Biochemical Pharmacology, Vol. 60, pp. 1027–1032, 2000.

ISSN 0006-2952/00/$–see front matter
PII S0006-2952(00)00416-0

Regulation of the Peroxisomal β-Oxidation-Dependent Pathway by Peroxisome Proliferator-Activated Receptor α and Kinases

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ABSTRACT. The first PPAR (peroxisome proliferator-activated receptor) was cloned in 1990 by Issemann and Green (*Nature* **347:**645–650). This nuclear receptor was so named since it is activated by peroxisome proliferators including several drugs of the fibrate family, plasticizers, and herbicides. This receptor belongs to the steroid receptor superfamily. After activation by a specific ligand, it binds to a DNA response element, PPRE (peroxisome proliferator response element), which is a DR-1 direct repeat of the consensus sequence TGACCT × TGACCT. This mechanism leads to the transcriptional activation of target genes (Motojima *et al., J Biol Chem* **273:**16710–16714, 1998). After the first discovery, several isoforms were characterized in most of the vertebrates investigated. PPARα, activated by hypolipidemic agents of the fibrate family or by leukotrienes; regulates lipid metabolism as well as the detoxifying enzyme-encoding genes. PPARβ/δ, which is not very well known yet, appears to be more specifically activated by fatty acids. PPARγ (subisoforms 1, 2, 3) is activated by the prostaglandin PGJ2 or by antidiabetic thiazolidinediones (Vamecq and Latruffe, *Lancet* **354:**411–418, 1999). This latter isoform is involved in adipogenesis. The level of PPAR expression is largely dependent on the tissue type. PPARα is mainly expressed in liver and kidney, while PPARβ/δ is almost constitutively expressed. In contrast, PPARγ is largely expressed in white adipose tissue. PPAR is a transcriptional factor that requires other nuclear proteins in order to function, i.e. RXRα (9-*cis*-retinoic acid receptor α) in all cases in addition to other regulatory proteins. Peroxisomes are specific organelles for very long-chain and polyunsaturated fatty acid catabolism. From our results and those of others, the inventory of the role of PPARα in the regulation of peroxisomal fatty acid β-oxidation is presented. In relation to this, we showed that PPARα activates peroxisomal β-oxidation-encoding genes such as acyl-CoA oxidase, multifunctional protein, and thiolase (Bardot et al., *FEBS Lett* **360:**183–186, 1995). Moreover, rat liver PPARα regulatory activity is dependent on its phosphorylated state (Passilly *et al., Biochem Pharmacol* **58:**1001–1008, 1999). On the other hand, some signal transduction pathways such as protein kinase C are modified by peroxisome proliferators that increase the phosphorylation level of some specific proteins (Passilly *et al. Eur J Biochem* **230:**316–321, 1995). From all these findings, PPARα and kinases appear to play an important role in lipid homeostasis. BIOCHEM PHARMACOL **60**;8:1027–1032, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. PPARα; phosphorylation; peroxisome; fatty acids; PKC; PI3-kinase

It is well established that fatty acid catabolism provides cell energy (in the form of ATP) by producing reducing equivalent (NADH). Moreover, β-oxidation produces acetyl-CoA, a precursor of several important pathways including the cholesterol synthesis route, which is also the pathway for producing farnesyl pyrophosphate and geranyl geraniol pyrophosphate (needed for ras protein membrane anchor) and dolichol (glycosylation) and cholesterol as well. In addition, fatty acid β-oxidation (especially that of peroxisome) regulates the level of arachidonic acid, which is the precursor of prostanoids. Peroxisomes are ubiquitous organelles originally discovered by Rhodin in 1954 [1] and first isolated and characterized by De Duve's group in 1963 [2]. They appear as membrane-surrounded particles and contain numerous catabolic and anabolic enzymes. Impairment of peroxisome function is the result of severe genetic diseases (Refsum's disease and Zellweger syndrome) [3]. The almost exclusive dependence of fatty acid β-oxidation in yeast on peroxisomes is well known. In contrast, in mammals, it is only recently that we have come to better appreciate the contribution of peroxisomes in breaking down long- and very long-chain fatty acids as compared to mitochondria [4]. The peroxisome contribution to whole fatty acid beta-oxidation can be up to 50% in rat liver for fatty acids longer than 16-carbon length.

It has been known for many years that rat or mice liver peroxisomes proliferate to an enormous degree [5]. This unique property is triggered by chemicals of the peroxisome

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proliferator family, including hypolipidemic agents (fibrates), plasticizers (phthalates), and herbicides (phenoxyacetate derivatives). All these compounds exhibit common structural and physicochemical properties, e.g. halogenated, substituted, aromatic weak organic acid with lipophilic solubility. Like xenobiotic molecules, natural compounds show similar peroxisome proliferative properties. In mammals, the phenomenon of peroxisome proliferation is largely species-specific, being almost restricted to rodents since primates (including human) are quite resistant [6].

However, parallel to or separate from peroxisome proliferation, fibrates stimulate the transcription of encoding genes for numerous enzymes of lipid metabolism, especially fatty acid β-oxidation [7]. In this survey, it will be shown that fibrate-mediated regulation is PPARα*-dependent. It will also be demonstrated that PPAR function is modulated by its phosphorylation state. Finally, it will be discussed how the response to peroxisome proliferators is dependent on the signaling cascade, starting from the membrane and going to the nucleus. Thus far, several kinase pathways within the impressive puzzle of phosphorylation–dephosphorylation reactions have been identified as regulating peroxisomal fatty acid β-oxidation, including PKC, PI3-kinase, and MAPK. The consequence would be a possible opposite cross-regulation between fatty acid and carbohydrate metabolism.

STRATEGY

Biological materials and methodologies have been largely described in previous papers: Fao hepatic-derived cell lines [8], PPARα cDNA and related plasmids [9], antibody anti-PPARα [9], cDNA of fatty acid β-oxidation enzyme-encoded genes (acyl-CoA oxidase) [10], multifunctional enzyme [11], thiolase [12], protein kinase C assay [13], the PI3-kinase subunit p85 [14], transfection assay [12], and Northern blotting [11].

TRANSCRIPTIONAL ACTIVATION OF PEROXISOMAL FATTY ACID β-OXIDATION ENZYME-ENCODING GENES

In rat liver, the level of these specific mRNAs is greatly increased after treatment with the peroxisome proliferator ciprofibrate [7]. Indeed, this high mRNA level is observed for the whole metabolic pathway, including acyl-CoA synthetase, the adrenoleukodystrophy-related protein (an ABC transporter superfamily protein), acyl-CoA oxidase, the multifunctional enzyme (hydratase–dehydrogenase), 3-ketoacyl-CoA thiolase, and acetyl/octanoyl carnitine transferase. This overall transcription increase is somewhat reminiscent of bacterial operon induction.

* *Abbreviations:* MAPK, mitogen-activated protein kinase; PI3-kinase, phosphatidylinositol 3-kinase; PKC, protein kinase C; PPAR, peroxisome proliferator-activated receptor; and PPRE, peroxisome proliferator response element.

PROPERTIES OF PPARα

The first PPAR was discovered 10 years ago by Issemann and Green [15]; it was subsequently called PPARα, since Dreyer *et al.* in 1992 cloned tow other members of this subfamily (β/δ and γ) belonging to the steroid receptor superfamily. Their tissue distribution has been well documented; PPARα is largely expressed in the liver and hardly or not at all in other tissues [17]. In contrast, PPARγ is mainly expressed in the adipose tissue or in the intestine, whereas PPARβ,δ can be considered as a constitutive transcription factor. They were later shown to be activated by the binding of specific ligands, including peroxisome proliferators [18]. More precisely, PPARs are activated either by pharmacological or physiological ligands [19]. Pharmacological ligands are the fibrates (hypolipidemic drugs) for PPARα and the thiazolidinediones (antidiabetic drugs of Type 2 diabetes) for PPARγ. The biological ligands mostly belong to signaling messagers produced from the arachidonic cascade pathways, e.g. leukotriene (LTB4) from the lipoxygenase route for PPARα or prostaglandin Δ15PGJ2 from the cyclooxygenase route for PPARγ. On the other hand, PPARβ/δ would appear to be preferentially activated by unsaturated fatty acids [20]. The PPAR–DNA response element is a DR-1 motif with a consensus sequence TGACCT T/A TGACCT [21]. This PPRE is found in the promoter of all peroxisomal fatty acid β-oxidation enzyme-encoding genes.

The PPAR–PPRE interaction as shown by "gel shift" assay leads to transcriptional activation [12, 22]. For this to occur, PPAR must be heterodimerized with RXRα, another transcription factor activated by 9-*cis*-retinoic acid. Such an effect can be seen by cotransfecting cells with a plasmid encoding for the luciferase gene reporter containing the PPRE at the promoting region in the presence of plasmids encoding for PPARα and RXRα [12, 22].

MODULATION OF PPARα ACTIVITY BY SIGNAL TRANSDUCTION

In addition to the several possibilities of protein modulation (transcriptional and translational regulation, ligand-mediated allostery, protein–protein interactions, et cetera), the phosphorylation/dephosphorylation process is an important mechanism of regulation which is also valid for transcription factors [23]. Indeed, PPARα is phosphorylated in rat Fao hepatic-derived cells. This was shown by *in vivo* cell pulse labeling with [^{32}P]orthophosphate followed by an immunoprecipitation with a specific anti-PPARα antibody [9]. This phosphorylation is enhanced by cell treatment with ciprofibrate, a peroxisome proliferator. Other papers reported that the phosphorylation occurs at seryl residue 12 located in the N-terminus region A/B transactivating domain. Moreover, the phosphorylation of PPARα is insulin-dependent [24]. On the other hand, PPARα activity is modulated by an inhibitor under the control of JAK2/STAT5b, i.e. Janus kinase 2/signal trans-

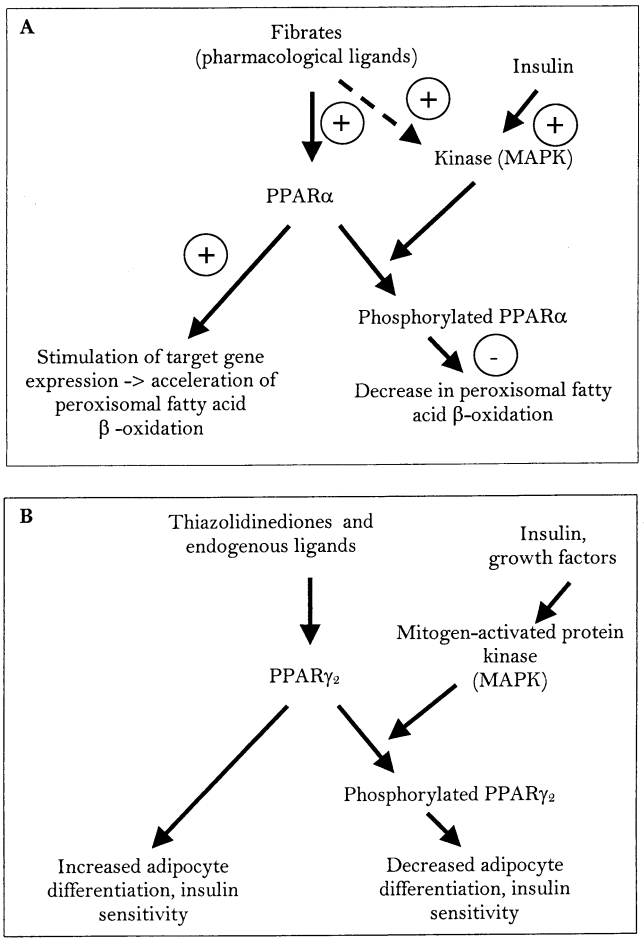

FIG. 1. Relationships between structure and functional roles of PPARα and PPARγ. (A) Role of PPARα in peroxisomal fatty acid β-oxidation and regulation by phosphorylation (from Refs. 9 and 24–26). (B) Role of PPARγ$_2$ and its regulation by phosphorylation (adapted from Ref. 21).

ducer and activator of transcription 5b, a kinase complex regulated by growth hormone [25]. Finally, human PPARα is phosphorylated at Ser12 and Ser21 by MAPK [26].

We attempted to determine the consequence of PPARα phosphorylation on a peroxisome marker, namely acyl-CoA oxidase encoded by a PPARα target gene, by treating Fao cells with okadaic acid and vanadate, two potent inhibitors of phosphoprotein phosphatases. The treatment, which would maintain PPARα in a high-level phosphorylated form, leads to a decrease in acyl-CoA oxidase activity [9]. The different data are summarized in Fig. 1A. Interestingly, preadipocytes and adipocytes show a similarity in the phosphorylation of PPARγ (Fig. 1B) where PPARγ phosphorylation is stimulated by insulin and is dependent on MAPK [27]. Moreover, the phosphorylation of PPARγ leads to a decrease in PPARγ-mediated preadipocyte differentiation into adipocytes. Other data reported on PPARγ and PPARβ/δ are the following: the Ser112 mutation prevents PPARγ2 phosphorylation [28]; Ser84 is phosphorylated by ERK2 (extracellular signal-regulated kinase) and JNK (c-jun N-terminal kinase) leading to a decrease in PPARγ-mediated adipogenic differentiation [29]; MAPK-dependent PPARγ1 phosphorylation at Ser82 is stimulated by EGFR (i.e. epidermal growth factor receptor) [30]; MAPK-dependent PPARγ phosphorylation is inhibited by PGF2 (prostaglandin F2) [31]; the Ser114 mutation stimulates dephosphorylated human PPARγ2 form-dependent adipocyte differentiation (It is to be noted that the Ser residues at positions 112 and 114 of PPARγ2 may be the same, as might the Ser residues at positions 82 and 84 of PPARγ1) [32]; the phosphorylation of PPARγ A/B domain decreases both ligand binding and transcriptional activity [33]; PPARγ1 is also phosphorylated by stress protein kinases (JNK/SAPK, i.e. c-jun N-terminal kinase/stress-activated protein kinase) in a MAPK-independent pathway, leading to a decrease in transcriptional activity [34]; PPARβ/δ could be phosphorylated by a MAPK-independent pathway [30]; the dephosphorylation of COUP-TF (chicken ovary upstream promoter-transcription factor) induced by TCDD (2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin) and RA (retinoic acid) prevents binding to PPRE [35]. Finally, peroxisome proliferators stimulate ERK1 and ERK2 phosphorylations at tyrosyl residues [36].

PROTEIN KINASE C AS A SIGNALING PATHWAY COMPONENT FOR MEDIATING THE PEROXISOME PROLIFERATOR EFFECT

Until recently, there was little information on peroxisome proliferator/transduction interference. Several years ago, a stimulation of EGF receptor autophosphorylation [37] and an *in vitro* stimulation of PKC by peroxisome proliferators [38] were reported. More recently, we showed that several cell phosphoproteins were overphosphorylated in the presence of ciprofibrate [39]. Now, by using Fao-permeabilized cells (employing streptolysin O), we report that ciprofibrate *in vivo* stimulates the PKC-dependent phosphorylation of a specific substrate [13]. On the other hand, in ciprofibrate pretreated cells, a particulate fraction contains both kinase activity and kinase substrate, since *in vitro* ^{32}P labeling using [γ-^{32}P]ATP leads to an overphosphorylated band of 85 kDa. The use of several biochemical or technical conditions to characterize the kinase responsible for the labeling of this 85-kDa protein is consistent with a PKC involvement: phosphorylation at sery1/threonyl residues (as shown by SDS–PAGE gel treatments leading to the resistance of the phospholabeling band under acidic conditions but to hydrolysis upon alkaline exposure); phospholabeling sensitive to calcium (but not to calmodulin); and disappearance of the P85-labeled band after particulate fraction treatment with PKC inhibitor (staurosporine, H7, or GF 109203 X, i.e. bisindolylmaleimide) [13].

Attempts to identify p85 polypeptide strongly suggest an identity with a regulatory component of the PI3-kinase having a molecular weight of 85. Indeed, this protein is present in the Fao cells.* Two p85 regulatory components

* Latruffe N, Rieusset J and Vidal H, unpublished results.

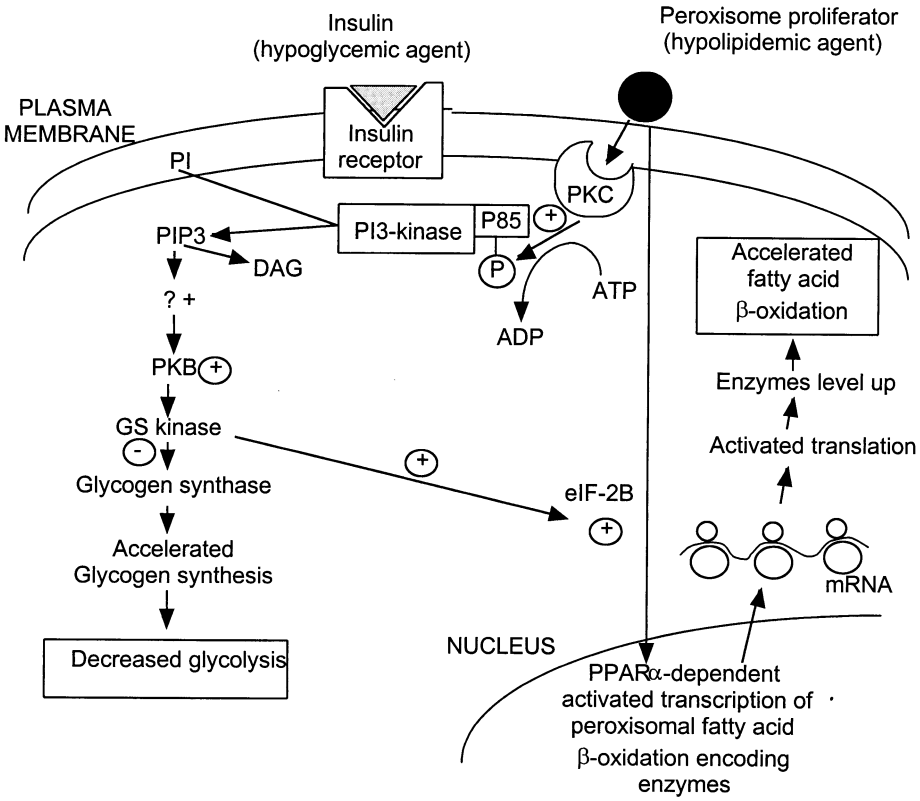

FIG. 2. Possible implication of the phosphoinositide signaling pathway in the peroxisome proliferator-mediated cross-regulation of lipid and carbohydrate metabolisms. GS: glycogen synthase; PKB: protein kinase B; PI: phosphatidylinositol; DAG: diacylglycerol; eIF: translation initiation factor-2B; PIP3: phosphatylinositol triphosphate.

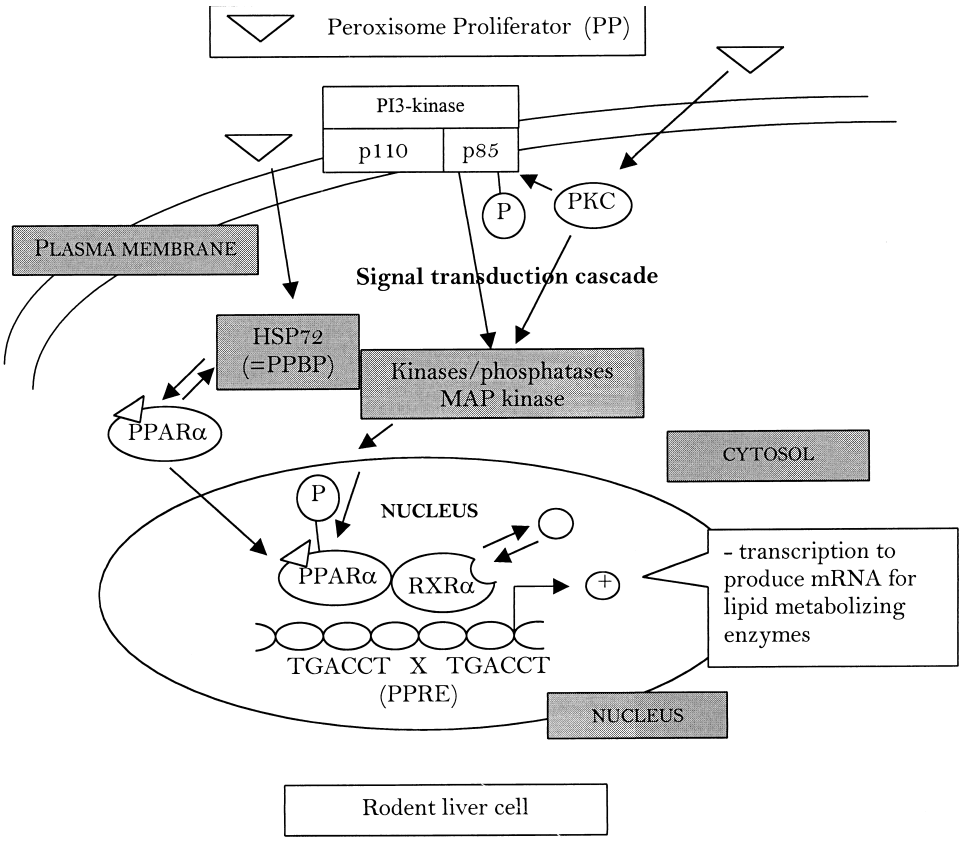

FIG. 3. Possible mechanism for PPARα and kinase dependence on fatty acid catabolism in rodent liver cells upon exposure to a peroxisome proliferator. Abbreviations and symbols used: PPBP: peroxisome proliferator-binding protein; P110: PI3-kinase catalytic subunit; p85: PI3-kinase regulatory subunit; PKC: protein kinase C; HSP72 = heat-shock protein 72; PPRE = DNA consensus sequence; ◯ = 9-*cis*-retinoic acid, Ⓟ = phosphorylation site.

of the PI3-kinase have been reported (α, β). In T cells, activation of PKC does not change the basal serine phosphorylation of the p85α subunit, whereas it results in a rapid increase in phosphorylation of the p85β subunit on threonine residues. This would be in agreement with a PKC-dependent phosphorylation of the p85β subunit [40, 41]. In this case, the ciprofibrate-dependent activated PKC would recruit both p85 (regulatory subunits) and P110 (catalytic subunit) of PI3-kinase into the membrane to allow protein kinase B (PKB = Akt) activation, leading to the triggering of the phosphoinositide signaling cascade. A possible mechanism is proposed in Fig. 2, one that would be consistent with a concerted and opposite cell regulation of energetic metabolism between glucose and fatty acid metabolisms. In this case, while fatty acid β-oxidation reactions would be stimulated through fibrate-activated PPARα dependency, a fibrate would orient glucose metabolism to glycogenogenesis through the PI3-kinase cascade including the fibrate → PKC → p85-dependent route [42].

Finally, there is additional evidence that fibrate-modulated-PKC could play an important role in peroxisomal fatty acid β-oxidation, since the level of ciprofibrate-stimulated thiolase mRNA is largely decreased when cells are exposed to PKC inhibitors (staurosporine, H7, genistein, etc. [43]).

In conclusion, the peroxisomal fatty acid β-oxidation pathway is regulated by peroxisome proliferators, especially by hypolipidemic agents of the fibrate family at at least two levels: through the PPARα, which can be modulated by a ligand-activated process but also at the phosphorylation chemical modification; and through signal transduction mechanisms where only a few kinase components have been identified thus far: MAPK, PKC, and possibly PI3-kinase (see Fig. 3).

We wish to thank the former colleagues/collaborators and external collaborators who participated in some parts of this study (see references). This program is supported by the Regional Council of Burgundy and the Cancer League of Burgundy.

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