration of PPARβ action in the central nervous system remains sketchy. The lipid content alteration observed in PPARβ null brains and the positive action of PPARβ agonists on oligodendrocyte differentiation, a process characterized by lipid accumulation, suggest that PPARβ acts on the fatty acids and/or cholesterol metabolisms in the brain. PPARβ could also regulate central inflammation and antioxidant mechanisms in the damaged brain. Even if not fully understood, the neuroprotective effect of PPARβ agonists highlights their potential benefit to treat various acute or chronic neurological disorders. In this perspective, we need to better understand the basic function of PPARβ in the brain. This review proposes different leads for future researches.

1. INTRODUCTION

Nuclear receptors (NRs) represent the largest family of transcription factors [1]. Up to today, 48 nuclear receptors have been described in humans and 49 in mice [2]. Most of them are ligand-dependent receptors; their specific ligands correspond to a diversity of hormones, steroids, fat-soluble vitamins, fatty acids, oxysterols, bile acids, and dietary lipids [3]. This broad range of ligand diversity and capacity to regulate gene expression makes the NRs key regulators of many pathways involved in reproduction, metabolism, and development. The central nervous system (CNS) expresses nearly all the NRs [2], but for most of them we are still missing in-depth knowledge of their role in brain development, cognition, behavior, and neurological or psychiatric disorders [4]. Among the NR superfamily, the peroxisome proliferator-activated receptors (PPARs), which are described as lipid sensors, are the focus of intense interest, particularly in the context of metabolic disorders and the associated search for new therapies. Amazingly, in addition to its metabolic activity in peripheral organs [5], the PPAR beta/delta isotype is highly expressed in the brain [6] and its deletion in mice is associated with a brain developmental defect [7].

In vertebrates, the PPAR family is composed of three different isotypes. They are known as PPARα (NR1C1), PPARβ (NR1C2) also named PPARδ , and PPARγ (NR1C3) [8, 9]. PPARs regulate whole body metabolism in response to dietary lipid intake, by directing their subsequent metabolism and storage. Among their endogenous ligands are poly-unsaturated fatty acids and lipid derivatives such as eicosanoids. However, the search for specific ligands interacting with the three individual receptors of the family has been difficult, owing to their relatively low affinity interactions and broad ligand specificity. PPAR: retinoid-X-receptor (RXR) heterodimers represent the functional entities and bind to conserved regulatory DNA elements and termed peroxisome proliferator response elements (PPREs). PPREs correspond to a repetition of two hexamers, derived from the AGGTCA consensus motif, separated by one nucleotide, and we still understand little on the binding selectivity of the three PPAR isotypes according to the nucleotide sequence of these response elements. PPAR-mediated transcriptional activity is a multistep process. In the absence of ligands, PPAR is

associated with corepressors. Upon ligand binding, they are replaced by coactivators, which recruit the basal transcriptional machinery. Thus, PPAR transcriptional activity is depending on a combination of ligand availability, RXR expression, and numerous cofactor interactions. This complexity together with a relatively specific tissue expression of PPARα and PPARγ contributes to the selective PPAR isotype activity.

PPARβ is an intriguing member of the PPAR family. It presents a fairly ubiquitous expression pattern from early embryonic up to adult stages. Its near-ubiquitous expression raised early speculation that it may have a "general housekeeping role" [10]. The phenotype of PPARβ-null mice highlights its role in development. PPARβ deletion induces a high rate of embryonic mortality around early embryonic day 10.5 (E10.5) due to a placental defect [7, 11, 12]. The phenotype of the surviving PPARβ-null mice is rather mild. They present a reduction of adipose tissue [7], an altered skin inflammatory response [7, 13], a decreased number of Paneth cells in the intestine [14], some discrete metabolic modification in muscle [7, 15], and impaired wound healing [16]. PPARβ-null mice also present a myelin alteration [7] but exploration of the PPARβ function in the brain remains sketchy. In this review, we highlight the few known facts and propose some hypotheses.

2. EVIDENCE FOR PPARβ ACTIVITY IN THE BRAIN

During development, PPARβ expression starts at midgestation, around E10.5 days in rats, and then reaches a peak in all neural tissue between E13.5 and E15.5 [17]. Even though it then fades slightly, it remains high all through development and adult life. In the adult brain, PPARβ is expressed ubiquitously, with high levels found in the cerebral cortex, thalamus, cerebellum, and brain stem [4, 18, 19] (Figure 1).

Most brain cell types appear to express PPARβ. Immunostaining, western blots, and RT-PCR confirmed their expression in primary cultures of embryonic cortical neurons [20]. Analyses of adult brain sections enabled more detailed observations [21] revealing that pyramidal cells of the cerebral cortex, neurons of the hypothalamus, and accumbens nuclei show high PPARβ expression. In situ hybridization coupled to immunolocalization revealed PPARβ mRNA and protein expression within the oligodendrocytes of the corpus callosum [19, 22]. PPARβ is also expressed in primary cultures of rat cortical and cerebral astrocytes, as well as in mouse cortical astrocytes [23, 24], even if, in vivo, astrocytes appear negative to PPARβ immunostaining, at least in the hippocampal commissure [19, 22].

Thus, the expression of PPARβ is documented in the three main neural cell types: neurons, astrocytes, and oligodendrocytes, whereas we still have no information for microglia cells. Interestingly, PPARβ mRNA is also expressed in the rat brain capillary endothelial cells [25], suggesting that it plays a role in the brain-blood barrier.

Concerning its cellular localization, PPARβ immunostaining is detected in the cytoplasm and neurites of some neurons [21], raising the question of PPARβ nongenomic effects in these specific cells. However, its main localization is nuclear, as revealed by its exclusive detection in the nuclear fraction of whole brain protein extracts [26].

PPARβ expression pattern suggests that it is involved in basic physiological functions in the brain. However, the brain phenotype of PPARβ-null mice is poorly documented. In one study, the authors noted that PPARβ-null mice brain diameters are significantly smaller than in wild-type mice, most likely due to their relatively smaller body size [7]. Histological examination revealed alterations in the extent of myelination in the corpus callosum, more often in female than in male mice (three of five females; two of seven males). This defect is absent in other parts of the brain, including the cerebellum and brain stem. The two main proteins playing a role in myelin organization, myelin basic protein (MBP) and proteolipid protein (PLP), are not differentially expressed in the corpus callosum of PPARβ-null mice, despite a putative PPRE in the PLP promoter [27].

Thus, the full functional exploration of PPARβ activity in the brain remains to be performed. In the following sections, we summarize and comment on studies that chart the first leads in this domain.

3. PPARβ AND LIPID METABOLISM IN THE BRAIN

The best-known role of PPARβ, with possible consequences on the whole organism, is to increase lipid oxidative metabolism in muscles, in particular fatty acid peroxisomal-β oxidation [28]. Along this line, long-term treatment of obese animals with the PPARβ agonist GW501516 causes significant weight loss accompanied by improvement of the lipoprotein profiles and metabolic parameters [29, 30]. Interestingly, the brain lipid content of PPARβ-null mice is altered in females: they present a 24% and 17% increase in plasmenylethanolamine and phosphatidylserine, respectively, and a 9% decrease in the level of phosphatidylinositol when compared to controls animals [31]. The altered phospholipid composition in female PPARβ-null brains could result from a defect in brain peroxisomal acyl-CoA utilization. If true, this could explain the altered myelination observed in PPARβ-null mice, as inactivation of peroxisomal β oxidation function induces demyelination in human and mouse brain [32]. The fact that a PPARβ selective agonist (L165041) increases the expression of AcylCoA synthtase 2 (ASC2) in rat brain cell cultures [33] supports a direct role of PPARβ on brain lipid metabolism. ASC2 turns fatty acids into fatty acyl-CoA, a modification required for their metabolism. However, in unchallenged conditions, ASC2 expression is not changed in the adult brains of PPARβ-null mice compared to wild-type mice [7]. If confirmed, the modification of brain lipid composition in PPARβ-null mice may have multiple impacts, including a modification of membrane plasticity or an alteration of pathways requiring lipid post-translational modifications. For example, acylation is a common post-translational modification of myelin proteins [34] such as PLP, which is crucial for the stabilization of myelin sheets. Another example is the processing of Shh, which undergoes cholesterol addition and palmitoylation to contribute to

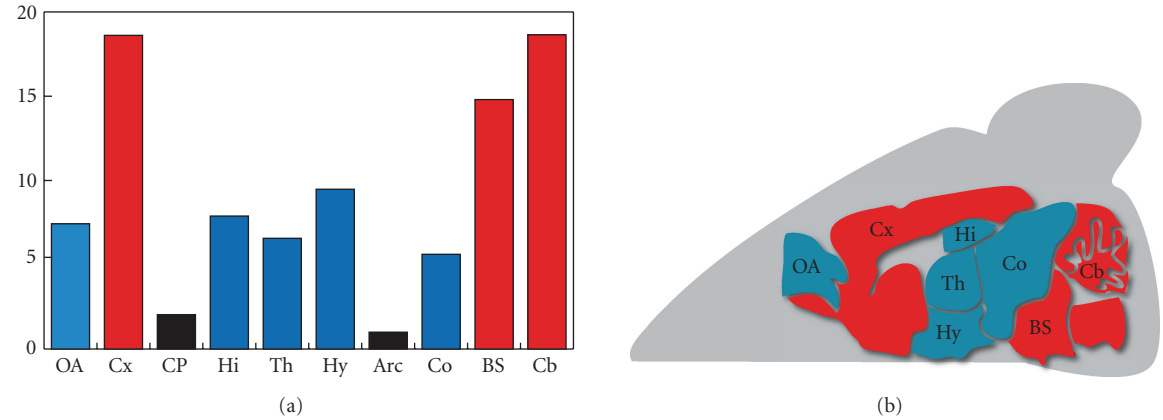

FIGURE 1: *Expression of PPARβ in the adult mouse brain.* Quantitative and spatial expression of PPARβ in the mouse brain: (a) Quantitative RT-PCR data, (b) Brain sagittal section. Moderated expression levels are in red and weak expression in blue. OA: olfactory areas, Cx: cerebral cortex, CP: caudate putamen, Hi: hippocampus, Th: thalamus, Hy: hypothalamus, Arc: arcuate nucleus, Co: colliculus, BS: brain stem, Cb: cerebellum. (MousePat: http://www-mci.u-strasbg.fr/mousepat/- consulted 8-08-2008).

forebrain patterning [35]. Finally, an alteration of the lipid metabolism can directly perturb neural differentiation, as discussed above.

Numerous recent papers have highlighted the role of PPARβ in cholesterol metabolism [36–40]. Even though the CNS accounts for only 2.1% of body weight, it contains 23% of the sterols present in the whole body pool. In PPARβ-null mice, the total cholesterol content of the brain is not changed compared to that of wild-type mice [31]. Nevertheless, it does not imply that brain cholesterol metabolism is not impaired. Cholesterol in the CNS comes almost entirely from in situ synthesis with little or no transfer from the blood into the brain, whereas cholesterol can leave the brain and pass into the general circulation in the form of 24-hydroxycholesterol. Inside the brain, a large amount of the cholesterol turnover is between glial cells and neurons during CNS development, but also occurs in the context of neuronal repair and remodeling. This internal recycling involves the cellular exchange of cholesterol through intermediate binding to apolipoproteins E and A1. Interestingly, alteration of the cholesterol balance across the whole body may alter sterol recycling and apolipoprotein E expression within CNS, thereby affecting neuron and myelin integrity [41]. Altogether, these observations are a strong incitement to exploring whether PPARβ acts on the fatty acids and cholesterol metabolisms in the brain.

4. PPARβ AND NEURAL CELL FATE

In different models, PPARβ has a prodifferentiation activity, observed for various cell types such astrophoblast giant cells [12], adipocytes [42, 43], sebocytes, Paneth cells in the intestine [14], and keratinocytes under normal and inflammatory conditions [13, 44]. There is now some evidence that PPARβ favors neural cell differentiation (Figure 2). However, observations vary according to the models investigated and many questions must be further addressed.

Oligodendrocytes are the myelin-producing cells in the CNS. The timing of oligodendrocyte differentiation depends on an intrinsic clock in oligodendrocyte precursor cells (OPC) that counts time or cell divisions and limits precursor cell proliferation. The timing of oligodendrocyte differentiation depends on hormonal signals such as thyroid hormones, glucocorticoids, and retinoic acid, which bind and activate their cognate nuclear receptor [48]. Two facts suggest a role of PPARβ in OPC differentiation: first, the strong expression of PPARβ in these cells [47], and second the partial alteration of the corpus callosum myelination in the brain of PPARβ-null mice [7]. Cell culture experiments support this hypothesis. In primary glial cell cultures and oligodendrocyte enriched cultures prepared from neonatal mouse brains, different PPARβ agonists accelerated OPC differentiation within 24 hours [46]. These treatments induced by two- to three-fold the number of oligodendrocytes with processes and huge membrane sheets. They also increased the expression of some differentiation markers, such as MBP and PLP, at the mRNA and protein levels [46]. While this prodifferentiation activity remains to be further documented in vivo, it suggests that PPARβ contributes to the dietary lipid activity in accelerating myelinogenesis [49, 50].

At the present time, we have few clues for how PPARβ acts on oligodendrocyte differentiation while not affecting oligodendrocyte precursor proliferation [46]. Oligodendrocytes synthesize myelin and thus are the major lipid producing cells in the CNS. Interestingly, a majority of the cells that are sensitive to PPARβ during their differentiation (adipocytes, trophoblast giant cells, sebocytes, and keratinocytes) are characterized by lipid accumulation during differentiation. Indeed, disruption of PPARβ resulted in an alteration of mouse adipose tissue development [7]. In 3T3-L1 and 3T3-F442A cell lines which replicate in vitro adipocyte differentiation, PPARβ is one of the early activated genes [51]. In contrast to this early implication in adipocyte differentiation, PPARβ regulates the late stages of sebaceous cell differentiation [52]. It is also the most

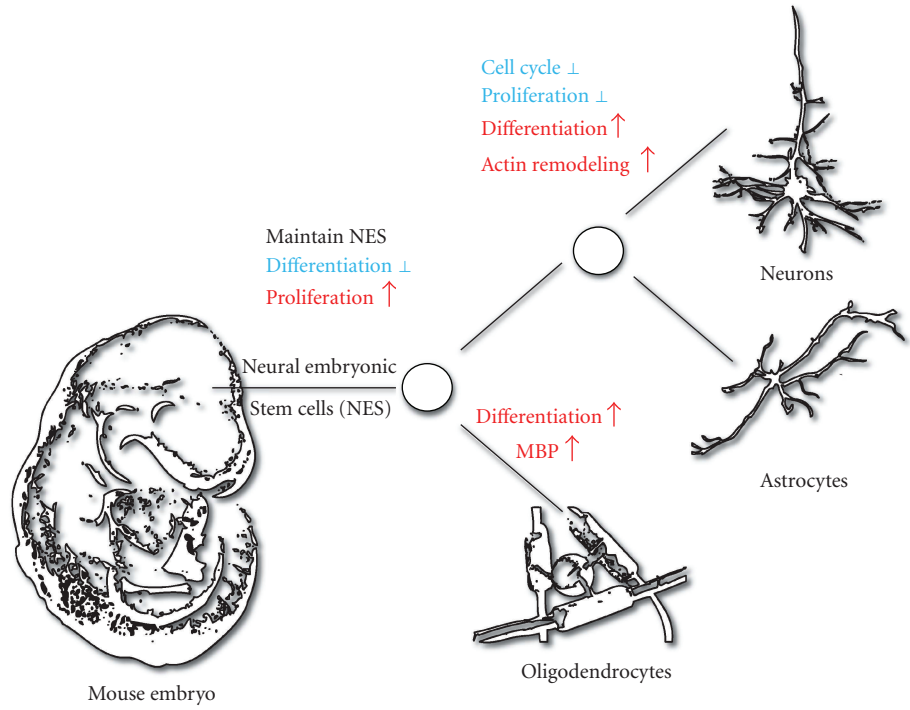

Figure 2: *PPARβ potential role in neural cell differentiation during development.* PPARβ is expressed in the embryo and in the differentiating cells of the CNS and plays important role for the maintenance and/or differentiation of neural stem cells (NSC). PPARβ maintains NSC in an undifferentiated proliferative status [45]. Once the NSC cells have started to differentiate, PPARβ could play a role in (1) neuronal differentiation, inducing the morphological characteristics and the gene expression patterns of neuronal cells, (2) promoting the oligodendrocyte precursor cells (OPC) differentiation [46], strongly expressing PPARβ [47], to mature oligodendrocytes [7]. In promoting differentiation, PPARβ also influences cell cycle and proliferation rate. Up to today no role has been clearly established for PPARβ in astrocyte differentiation. (↑ Activation in red, ⊥ Repression in blue).

effective PPAR isotype in stimulating lipid accumulation in keratinocytes [53]. Finally, the differentiation of giant cells in mouse placenta is accompanied by a PPARβ-dependent accumulation of lipid droplets and an increased expression of the adipose differentiation-related protein (ADRP, also called adipophilin), which may participate in lipid metabolism and/or steroidogenesis [12]. While the specificity of each of these contexts suggests that PPARβ acts on different programs of differentiation [54], this contribution of PPARβ to lipid synthesis may well also apply in oligodendrocytes, accelerating their differentiation from precursor to fully mature cells.

PPARβ action on neuronal differentiation is still under investigation. In primary cultures of embryonic cortical neurons, PPARβ is expressed in the neuron nuclei and increases in relation to the degree of maturation of these cells, in correlation with its heterodimer partners RXR β and γ. The concomitant induction of the PPARβ target gene ACS2 suggests that PPARβ is activated [20]. With respect to neuronal differentiation per se, the available data are limited to human neuroblastoma cell lines. Exposure of these PPARβ expressing cells to PPARβ agonists, either oleic acid or the GW610742X, triggers neuronal differentiation [20], characterized by neuritis outgrowth. Both compounds also promote morphological modifications of the actin filaments [55] and induce the expression of a series of neuronal differentiation markers such as growth associated protein 43 (GAP-43), neural cell adhesion molecule (N-CAM), and neurofilament-200 [55]. As the cells undergo differentiation, their proliferation rate, cellular migration, and invasiveness are slowed down by oleic acid or GW610742X treatments. In parallel, oleic acid or the GW610742X agonist increases the expression of the cyclin inhibitor p16, indicating that PPARβ activation may be able to promote cell cycle arrest [55]. All GW610742X effects are related to PPARβ, as demonstrated by the use of siRNA silencing. In contrast, the oleic acid effects were never fully reverted thereby indicating that they are only partially mediated by PPARβ.

These observations however need to be confirmed in more physiological models, such as primary cultures of neurons and mouse models. In fact, they contrast with other data suggesting that PPARβ rather maintains neural stem cells in an undifferentiated, proliferative status [56]. In the model of neurospheres cultures, prepared from the periventricular tissue of the adult mouse brain, western-blot and RT-PCR analysis demonstrated that PPARβ is expressed

in undifferentiated neurospheres (S0) and decreases in differentiated neurospheres (S10) [45]. In line with this, the expression of PPARβ in primary cultures of mouse cortical astrocytes also decreases between 14 and 21 divisions, possibly in relation to the decreased astrocyte proliferation at confluence [45]. In these studies, PPARβ activity correlates with the expression of genes involved in cell cycle [20, 45, 57]. Nevertheless, PPARβ action on cell proliferation is highly dependent on the cell type. For example, it exerts a proproliferative action on preadipocytes [58, 59] and an antiproliferative action on keratinocytes [60]. Moreover, PPARβ proproliferative activity on neural stem cells does not necessarily exclude a prodifferentiation activity.

After this tour of the possible functions of PPARβ in the brain, ranging from metabolism to neural cell fate, the next sections of this review highlight the consequent roles of PPARβ in brain alterations and repair.

5. PPARβ IN BRAIN ISCHEMIA

The role of PPARβ in brain repair was first addressed in a model of focal cerebral ischemia, with a middle cerebral artery occlusion. Compared with wild type, PPARβ-null mice exhibited a significant increase in the infarct size [61, 62], suggesting that PPARβ exerts a neuroprotective activity. Intriguingly, the difference in infarct size between wild-type and PPARβ-mutant mice was detected by RMN as early as 30 minutes after performing the ischemia [62], suggesting that PPARβ plays a role in the very early events. Reciprocally, in a transient middle cerebral artery occlusion, intracerebral infusion of L-165041 or GW501516 in rat ventricle significantly attenuated the ischemic brain infarct size 24 hours after reperfusion [63]. Several hypotheses concerning the molecular mechanism of this neuroprotective activity are discussed below.

An important activity of PPARβ is to promote cell survival under stress conditions, as demonstrated in keratinocytes during skin wound healing [50] and in primary keratinocyte exposed to inflammatory signals [64]. PPARβ activation also promotes renal cell survival following hypertonic stress [65] as well as oxidative stress [66]. Neural cells also seem to be sensitive to PPARβ activation under stress conditions. In primary cultures of rat cerebellar granule neurons, treatment with GW0742 significantly reduced cell death during a 12-hour exposure to low-KCl media. However, prolonged incubation (48 hours) with GW0742 produced significant inherent toxicity [67]. In a different context, human neuroblastoma SH-SY5Y cells were exposed to a variety of chemicals provoking cell death, such as thapsigarginand the endoplasmic reticulum calcium ATPase inhibitor, 1-methyl-4-phenylpyridinium. Treatment with two PPARβ agonists, L-165041 or GW501516, significantly attenuated cell death in a concentration-dependent manner [63]. In vivo, in a model of middle cerebral artery occlusion, an increase of malondialdehyde and a decrease of glutathione and manganese superoxide dismutase in PPARβ-null mice argue for increased brain oxidative stress. This phenotype was associated with a relative increase in interferon γ but a lack of TNFα production [61]. Thus, PPARβ could regulate central inflammation and antioxidant mechanisms in the damaged brain. Some known PPARβ-regulated genes could explain these observations, including COX2 [68], which promotes inflammatory reactions by prostaglandins synthesis [69]. Nevertheless, in vivo or in vitro treatment of T cells with GW0742, a PPARβ selective agonist, did not reduce IFNγ production [70]. Alternatively, PPARβ could also act via the regulation of IL-1β to reduce astroglial and microglial inflammatory activation, as suggested in experimental autoimmune encephalomyelitis (EAE) [70]. PPARβ inflammatory response could also involve a direct interaction between PPARβ and the inflammatory suppressor protein, BCL-6, as in macrophages [71].

While we can reasonably hypothesize that PPARβ may indeed play a role in modulating inflammation and controlling oxidative damages, thereby contributing to moderate ischemia lesion, other hypotheses may also contribute to understanding the increased ischemic lesion in PPARβ-null mice. An interesting one concerns the role of PPARβ in the vascular system. For example, a different patterning of vascular territories would result in a different infarct size occurring at the very first time point postischemia. An experiment designed to visualize the vascular tree would then provide an important control. Local conditions of blood flow might also affect the outcome of an ischemia experience. However, no data so far have been published concerning hemodynamic parameters in the brain of PPARβ-null mice. Finally, angiogenesis itself might be concerned. This is supported by investigations performed on an unrelated model, using subcutaneous inoculation of lung carcinoma cells carrying the two PPARβ wild-type alleles in a PPARβ-null mutant mouse. In this model, the tumor growth was impaired, due to the absence of PPARβ in the stroma cells surrounding the developing tumor. This led to a diminished blood flow and a reduced development of hyperplastic microvascular structures [72]. Thus, PPARβ deletion could also affect the delayed response to ischemia by impairing angiogenesis [73].

An interesting cellular property that may be overlooked in the search for functional disturbances linking PPARβ and cerebral ischemia is cell-cell adhesion and matrix-cell adhesion. As proposed by del Zoppo et al. [74], matrix cell adhesion receptors might be essential for the maintenance of the integrity of the blood-brain permeability barrier, challenged upon local injury. In particular, focal ischemia suddenly alters the matrix constituents and changes the expression of cell adhesion receptors, locally increasing vascular permeability [75]. We summarize below some indirect evidence for a role of PPARβ in cell adhesion, which may contribute to its neuroprotective activity.

In an in vitro study, modulation of PPARβ activity in F9 teratocarcinoma cells positively correlated with modulation of neural cell adhesion molecule (NCAM) expression. In fact, F9 cells treated with valproic acid increased the expression of PPARβ, but not that of PPARα or PPARγ, while also enhancing the expression of cell adhesion molecules such as NCAM and PST1. Reciprocally, overexpression of a dominant-negative PPARβ reduced the NCAM induction [76]. In endothelial cells of human umbilical, PPARβ

directly regulated a few key cell-adhesion genes. PPARβ agonists GW0742 and GW501516 significantly inhibited TNFα induced expression of vascular cell adhesion molecule-1 and E-selectin, and the ensuing endothelial-leukocyte adhesion [77]. Chromatin immunoprecipitation assays showed that GW0742 switched the interaction of BCL-6, a transcription repressor, from PPARβ to the vascular cell adhesion molecule-1 (VCAM-1) gene promoter. Evidence for a role of PPARβ in cell adhesion/migration also stems from PPARβ activity in wound healing, where it regulates the intracellular pathways activated during keratinocyte directional sensing, polarization, and migration [16]. In these events, redistribution of integrins requires Akt1 activity while NF-κB stimulates the production of the metalloproteinase MMP-9, which allows the digestion of extracellular matrix, a process required for cell migration. Since PPARβ concomitantly regulates both Akt1 and NF-κB [64], it would be of great interest to study these two pathways in the damaged brain.

In a more general way, cell adhesion is a key developmental process, as it determines the location of a cell by regulating its capacity to move in a tissue or to be restricted to a defined area. It is also crucial for neural cell-cell interactions, including axon guidance and synapse formation, processes tightly connected to the functioning of the brain itself, such as learning and memory. Taking these observations together points to the particular need for a better understanding of PPARβ action on neural cell adhesion.

6. USING PPARβ AGONIST/ANTAGONIST TO TREAT BRAIN DISEASES

Whereas many studies have explored the possible benefits of targeting the ever popular PPARγ isotype with regard to its neuroprotective effect [78], an interest in the more ubiquitous PPARβ isotype is recently emerging. In this last section, we will therefore review studies that have explored PPARβ targeted therapeutics for a variety of brain diseases.

Experimental autoimmune encephalomyelitis (EAE) [79, 80] is a T-cell mediated autoimmune disease that involves inflammatory activation of brain glial cells, and is used as a model for multiple sclerosis. Oral administration of PPARγ agonists reduces the incidence and severity of clinical, histological, and biochemical symptoms in EAE. The GW0742 PPARβ agonist has also beneficial effects, demonstrated in a mouse model of EAE, in which mice were immunized with an encephalitogenic myelin oligodendrocyte glycoprotein (MOG) peptide [70]. When given at the time of immunization, GW0742 had only a moderate effect on the appearance and severity of clinical symptoms. Nevertheless, prolonged treatment of mice already exhibiting signs of the disease improved their clinical status. Intriguingly, the clinical improvement of cortical lesions contrasts with no significant reduction of the cerebellar lesions. The mechanism does not involve T cell activation, oligodendrocyte maturation, or survival, but probably a reduction of astrocyte and microglial inflammatory responses [70]. Thus, PPARβ and PPARγ agonists differ both in the timing of treatment efficiency and the molecular mechanism involved [81].

The absence of PPARβ agonist action on oligodendrocyte maturation or survival in this model of EAE is surprising (see Section 4). It is thus tempting to explore how the PPARβ agonist could alter the course of a nonautoimmune demyelinating disease, such as a diabetes complication or leukodystrophy. In particular, adrenoleukodystrophy is a rare inherited disorder that leads to progressive failure of the adrenal gland, brain damage, and eventually death. In this disease, the mutation of the ATP-binding cassette subfamily D member 1 (ABCD1) gene leads to a reduction of beta oxidation in peroxysomes with the accumulation of very long chain fatty acids in the adrenal cortex and brain, causing a progressive inflammatory demyelination. Because of its crucial role in peroxisome proliferation and fatty oxidation, PPARα was the prime target tested in a therapeutic approach. However, no direct effect of PPARα agonists could be seen in modulating ABCD2, the closest relative of ABCD1, in the brain [82]. It would still be interesting to test a PPARβ agonist because of its combined role in fatty acid oxidation and in oligodendrocyte maturation.

Alzheimer disease is a neurodegenerative disorder characterized by cognitive and memory deterioration, progressive impairment of activities, and a multiplicity of behavioral and psychological disturbances. While not fully understood, the mechanism of the alteration includes an extracellular accumulation of amyloidal plaque formed by oligomerisation of the amyloidogenic peptide Aβ 1–42, and the accumulation of the Tau protein responsible for neurofibrillar degeneration. Interestingly, the noradrenalin (NA) neurotransmitter protects neurons from inflammation [83], via a mechanism that is partially PPARβ dependent. In fact, in the model of primary cultures of rat cortical neurons exposed to oligomeric amyloid beta, NA partially reduced neuronal damage and toxicity, as assessed by a reduction in the release of LDH. Interestingly, there was a concomitant two fold induction of PPARβ mRNA and protein levels. The NA neuroprotective effects were partially blocked by cotreatment with a PPARβ selective antagonist. Moreover, the selective PPARβ agonist GW742 reduced LDH release to the same extent as did the NA, suggesting that PPARβ is the main mediator of NA action [84]. Nevertheless, high concentrations of GW742 (50 μM) are required in order to observe this effect [85], and further in vivo studies are required to evaluate the true potential of PPARβ agonists as therapeutic tools for Alzheimer disease.

Finally, PPARβ agonist treatment could be beneficial in another notorious neurodegenerative disorder: Parkinson disease, characterized by the disappearance of the dopaminergique neurons with alteration of the nigrostriatal pathways. Beside the classic occurrence of Parkinson disease, whose etiology is mainly unknown, the synthetic opiate 1-methyl-4-phenyl-1,2,3,6-tetrahydrodropyridine (MPTP) causes Parkinsonism in young drug-addicted individuals. Iwashita et al. showed that an L-165041or GW501516 intracerebroventricular infusion 48 hours before the first injection of MPTP protects against depletion of striatal dopamine and its metabolites [63].

These different studies highlight the need to characterize and optimize some PPARβ agonists for their capacity to cross

the blood-brain barrier in order to treat various acute and chronic brain disorders.

7. CONCLUSION

This review highlights the few data available on PPARβ activity in the brain. While sometimes highly speculative, it underlines the good reasons to pursue dedicated research in this domain. The brain is the second most lipid-enriched tissue after adipose tissue. Products of fat metabolism, free-fatty acids, ketone bodies, and glycerol dominate metabolic pools in early development, as a consequence of the milk diet. The high expression of the PPARβ lipid sensor during brain maturation suggests that it is a key regulator of brain metabolism during neurodevelopment. Moreover, PPARβ anti-inflammatory and prosurvival activities may play a prominent role in the acute phase of brain injury. In addition, PPARβ prodifferentiation activity, in particular on oligodendrocyte lineage, is of potential benefit in the treatment of neurodegenerative diseases. Therefore, in view of its potentially wide therapeutical use, it appears crucial to carry out in depth studies of the basic mechanism of PPARβ activity, in order to understand the molecular network driven by this receptor in the brain.

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Review Article

The Potential Applications of Peroxisome Proliferator-Activated Receptor δ Ligands in Assisted Reproductive Technology

Jaou-Chen Huang

Division of Reproductive Endocrinology and Infertility, Department of Obstetrics, Gynecology & Reproductive Sciences, University of Texas Health Science Center at Houston, Houston, TX 77030, USA

Correspondence should be addressed to Jaou-Chen Huang, jaou-chen.huang@uth.tmc.edu

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Peroxisome proliferator-activated receptor δ (PPARδ, also known as PPARβ) has ubiquitous distribution and extensive biological functions. The reproductive function of PPARδ was first revealed in the uterus at the implantation site. Since then, PPARδ and its ligand have been discovered in all reproductive tissues, including the gametes and the preimplantation embryos. PPARδ in preimplantation embryos is normally activated by oviduct-derived PPARδ ligand. PPARδ activation is associated with an increase in embryonic cell proliferation and a decrease in programmed cell death (apoptosis). On the other hand, the role of PPARδ and its ligand in gamete formation and function is less well understood. This review will summarize the reproductive functions of PPARδ and project its potential applications in assisted reproductive technology.

1. INTRODUCTION

Assisted reproduction uses a spectrum of technologies to enhance fertility. In vitro fertilization (IVF) is the most advanced and most sophisticated assisted reproductive technology (ART). Since the first "IVF baby", Louise Brown, was born in 1978, IVF has gradually been accepted by the general public. Nowadays, IVF is a routine procedure to treat the infertile couples. Recent data shows more than 45 000 IVF babies were born in the US each year (http://www.cdc.gov/ART/ART2005/section1.htm) and there are more than three millions "IVF babies" in the world (http://news.bbc.co.uk/1/hi/health/5101684.stm).

Compared with embryos in natural pregnancies, IVF embryos have low implantation potential (about 15–20% per embryo, http://apps.nccd.cdc.gov/ART2005/nation05.asp). In order to increase IVF success, it is common to transfer two or more embryos to the uterus. This practice not only increases the odds of pregnancy but also increases the chance of multiple pregnancy. Compared with natural conception, IVF pregnancies are thirty times more likely to be multiple (32% versus 1%, http://www.cdc.gov/ART/ART2005/sect2_fig5-15.htm#Figure 8). Furthermore, 13% of the IVF multiple pregnancies are "high-order" multiple pregnancies, that is, triplets or more. These pregnancies are prone to develop obstetrical complications and pose great risks to mothers and infants. As a result, some infants suffer short-term complications and life-long sequels. Therefore, one of the most urgent tasks in ART is to enhance the implantation potential of IVF embryos so that transferring single embryo yields an acceptable chance of success.

It has long been observed that, compared with in vivo embryos, embryos cultured in simple media develop at a slower pace [1, 2] and have more apoptosis [3]. It is generally accepted that, compared with in vivo embryos, IVF embryos are in a less optimal environment—they are not in the supportive and protective environment of the oviduct. As a result, IVF embryos do not develop to their full potential and do not implant as well as their in vivo counterparts. It has been proposed that modifying embryo culture conditions, making them similar to those of the oviduct, may improve embryo development and enhance IVF success. Recent reports show embryos express

PPARδ and that PPARδ activation by oviduct-derived ligand enhances embryo development and implantation (more below). Thus PPARδ is a novel pathway that could be exploited to enhance ART outcome. This article will review the current literature regarding PPARδ and reproduction and outline the potential applications of its ligands in ART. Because PPARδ interacts with PPARα and -γ, relevant information regarding PPARα and -γ will also be provided.

2. PPARS AND THEIR LIGANDS

Peroxisomes are organelles in eukaryotes that participate in fatty acid oxidation. In 1990, the first PPAR (PPARα) was discovered in the mouse [4]. Two years later, PPARα and two additional PPAR isotypes, PPARβ (also known as PPARδ) and PPARγ, were discovered in the *Xenopus* [5]. Subsequently, all three isotypes were found in mouse and many species including human. PPARδ was originally discovered in a human osteosarcoma cell line [6] and later found to be the human homolog of PPARβ in the *Xenopus*.

PPARs are ligand-activated transcription factors. They form heterodimers with another nuclear receptor, retinoid X receptor (RXR), which also has three isotypes: RXRα, β, and γ [7]. The functions of PPAR-RXR complexes are determined by PPAR isotypes. Although either PPAR or RXR ligand can activate PPAR-RXR complexes, simultaneous PPAR and RXR binding yields more potent activities [8]. Whereas PPARα and PPARγ activate genes related to glucose and lipid metabolism, only a few genes are reported to be directly regulated by PPARδ. PPARδ reportedly upregulates PDK1, ILK [9] and 14-3-3ε [10], and downregulates PTEN [9] and 11 beta hydroxysteroid dehydrogenase type 2 gene [11]. The functions of these genes, unlike those regulated by PPARα and PPARγ, are not limited to energy homeostasis. They include carbohydrate homeostasis (PDK1), cell migration, proliferation and adhesions (ILK), signal transduction (ILK) and its modulation (14-3-3ε) and, finally, tumor suppression (PTEN).

Unlike PPARα and PPARγ, the outcome of PPARδ activation is not limited to the transcriptional activities of genes directly regulated by PPARδ because PPARδ also modulates the transcriptional activities of PPARα, PPARγ, other nuclear receptors (such as estrogen receptor), and BCL-6 (a transcriptional repressor). A recent report shows that binding of PPARδ by its ligand allows full transcriptional activities of PPARα and PPARγ, which is normally inhibited by nonliganded PPARδ [12]. In addition, binding of PPARδ by its ligand releases a transcription repressor BCL-6 [13] which targets a group of genes with diverse activities including transcription regulation ($n = 18$), protein binding ($n = 11$), signal transduction ($n = 10$), catalysis ($n = 8$), structural molecule activity ($n = 3$), enzyme activity regulation ($n = 3$), protein transportation ($n = 2$), cell movement ($n = 2$), chaperone ($n = 1$), and unknown function ($n = 3$) [14]. Thus PPARδ interacts with an extensive array of intracellular proteins to regulate cellular functions.

A diverse group of chemicals including hypolipidemic drugs, herbicides, and industrial plasticizers causes liver tumors in the rodents. They induce peroxisome proliferation and led to the discovery of PPARα [4]. Fatty acids, particularly the unsaturated fatty acids, and certain eicosanoids bind to PPARα, -γ, and -δ with varying affinities [8, 15]. Although all PPAR isotypes bind to unsaturated fatty acids, PPARα has the highest affinity. Eicosanoids from the lipoxygenase pathway (such as leukotrienes and hydroxyeicosatetraenoic acids—HETEs) and the cyclooxygenase pathway (such as prostaglandins—PGs) bind to PPARs: leukotriene B4 and 8(S)-HETE are PPARα ligand, 15-deoxy-Δ12,14-PGJ_2 (a PGD_2 derivative) is a PPARγ ligand, and PGI_2 is a PPARδ ligand [15]. In addition to the natural ligands, PPARs also respond to synthetic ligands. Some of the synthetic PPAR ligands are currently used to treat metabolic diseases: fibrates, which bind to PPARα, are hypolipidemic agents; thiazolidinediones (TZDs), which bind to PPARγ, are insulin sensitizers. A recent report shows that retinoic acid, depending on the ratio of cellular retinoic acid binding protein 2 (CRABP-II) and fatty acid binding protein 5 (FABP5), may function as a PPARδ ligand [16].

3. BIOLOGICAL FUNCTIONS OF PPARδ

The roles of PPARα and PPARγ in energy homeostasis are relatively easy to understand because the former is predominantly expressed in the brown adipose tissue and liver, and the latter, the adipose tissue [8, 17]. While PPARα catabolizes lipid in the liver, PPARγ facilitates fatty acid storage in adipose tissue by inducing the maturation of preadipocyte to fat cells.

The functions of PPARδ, on the other hand, are not as easy to ascertain because PPARδ has a ubiquitous distribution (including high levels of expression in the gut, kidney, and heart, and a lower level of expression in the liver) and interacts with extensive arrays of proteins in the cells (more in Section 2). Reported functions of PPARδ include the formation of intestinal adenoma [18] and colon cancer [19], the healing of skin [20], the development of hair follicles [21], and the protection of cells against noxious stimuli [10, 22]. The reproductive function of PPARδ was revealed for the first time during the investigation of cyclooxygenase-2 knockout mouse [23].

4. PPARδ AND REPRODUCTION

In primates, including humans, mature eggs are picked up by the fimbria and become fertilized in the ampulla. The zygotes remain in the oviduct for 72 hours; develop to morula/early blastocyst stage embryos before entering into the uterus. During this period, the oviduct produces soluble factors to promote embryo development and protect the embryo.

As mentioned earlier, the link between PPARδ and reproduction was first revealed at the implantation site of cyclooxygenase-2 knock out mice [23]. Since then, it was learned that embryos express PPARδ and that oviducts and embryos produce PGI_2. Recent studies also show that exogenous PPARδ ligand promotes the development of embryos and enhances their implantation potential (more in Section 4.4).

4.1. Female reproductive tract and embryos produce PPARδ ligand

We analyzed the metabolites of arachidonic acid by human [24] and mouse [25] oviducts and found substantial amount of PGI_2. Further analysis shows that PGI_2 production by the oviducts varies according to the estrus cycle. It peaks shortly after ovulation, coincides with the presence of cleaving zygotes in the oviduct and the "window" of embryonic responsiveness to PGI_2 [25]. Oviducts posses PGI_2 synthase and cyclooxygenase-2; the latter is the rate limiting enzyme of PG synthesis. The increased PGI_2 production is due to upregulation of the cyclooxygenase-2 gene. Oviduct also produces retinoic acid [26], the effects of oviduct-derived retinoic acid on embryo development is controversial (details below).

Similar to oviducts, embryos also metabolize arachidonic acid via cyclooxygenase and lipoxygenase pathways. PGI_2 is the most abundant metabolites of arachidonic acid by mouse embryos [27]. Preimplantation embryos express PGI_2 synthase, and cyclooxygenase-1 and -2; all are expressed in early stage and throughout the preimplantation period. The preimplantation embryos also produce retinoic acid [28] but its role in embryo development is yet to be determined.

The uterus is known to produce PGI_2 [29] but its central role in assisting embryo implantation was not revealed until twenty years later [23]. The uterus produces retinoic acid [30] but its biological role is unclear. Similarly, the ovary produces retinoic acid [31] and PGI_2 [32] but the extent to which they interact with PPARδ to influence oocyte maturation is not clear.

4.2. Testes express PPARδ

All three PPAR isotypes are present in Sertoli and Leydig cells of the testes: PPARα and -δ transcript and protein are expressed in Leydig cells and Sertoli cells of rat [33], PPARγ1 transcript is detected in human testis [34], both PPARα and -γ transcripts and proteins are expressed in mouse Sertoli cells [35]. In addition, mouse spermatids and spermatocytes express PPARδ [36]. The functionality of PPARδ in the testes is supported by the presence of *Ssm*, a novel PPARδ target gene in mouse testes [37]. Thus PPARδ may directly or indirectly (i.e., via PPARα or -γ) affect spermatogenesis. Information regarding PPARδ expression and action in mature sperms is limited. We previously report that iloprost (a PGI_2 analog) does not affect sperm activity [38]. However, the response of mature sperms to synthetic PPARδ ligand or retinoic acid has not been reported.

4.3. Ovaries express PPARδ

Similar to testes, the ovary expresses all three PPAR isotypes [39]. While PPARδ is expressed throughout the ovary, PPARα is mainly expressed in the theca and the stroma, and PPARγ, in the granulosa cells (of human, pig, rodents, and sheep) and the oocytes (of cattle and zebrafish). Of the three PPAR isotypes in the ovary, only PPARγ shows cyclic changes thus implying its role in follicular genesis and/or oocyte maturation. Since PPARδ may regulate the transcriptional activity of PPARγ, the growth of follicles or oocytes may be indirectly modulated by PPARδ ligand. The expression of PPARδ and the effect of its activation on the oocytes remain unclear at the moment.

4.4. Preimplantation embryos express PPARδ

Compared with PPARγ [28, 40] or PPARα [28, 40], there is more information regarding the expression of PPARδ and the outcome of its activation on preimplantation embryos [28, 40]. Mouse embryos express PPARδ at an early stage [40, 41] and throughout the preimplantation period. Blastocyst stage embryos express PPARδ in the inner cell mass and the trophectoderm [40]. PPARδ activation is associated with embryonic cell proliferation and improved embryo development [40]. Supplementing L-165,041 (a synthetic PPARδ ligand) or iloprost (a PGI_2 analog) to culture media enhances embryo hatching [38, 40–42]. Pretreatment with iloprost also increases the potentials of implantation and live birth of mouse embryos [40, 43].

The reported effects of retinoic acid on embryo development are inconclusive: some show it is beneficial to embryo development [44, 45], others show it is detrimental [46, 47]. A recent report shows LG100268 (a synthetic RXR ligand) reduces trophectoderm cell proliferation in a concentration-dependent manner, but enhances the development of bovine blastocysts at 0.1 μM [48]. Since retinoic acid binds to retinoic acid receptor (RAR), RXR, and (depending on the balance of intracellular CRABP-II and FABP5) PPARδ, the effect of retinoic acid on embryo development is likely to depend on its concentration as well as receptor availability. The latter is likely to change according to the developmental stage of the embryo. More studies are needed to resolve this complex issue.

5. CLINICAL APPLICATIONS OF PPARδ LIGAND IN ART

PPARδ can be activated via one of the three methods: PGI_2 (either stable analog or natural PGI_2), synthetic PPARδ ligand, or retinoic acid. The data presented above supports the notion that embryonic PPARδ activation by iloprost or synthetic PPARδ ligand may enhance ART outcome. However, using PPARδ ligand in ART should be approached with caution. A PPARδ ligand suitable for ART use should have passed extensive reproductive toxicology studies involving laboratory animals as well as domestic species to assure the health of progeny. The potential applications of PPARδ ligand in ART are listed below.

5.1. Using PPARδ ligand to enhance gamete function

The potential of PPARδ ligand in enhancing male gamete function or production is unknown because there is limited information on the effects of PPARδ ligand on the spermatogenesis and sperm activities. PPARδ may have no effect on sperm function because PGI_2 analog does not seem to affect the motility of human sperms [38]. However, synthetic

PPARδ ligand or retinoic acid was not tested in the previous report.

It is not clear the extent to which PPARδ ligand affects the oocytes either directly or indirectly (through granulosa cells). At the molecular level, PPARδ ligand has the potential to influence follicular genesis and/or oocyte maturation via PPARδ or PPARγ. This influence depends on the species-specific expression of PPARγ and PPARδ in the oocytes and the granulosa cells [39]. Recent reports show PPARγ ligand enhances the meiotic resumption of mouse oocytes [49] and reverses the adverse effects of diet-induced obesity on the oocytes [50]. More research is needed to understand the potential targets of PPARδ ligand in the ovary, that is, oocyte versus granulosa cells and PPARδ versus PPARγ.

5.2. Using PPARδ ligand to enhance embryo development

Three independent laboratories using different strains of mice show iloprost (a PGI_2 analog) enhances embryo development [10, 38, 41]. A recent report, based on 60 cryopreserved human embryos donated by patients for research, confirmed the enhancing effects of iloprost on human embryos [51]. Mouse embryos previously exposed to iloprost produce more implantation sites and yield more live pups [43]. These positive findings support the use of PPARδ ligand to enhance IVF outcome.

Whereas no synthetic PPARδ ligand has been approved by the FDA for clinical use, iloprost (which activates PGI_2 receptor and PPARδ) is approved by the FDA to treat pulmonary hypertension and peripheral vascular disease. Iloprost has undergone rigorous toxicology test and shows no teratogenicity [52]. Various animals (including rats, rabbit, and monkeys) exposed to iloprost during the peri-implantation period and throughout the pregnancy produce normal offspring. Based on four positive results from independent sources (three involving mouse embryos and one involving human embryo described above) and reassuring reproductive toxicology profile, a small scale, phase I/II clinical trial using iloprost may be considered. Supplementing iloprost to IVF embryos mimics the environment of the oviduct which provides a PGI_2 rich environment surrounding the embryos [24, 25].

There is a long way before retinoic acid is ready for use in ART. Given that PPARδ activation by retinoic acid is dependent on the ratio of CRABP-II and FABP5, the extent to which retinoic acid functions as a PPARδ ligand in the embryos is likely to vary based on the developmental stage of the embryos. More research is needed to ascertain the stage-dependent response of embryos to retinoic acid.

5.3. Using PPARδ ligand to augment PPARδ system at the implantation site

The uterus, being the site of implantation, is as important as the embryos in ensuring a good ART outcome. Therefore, although uterus is not an area served by ART, a discussion about PPARδ ligand and ART is not complete without a brief discussion of the uterus.

It is PPARδ at the implantation site that establishes the first link between PPAR and reproduction [23]. Recent evidence suggests that a host of signaling pathways involved in decidualization, implantation, and placentation converge on PPARδ: maternal PPARδ is important for decidualization and implantation; embryonic PPARδ is important for placentation [53]. Thus enhancing the PPARδ system of the uterus may be a novel method to ensure ART success. Activating PPARδ system at the implantation site is different from activating PPARδ system in the preimplantation embryos. The former involves administering PPARδ ligand to potential mothers during the peri-implantation period; the latter involves exposing IVF embryos to PPARδ ligand prior to embryo transfer. The former may require days of treatment; the latter only takes 18–24 hours (i.e., during the eight cell to morular stage transition). In addition, more information regarding genes and pathways activated by PPARδ ligand in the uterus and their impacts on the progeny needs to be obtained before it can be used to target the uterus.

5.4. Using PPARδ ligand to improve oocyte cryopreservation outcome

One important aspect of ART is the cryopreservation of oocytes. Oocyte cryopreservation is considered as a solution to ovarian aging in women who wish to defer raising their family; it is also viewed as one of the methods to preserve fertility in young women who are about to receive chemotherapy or irradiation. The outcome of oocytes cryopreservation is, however, far from satisfactory [54]; its success is hampered by freezing injury [55]. Lipid in the egg membrane and inside the cytoplasm is believed to be one of the contributing factors. Modifying membrane lipid [56] or removing excess cytoplasmic lipid [57] reportedly enhances oocyte survival after cryopreservation. Since PPARδ regulates lipid metabolism, it may mobilize lipid and augment the viability and the developmental competence of cryopreserved oocytes. The suitability of the above method in human ART requires more research because lipid content in the oocytes varies among species [58].

6. SUMMARY

Competent gametes, quality embryos, and a receptive endometrium are essential elements for a viable pregnancy. The outcome of ART may thus be enhanced by improving any or all of the above elements. While it is high time to consider using PPARδ ligand such as iloprost to enhance embryo development, the application of PPARδ ligand in other areas of ART requires more research. Continuing research on the reproductive functions of PPARδ and the safety of its ligand will ensure a smooth translation of basic science to clinical medicine.

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