

MINIREVIEW

Activation of Mitogen-Activated Protein Kinases by Peroxisome Proliferator-Activated Receptor Ligands: An Example of Nongenomic Signaling

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ABSTRACT

Peroxisome proliferator-activated receptors (PPARs) are a subfamily of nuclear hormone receptors that function as ligand-activated transcription factors to regulate lipid metabolism and homeostasis. In addition to their ability to promote gene transcription in a PPAR-dependent manner, ligands for this receptor family have recently been shown to induce mitogen-activated protein kinase (MAPK) phosphorylation. It is noteworthy that the transcriptional changes induced by PPAR ligands can

be separated into distinct PPAR- and MAPK-dependent signaling pathways, suggesting that MAPKs alone mediate some of the effects of PPAR agonists in a nongenomic manner. This review will highlight recent studies that elucidate the nongenomic mechanisms of PPAR ligand-induced MAPK phosphorylation. The potential relevance of MAPK signaling in PPAR biology is also discussed.

Peroxisome proliferator-activated receptors (PPARs) comprise a three-member subgroup (α , γ , and β/δ) within the nuclear hormone receptor family of ligand-activated transcription factors (Dreyer et al., 1992). As physiological lipid sensors and regulators of lipid metabolism, PPARs have recently emerged as attractive targets in the development of pharmaceutical agents to treat metabolic disorders, hypercholesterolemia, diabetes, inflammation, and cancer (Vanden Heuvel, 1999; Chinetti et al., 2000; Michalik et al., 2004). Indeed, not only have ligands for PPARs become a leading treatment for patients with type II diabetes and dyslipidemia, but they also show promise as anti-inflammatory and antitumor drugs. Although receptor activation was thought to be the primary mechanism of action of PPAR ligands, the direct role of PPARs in mediating these therapeutic effects

has recently come under scrutiny. In particular, PPAR α and γ agonists affect growth in cell types that lack their respective receptor (Palakurthi et al., 2001; Pauley et al., 2002). Likewise, whereas PPAR γ ligands improve insulin sensitivity by simultaneous coordinated actions on adipose, muscle, and liver tissues (Picard and Auwerx, 2002; Evans et al., 2004), only adipose tissue expresses significant levels of PPAR γ (Vidal-Puig et al., 1996).

Although it is well documented that PPAR ligands induce transcription of target genes in a PPAR-dependent manner, recent research has revealed that these drugs also elicit “nongenomic” PPAR-independent effects. For example, PPAR ligands were shown to rapidly induce phosphorylation of mitogen-activated protein kinase (MAPK) family members (Rokos and Ledwith, 1997; Mounho and Thrall, 1999; Lennon et al., 2002; Teruel et al., 2003). This well-characterized kinase family plays a pivotal role in signal transduction by relaying a variety of signals from the cell surface to the nucleus (Johnson and Lapadat, 2002). MAPKs are specifi-

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ABBREVIATIONS: PPAR, peroxisome proliferator-activated receptor; MAPK, mitogen-activated protein kinase; RXR, retinoic acid receptor; Wy-14,643, pirinixic acid; ERK, extracellular signal regulated kinase; JNK, c-jun N-terminal kinase; MEK or MKK, mitogen-activated protein kinase kinase; PD98059, 2'-amino-3'-methoxyflavone; EGFR, epidermal growth factor receptor; EGF, epidermal growth factor; Pyk2, proline-rich tyrosine kinase; CaMKII, calcium/calmodulin-dependent kinase II; ER, endoplasmic reticulum; U0126, 1,4-diamino-2,3-dicyano-1,4-bis(2-aminophenyl)butadiene; PKR, double stranded RNA-activated protein kinase; eIF2 α , eukaryotic initiation factor 2 α .

cally involved in promoting cell growth and differentiation as well as coordinating responses to cell stress. It is noteworthy that the ability of PPAR ligands to activate MAPKs could contribute to their pharmacological mechanism of action and thus help to explain their apparent receptor-independent effects. This review will give a brief history of the relationship between PPARs and MAPKs, highlight the mechanisms of MAPK activation by PPAR ligands, and explain the potential relevance of kinase signaling to PPAR biology.

Peroxisome Proliferator-Activated Receptors

Peroxisome proliferator-activated receptors were discovered as a class of receptors activated by a diverse group of rodent hepatocarcinogens (Issemann and Green, 1990). The name peroxisome proliferator-activated receptor was derived from the observation that these carcinogens induced proliferation of peroxisomes, the subcellular organelles primarily responsible for the oxidation of long-chain fatty acids and subsequent detoxification of hydrogen peroxide. Since the cloning of PPAR α , two additional PPARs have been characterized: PPAR γ and PPAR β/δ . PPAR γ has been further subdivided into PPAR γ_1 , PPAR γ_2 , and PPAR γ_3 , resulting from differential RNA splicing and alternative promoter use, giving rise to a total of five receptor isoforms (Fajas et al., 1997, 1998).

PPARs possess the two characteristic functional domains present in all nuclear hormone receptors (Fig. 1A), a superfamily that also includes receptors for estrogen, progesterone, vitamin D₃, and thyroid hormone among others (Evans, 1988; Issemann and Green, 1990). The N-terminal DNA-binding domain of PPARs contains two zinc fingers responsible for the recognition of specific hormone response elements within the promoter region of target genes, whereas the C-terminal domain controls ligand-dependent receptor activation and additional protein-protein interactions (Berg, 1989; Klug and Schwabe, 1995). The presence of these signature motifs indicates that PPARs function physiologically as nuclear hormone receptors or, more specifically, intracellular ligand-activated transcription factors. Agonist binding to PPARs induces the formation of PPAR/retinoic acid receptor (RXR) heterodimers (Kliwer et al., 1992; Miyata et al., 1994), leading to the recruitment of cofactor proteins that facilitate the initiation of transcription (Xu et al., 1999). The active PPAR/RXR transcription complex binds to peroxisome proliferator response elements, a direct repeat of two copies of a hexameric nucleotide sequence within the promoter region (Fig. 1B), leading to transcription of these target genes (Tugwood et al., 1992).

PPAR α . PPAR α was identified after a screen of a mouse liver cDNA library for nuclear hormone receptors that were activated by a class of chemicals known as peroxisome proliferators (Issemann and Green, 1990). In addition to liver, this receptor is expressed in other highly metabolic tissues, such as kidney, heart, skeletal muscle, and vascular smooth muscle cells, where it functions specifically in the regulation of genes responsible for cellular uptake and β -oxidation of fatty acids (Lee et al., 1995; Braissant et al., 1996). More than 70 compounds, including synthetic hypolipidemic fibrate drugs (Wy-14,643, nafenopin, clofibrate), phthalate plasticizers (monoethylhexylphthalate), chlorinated hydrocarbons, and herbicides along with endogenous hormones

and fatty acids (arachidonic acid), have been identified as PPAR α agonists (Table 1, Fig. 2) (Citron, 1995; Kliwer et al., 1997; Krey et al., 1997; Zhou and Waxman, 1998).

PPAR γ . Although no significant functional differences have been reported for the PPAR γ isoforms, expression of these variants is tissue-specific. Similar to PPAR α , PPAR γ_1 is expressed to some extent in highly metabolic tissues, whereas PPAR γ_2 is found nearly exclusively and at much higher levels in adipose tissue (Fajas et al., 1997). PPAR γ_3 is also expressed in adipose tissue as well as the colon and macrophages (Fajas et al., 1998). In addition to its ability to affect cellular energy homeostasis, PPAR γ is unique among this subfamily of nuclear hormone receptors in that its main physiological function is to regulate adipocyte differentiation as well as insulin sensitivity (Tontonoz et al., 1994; Masugi et al., 1999).

In addition to its ability to influence fat cell proliferation, growth inhibitory actions of PPAR γ have also been observed in breast cancer cells (Mueller et al., 1998), advanced liposarcoma (Demetri et al., 1999), and most notably colon cancer (Sarraf et al., 1998). The role of PPAR γ in maintaining glycemic control, however, was not fully appreciated until it was shown that the antidiabetic and insulin-sensitizing agents known as thiazolidinediones (e.g., ciglitazone and troglita-

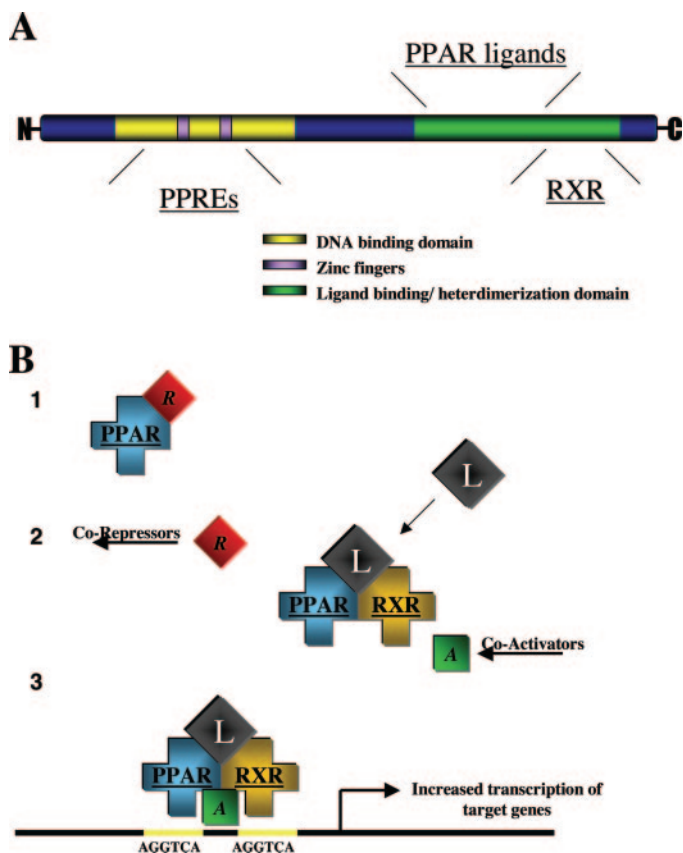


Fig. 1. PPARs as nuclear hormone receptors. A, functional domains of PPARs. B, mechanism of ligand-dependent PPAR/RXR heterodimerization and DNA binding. 1, in the absence of ligand (boxed L), nuclear hormone receptors are maintained in the inactive state via interactions with corepressors (boxed R). 2, ligand binding to PPARs results in a conformational change leading to dissociation of corepressors (boxed R), heterodimerization of RXR, and recruitment of transcriptional coactivators (boxed A). 3, the active transcription complex binds peroxisome proliferator response elements, two copies of a direct repeat hexanucleotide sequence, in the promoter region of target genes leading to increases in gene transcription.

zone) were high-affinity PPAR γ ligands (Fig. 3A) (Lehmann et al., 1995). The ability of thiazolidinediones to activate PPAR γ is thought to be a primary mechanism by which these agents exert their antidiabetic effects. In addition to the synthetic thiazolidinediones, PPAR γ is also activated by endogenous ligands such as the eicosanoid 15-deoxy- $\Delta^{12,14}$ -prostaglandin J₂ (Kliwer et al., 1997; Krey et al., 1997).

PPAR β/δ . Expression of this PPAR isoform is ubiquitous and often found at higher levels than PPAR α and γ (Braisant et al., 1996). The biological functions of PPAR β/δ are the least understood of the three PPARs, and no PPAR β/δ target

TABLE 1

PPAR α agonists are a diverse class of natural and synthetic compounds

Class and Name of Compound	Reference
Hypolipidemic fibrates	
Clofibrate	Krey et al., 1997
Gemfibrozil	Krey et al., 1997
Nafenopin	Krey et al., 1997
Wy-14,643	Issemann and Green, 1990
Industrial plasticizers	
Monoethylhexylphthalate	Issemann and Green, 1990
Diethylhexylphthalate	Issemann and Green, 1990
Industrial Solvents	
Trichloroacetate	Walgren et al., 2000
Dichloroacetate	Walgren et al., 2000
Eicosanoids	
8(S)-Hydroxyeicosatetraenoic acid	Krey et al., 1997
Docasaheptaenoic acid	Krey et al., 1997
Leukotiene B4	Devchand et al., 1996
Fatty Acids	
Linoleic acid	Krey et al., 1997
Linolenic acid	Krey et al., 1997
Arachidonic acid	Krey et al., 1997

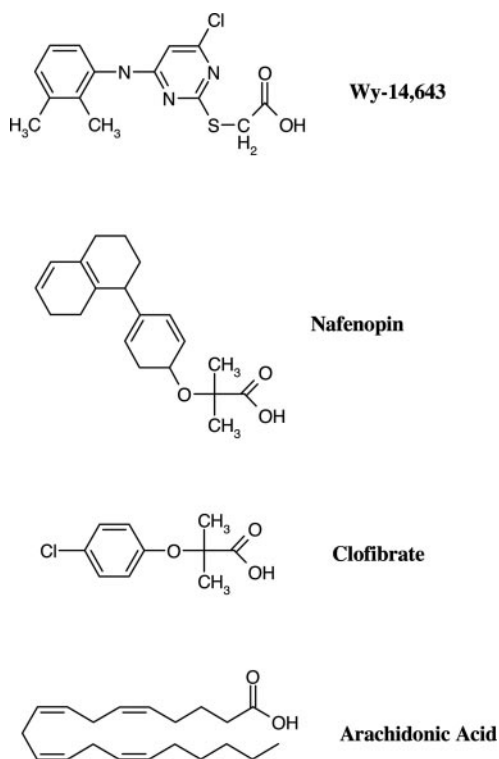


Fig. 2. PPAR α ligands. Chemical structures for synthetic PPAR α agonists such as Wy-14,643, nafenopin, and clofibrate, as well as arachidonic acid, a natural ligand, are shown. Despite noticeable structural diversity, most PPAR α agonists have a carboxylic acid group coupled to a large, hydrophobic side chain.

genes have been identified. Moreover, a PPAR β/δ -selective ligand has yet to be discovered. Evidence collected thus far suggests that PPAR β/δ , like PPAR γ , plays a critical role in colon cancer and potentiates PPAR γ -stimulated adipocyte differentiation (Harman et al., 2004; Matsusue et al., 2004). Targeted activation of PPAR β/δ in mice results in complete resistance to both high-fat diet-induced and genetically predisposed obesity (Wang et al., 2003), suggesting that this receptor isoform is also important in the regulation of lipid metabolism.

PPAR Ligands Induce Receptor-Independent Signaling

The presence of PPARs in numerous tissues, as well as the vast array of ligands capable of activating these receptors, suggests that PPARs play a critical role in maintaining normal cellular function on multiple levels. It is noteworthy that studies have shown that the effects of PPAR agonists are

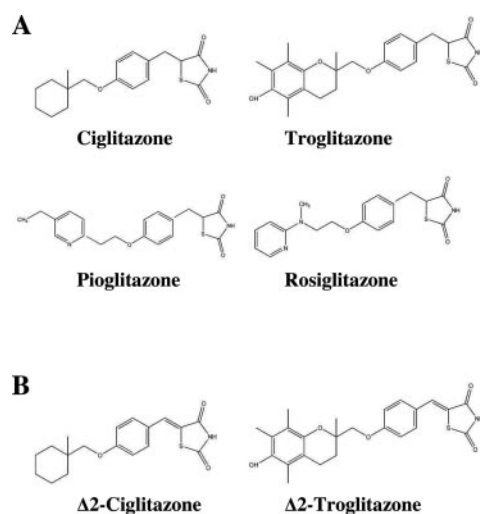


Fig. 3. The thiazolidinedione class of PPAR γ ligands and structurally inactive derivatives. A, thiazolidinediones are antidiabetic agents that increase insulin sensitivity in adipose, liver, and muscle tissue in animals and human patients with non-insulin-dependent diabetes. Ciglitazone, a clofibrate (Fig. 2) analog, was the first of these compounds to be synthesized (Sohda et al., 1982). Whereas both clofibrate and ciglitazone had potent lipid-lowering activity, ciglitazone was unique in that it also exhibited unexplained glucose-lowering properties (Fujita et al., 1983). This insulin-sensitizing effect was later attributed to the ability of thiazolidinediones to bind and activate PPAR γ (Lehmann et al., 1995). Indeed, the ability of thiazolidinediones to bind PPAR γ in vitro correlates well with their antidiabetic activity in vivo (Willson et al., 1996). Although the discovery of ciglitazone presented a promising treatment for type II diabetes, ciglitazone was abandoned as a potential therapy because of liver toxicity. Troglitazone (Rezulin; Pfizer, New York, NY), a derivative of ciglitazone, was the first thiazolidinedione to demonstrate clinical efficacy, yet was promptly withdrawn from the market because of idiosyncratic hepatotoxicity and other mechanisms that remain unclear (Watkins and Whitcomb, 1998; Kohlroser et al., 2000). Rosiglitazone (Avandia; GlaxoSmithKline, Uxbridge, Middlesex, UK) and pioglitazone (Actos; Eli Lilly & Co., Indianapolis, IN), which are at least 100-fold more potent ligands for PPAR γ than troglitazone (Willson et al., 1996), are currently available in the United States. Whereas the ability of all these agents to activate PPAR γ and promote effective glycemic control is clear, thiazolidinedione-specific effects have also been documented that may be a consequence of differing chemical structures and/or potency for PPAR γ . It is noteworthy that rosiglitazone and pioglitazone do not induce liver failure (Scheen, 2001). In addition, rosiglitazone is metabolized differently from pioglitazone and troglitazone, greatly decreasing the potential for unwanted drug interactions. B, structure of Δ^2 -ciglitazone and Δ^2 -troglitazone that completely lack PPAR γ binding capability (Shiau et al., 2005).

even more complex in that these compounds not only induce receptor-mediated transcription but also seem to exert PPAR-independent or “nongenomic” effects. For example, the carcinogenicity of PPAR α agonists does not always correlate with the degree of peroxisome proliferation (Marsman et al., 1988), a PPAR α -dependent event (Lee et al., 1995). In addition, PPAR α ligands were found to induce expression of immediate early genes in cell lines that did not express this receptor (Pauley et al., 2002).

Likewise, although treatment with the thiazolidinedione class of PPAR γ ligands has been directly associated with up-regulation of insulin sensitizing genes and coordinate inhibition of genes promoting insulin resistance (Hofmann et al., 1994; Ribon et al., 1998; Smith et al., 2001), knowledge about the mechanism by activation of PPAR γ improves insulin sensitivity remains unclear. For example, the specific target tissue(s) of thiazolidinediones is unknown; candidates include adipose, skeletal muscle, and liver tissues, as well as pancreatic β cells (Evans et al., 2004). Although each of these targets is significantly affected by thiazolidinediones, PPAR γ is expressed at disproportionately higher levels in adipocytes. Furthermore, the role of PPAR γ in mediating the growth inhibitory effects of thiazolidinediones has also been questioned for similar reasons. The PPAR γ agonists ciglitazone and troglitazone significantly inhibited cell growth to the same extent in PPAR $\gamma^{+/+}$ as well as PPAR $\gamma^{-/-}$ cell lines (Palakurthi et al., 2001).

Although these apparently receptor-independent effects of PPAR ligands are contradictory to the classic mechanism of nuclear hormone receptor action, results of recent studies have implied that a variety of other nuclear hormone receptor agonists exert similar nongenomic effects. For example, progesterone, estrogen, aldosterone, thyroid hormone, and vitamin D₃ have all been shown to evoke rapid changes in signal transduction pathways that contribute to their biological mechanism of action (Losel and Wehling, 2003). In addition to the aforementioned effects, PPAR α and γ agonists, like other nuclear hormone receptor ligands, were recently shown to activate members of the MAPK family at times too rapid to account for new protein synthesis (Rokos and Ledwith, 1997; Mounho and Thrall, 1999; Lennon et al., 2002; Teruel et al., 2003). Because MAPKs themselves are known transcriptional regulators (Johnson and Lapadat, 2002), this finding provides a possible mechanism explaining how PPAR agonists are able to induce cellular effects in a PPAR-independent manner.

Activation of MAPKs by PPAR Ligands

MAPKs alter the activity of a diverse array of transcription factors and the expression of their conjugate genes and represent one of the major cellular mechanisms effecting gene expression. There are currently four main groups of MAPKs in mammalian cells: extracellular signal regulated kinase (ERK), c-jun N-terminal kinase (JNK), p38, and ERK-5 [or Big MAP kinase-1 (BMK1)] (for review, see Lewis et al., 1998). Alternate splicing variants of pre-mRNAs results in a number of gene products and isoforms within each group. Activation of ERK occurs in response to mitogenic stimuli such as growth factors and hormones, whereas JNK and p38 predominantly activate in response to stress stimuli. ERK5 activation occurs in response to both stress stimuli and

growth factors (Kyriakis and Avruch, 2001). In addition to the direct regulation of transcription factors through phosphorylation, MAPKs additionally affect other kinases, including ribosomal S6 kinase, MAPK-interacting kinase, MAPK-activated protein kinase, and mitogen and stress-activated protein kinase, which in turn regulate gene expression through phosphorylation of histones and transcriptional regulatory proteins (Lewis et al., 1998; Kyriakis and Avruch, 2001). For a detailed list of transcriptional targets regulated by MAPK pathways, see Yang et al. (2003). The ability of PPAR ligands to activate MAPKs independent of their ability to modulate PPAR-dependent genes consequentially implies that numerous other target genes may be subject to transcriptional control by PPAR ligands.

Both PPAR α and γ ligands have been shown to activate MAPK family members in a variety of different cell types (Table 2). MAPK activation is thought to facilitate some of the pharmacological as well as toxicological effects associated with these agents. In particular, the PPAR α agonists Wy-14,643, monoethylhexylphthalate, and clofibrate induced mitogen-activated protein kinase kinase (MEK or MKK) and ERK phosphorylation in mouse liver cells (Rokos and Ledwith, 1997). In addition, nafenopin was demonstrated to activate ERK as well as p38 in primary rat hepatocytes (Cosulich et al., 2000). Inhibition of MAPK signaling in these models prevented both increases in immediate early gene expression and DNA synthesis, suggesting that MAPKs play an important role in promoting the hepatoproliferative effects of PPAR α ligands. Likewise, the PPAR γ agonist rosiglitazone was shown to increase uncoupling protein-1 expression in fetal rat brown adipocytes via a p38 MAPK-dependent pathway (Teruel et al., 2003). Uncoupling proteins are involved in the control of energy expenditure in response to nutritional status and are known PPAR target genes (Sears et al., 1996). Furthermore, the antiproliferative effect of troglitazone on colon cancer cell growth was demonstrated to require ERK (Kim et al., 2002; Baek et al., 2003). In particular, troglitazone-dependent induction of early growth response-1, a transcription factor linked to apoptosis, was prevented by ERK inhibition. Likewise, the MEK inhibitor PD98059 prevented troglitazone-induced p21^{Cip1/WAF1} translocation to the nucleus, an event correlated with reduced cell viability in this model. Troglitazone-induced hepatotoxicity in a human liver cell line was also shown to involve JNK (Bae and Song, 2003). The ability of PPAR ligands to activate MAPKs clearly plays an important role in mediating the biological effects of these compounds.

Mechanisms of MAPK Activation by PPAR Ligands

It is noteworthy that studies with MAPK inhibitors have revealed that some of the transcriptional changes induced by PPAR agonists can be dissociated into distinct MAPK- and PPAR-dependent pathways (Baek et al., 2003). This suggests that PPAR ligand-dependent PPAR activation alone cannot account for simultaneous MAPK phosphorylation. Additional evidence also suggests that MAPK and PPAR activation by PPAR agonists are separate events. For example, ciglitazone activated ERK, p38, and JNK in astrocytes, whereas rosiglitazone, a structurally similar PPAR γ ligand, failed to induce phosphorylation of any of these kinases in this model (Len-

non et al., 2002). Although kinase activation by PPAR agonists thus seems to play an important role in the mechanism of action of these compounds, few studies have investigated the potential nongenomic mechanism responsible for MAPK phosphorylation by PPAR ligands. An understanding of this mechanism is necessary because of the widespread and clinical use of these agents. MAPK expression is also ubiquitous; thus, these ligands have the potential to induce effects in multiple cell types regardless of PPAR expression.

The Epidermal Growth Factor Receptor. A classic mechanism for ERK activation is dependent upon the epidermal growth factor receptor (EGFR), a receptor tyrosine kinase (Prenzel et al., 2001). In general, an extracellular ligand (i.e., epidermal growth factor or EGF) binds to the EGFR, leading to receptor autophosphorylation on multiple tyrosine residues, which is followed by activation of downstream kinase signaling cascades (Ullrich and Schlessinger, 1990). In addition to its role in relaying EGF-dependent signals to the cytosol, the EGFR has recently emerged as a critical transducer of intracellular signals in the absence of physiological ligands via a mechanism termed EGFR "transactivation" (Carpenter, 1999; Zwick et al., 1999). Pauley et al. (2002) noted that a nonspecific EGFR kinase inhibitor blocked ERK activation by the PPAR α agonist Wy-14,643, suggesting possible cross-talk between the EGFR and PPAR ligand-induced signaling. Furthermore, it has been proposed that EGF and PPAR α ligands act synergistically to promote the clonal expansion of hepatocytes (James and Roberts, 1994). Together, these studies suggest a potential role for the EGFR in mediating MAPK activation by PPAR ligands.

Increases in Intracellular Calcium. In addition to the EGFR, increases in intracellular calcium are also associated with MAPK phosphorylation in that a number of protein kinases are directly affected by changes in calcium homeostasis. The calcium-regulated protein kinase C family of serine/threonine kinases can activate ERK through Raf-dependent as well as Raf-independent mechanisms (Cobb and Gold-

smith, 1995). Likewise, the calcium-activated proline-rich tyrosine kinase (or Pyk2) and calcium/calmodulin-dependent kinase II (CaMKII) are known to be upstream activators of p38 in glomerular mesangial cells and neurons, respectively (Pandey et al., 1999; Sorokin et al., 2001; Takeda et al., 2004). Moreover, expression of a calcium-dependent tyrosine kinase such as Pyk2 was shown to link calcium signals to JNK activation in rat liver epithelial cells (Zohn et al., 1995). It is noteworthy that exposure of different cell types to PPAR ligands was observed to cause increases in intracellular calcium. In particular, treatment of macrophages with the PPAR α agonist Wy-14,643 led to an influx of extracellular calcium (R. G. Thurman, unpublished observations), an ability that was correlated with Wy-14,643-dependent protein kinase C activation (Rose et al., 1999). The PPAR γ ligands ciglitazone and troglitazone were recently shown to increase intracellular calcium by directly promoting its release from the endoplasmic reticulum (ER), initiating a stress response (Palakurthi et al., 2001). For a detailed summary of ER stress, see review articles by Rutkowski and Kaufman (2004) and Zhang and Kaufman (2004). This ER-associated stress response has been coupled to activation of JNK (Nishitoh et al., 2002) as well as p38 (Yamamoto et al., 2003). Thus, although the ability of these agents to mobilize calcium is clear, whether such effects are important for PPAR ligand-dependent MAPK activation remains unknown.

PPAR-Independent Activation of MAPK Signaling Pathways by PPAR Ligands in Liver Epithelial Cells

Using GN4 rat liver epithelial cells, our laboratory has recently shown that PPAR α and - γ ligands activate two distinct kinase signaling cascades that culminate in either ERK or p38 MAPK phosphorylation (Gardner et al., 2003). Consistent with earlier studies suggesting a connection between the EGFR and MAPK signaling by PPAR α ligands (Orellana

TABLE 2

Activation of MAPKs, or non-genomic effects, by various PPAR ligands in different cell model systems

Ligand	Cell System	MAPK Activated	Reference
PPARα			
Docosahexaenoic acid	Rat VSMC	p38	Diep et al., 2000
Retinoic acid	Rat adipocytes	ERK, p38	Teruel et al., 2003
Linoleic acid	Rat aortic SMC	ERK	Rao et al., 1995
WY-14,643	ML457	ERK	Rokos and Ledwith, 1997
	Primary mouse hepatocytes	ERK	Mounho and Thrall, 1999
	Primary rat hepatocytes	ERK, p38	Pauley et al., 2002
Clofibrate	ML457	ERK	Rokos and Ledwith, 1997
Nafenopin	Primary rat hepatocytes	ERK, p38	Cosulich et al., 2000
PPARγ			
15d-PGJ ₂	Human mesangial cells	ERK, no effect on p38 or JNK	Wilmer et al., 2001
	Primary rat astrocytes	ERK, p38, JNK	Lennon et al., 2002
	PC-12	P38	Jung et al., 2003
	Rat VSMC	ERK	Takeda et al., 2001
	C ₂ C ₁₂	ERK	Huang et al., 2002
Ciglitazone	Human mesangial cells	No effect	Wilmer et al., 2001
	Primary rat astrocytes	ERK, p38, JNK	Lennon et al., 2002
	GN4	ERK, p38, JNK	Gardner et al., 2003
	C ₂ C ₁₂	ERK	Huang et al., 2002
Troglitazone	HCT-15	ERK	Kim et al., 2002; Baek et al., 2003
	Rat VSMC	ERK	Takeda et al., 2001
	HepG2	p38, JNK, no effect on ERK	Bae and Song, 2003
	GN4	p38, ERK, no effect on JNK	Gardner et al., 2005
	MCF-7	ERK, p38, JNK	Yin et al., 2004
Rosiglitazone	Fetal rat adipocytes	ERK, p38	Teruel et al., 2003

15d-PGJ₂, 15-deoxy- $\Delta^{12,14}$ -prostaglandin J₂.

et al., 1993; James and Roberts, 1994; Pauley et al., 2002), inhibition of EGFR kinase activity prevented ERK activation by both PPAR α and γ agonists. In addition to PPAR α ligands, these studies also provided the first evidence that PPAR γ agonists influence ERK activity through specific phosphorylation of the EGFR. Further investigation into this mechanism of EGFR-dependent ERK activation revealed that PPAR ligands transactivate the EGFR, a process that requires Src and reactive oxygen species (Gardner et al., 2003). Although other kinases, such as protein kinase C (Shah and Catt, 2002), as well as proteolytic cleavage of diffusible EGF-like ligands by metalloproteases (Prenzel et al., 1999), have been associated with EGFR transactivation, there was no evidence to suggest that these mechanisms were important for EGFR phosphorylation in response to PPAR ligands (O. S. Gardner, unpublished observations). Other mechanisms, such as the possible role of Pyk2 and changes in intracellular calcium concentration, remain undetermined. Furthermore, expression of a dominant-negative Ras as well as inhibition of MEK1/2 with U0126 blunted PPAR agonist-induced ERK phosphorylation without an observable effect on the EGFR. Together, this evidence suggests that PPAR α and γ ligands promote Src-dependent EGFR transactivation in GN4 cells, leading to downstream phosphorylation of Ras, MEK, and ultimately ERK (Fig. 4).

In addition to ERK, PPAR α and γ ligands also activated p38 GN4 cells (Gardner et al., 2003). It is noteworthy that inhibition of EGFR signaling had no effect on the ability of these agents to induce p38 phosphorylation, suggesting that

two signaling pathways facilitate PPAR agonist-dependent MAPK activation in this model (Fig. 5). This hypothesis is supported by the observation that PPAR ligand-dependent p38 activation requires increases in intracellular calcium, CaMKII, and MKK3/6, signaling components whose inhibition conversely did not prevent EGFR and ERK phosphorylation by these compounds (Gardner et al., 2005). Further examination into the mechanism responsible for calcium-dependent p38 activation revealed that PPAR γ ligands induce ER stress in GN4 cells. Together, these data not only suggest that the ER is the likely source of calcium necessary for PPAR agonist-dependent CaMKII and downstream p38 activation but also provide novel, mechanistic evidence that induction of ER stress and p38 phosphorylation are potentially tightly coupled signaling events. In particular, both CaMKII and the ER stress-sensitive kinase PKR or double-stranded RNA-activated protein kinase were required for PPAR γ ligand-induced p38 as well as eukaryotic initiation factor 2 α (eIF2 α) phosphorylation. Furthermore, the data suggest that CaMKII may regulate PKR, which then leads to downstream phosphorylation of both p38 and eIF2 α . Future studies are needed to characterize the specific role of CaMKII in PKR activation.

MAPK activation by PPAR agonists in liver epithelial cells was both rapid and transient; the onset of kinase phosphorylation was first observed as early as 5 min after exposure to these drugs, which supports previous ideas that nuclear hormone receptor ligands have nongenomic, PPAR-independent signaling effects (Losel and Wehling, 2003). Studies using the

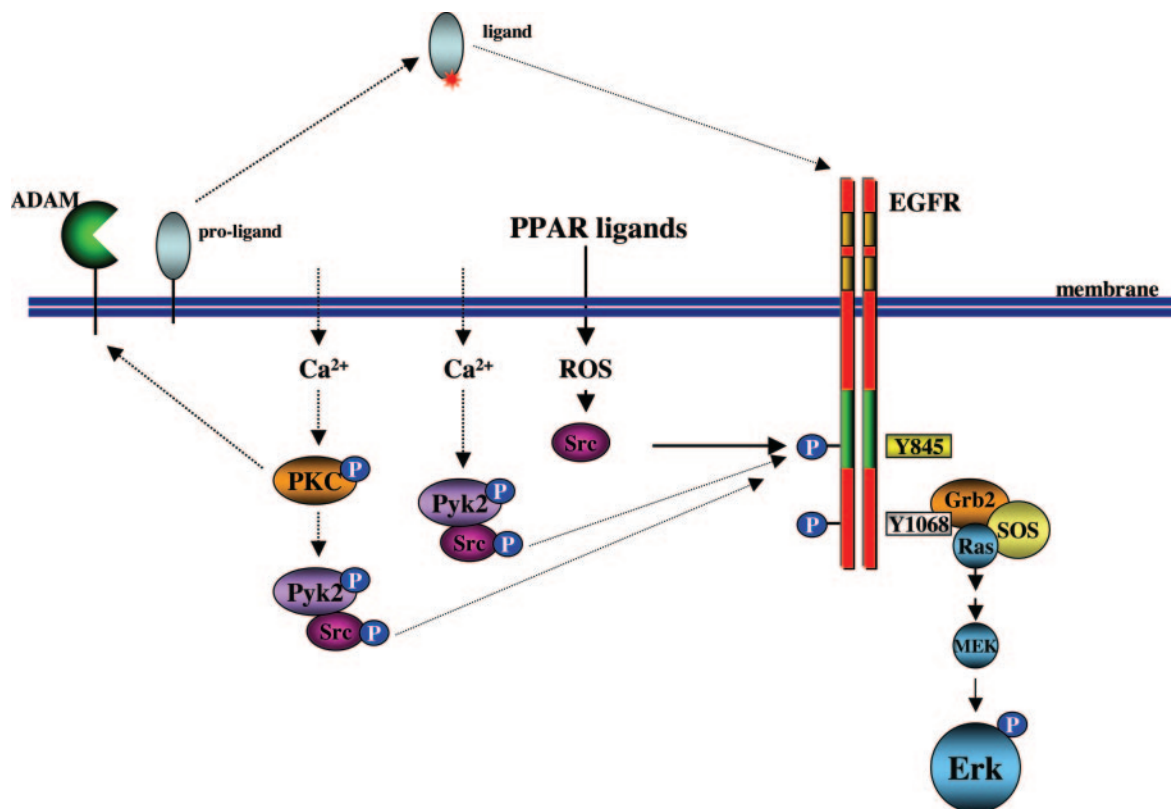


Fig. 4. Mechanisms of epidermal growth factor receptor transactivation leading to ERK activation. A diverse array of signals has been shown to induce EGFR transactivation. Work from our laboratory provides evidence for a PPAR α and γ ligand activation of ROS/Src-dependent transactivation of EGFR leading to ERK activation through Ras. There is currently no evidence to suggest that additional mechanisms such as protein kinase C activation or proteolytic generation of diffusible EGF-like ligands by metalloproteases is important for EGFR transactivation in response to PPAR ligands.

protein synthesis inhibitor cycloheximide, pharmacological antagonists of PPAR α and γ , and thiazolidinedione structural derivatives that lack receptor ligand-binding activity (Fig. 3B) ($\Delta 2$ -troglitazone, $\Delta 2$ -ciglitazone) were unable to demonstrate a necessary role for PPAR transcriptional activity in MAPK phosphorylation (Gardner et al., 2003, 2005). Moreover, the ability of certain thiazolidinediones (ciglitazone and troglitazone) but not other higher affinity PPAR γ ligands (rosiglitazone and pioglitazone) to selectively activate MAPKs in this model speaks to the PPAR-independent nature of these signaling events. Together, these data provide further evidence to support the current hypothesis that nuclear hormone receptor ligands possess unanticipated and diverse signaling capacity that must be recognized and appreciated to fully understand their mechanism of action.

Potential Biological Significance of MAPK Activation by PPAR Ligands

Because MAPKs are well known growth regulators (Johnson and Lapadat, 2002), the ability of PPAR α and γ ligands to activate members of this kinase family may be important for their effects on cell proliferation. Indeed, PPAR α agonist-dependent increases in immediate early gene expression were previously shown to require ERK (Rokos and Ledwith, 1997). Our work elaborates on this mechanism and identifies an essential role for the EGFR in triggering downstream ERK phosphorylation. In contrast to PPAR α ligands, the thiazolidinedione class of PPAR γ agonists promotes growth inhibition by a mechanism that is not well understood. It is

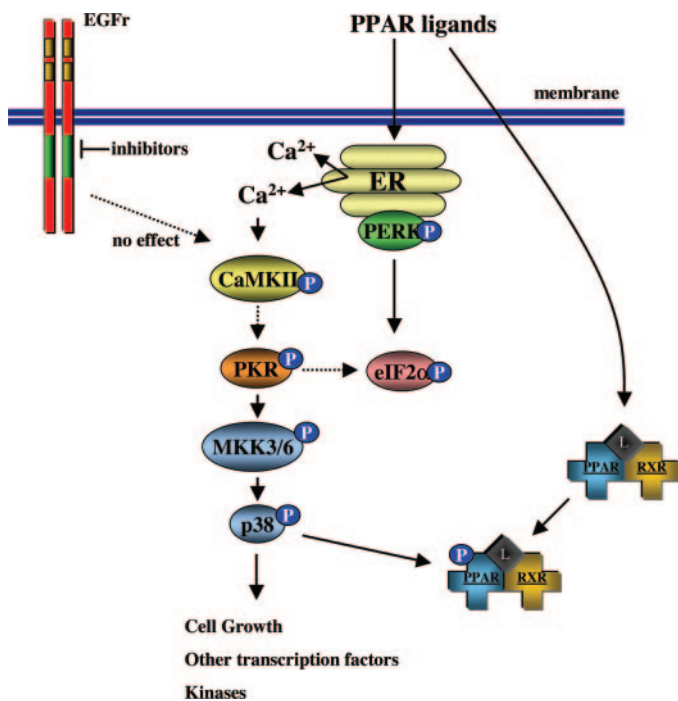


Fig. 5. Working hypothesis of thiazolidinedione-induced ER stress p38 activation. PPAR ligands stimulate the release of intracellular calcium and activation of CaMKII, followed by subsequent activation of PKR, MKK3/6, and p38. Increases in intracellular calcium correlate with PPAR ligand-induced ER stress as seen by the activation of known ER stress signals PERK and eIF2 α . PPAR ligand-induced p38 activation is not dependent upon EGFR transactivation events. Activation of MAPK by PPAR γ ligands may alter gene transcription because of phosphorylation of PPARs or by activation of downstream p38 effectors.

interesting that the ability of thiazolidinediones to promote ER stress as well as down-regulate G₁ cell cycle regulators was previously correlated with decreased cell viability (Palakurthi et al., 2001). Our work not only supports these earlier findings but also suggests that ER stress and p38 signaling are part of the same pathway in liver epithelial cells. It is thus possible to speculate that MAPKs play a role in growth inhibition by PPAR γ ligands. Future studies are necessary to determine a causal role for p38 in this mechanism.

In addition to their potential role in influencing PPAR ligand-dependent changes in cell proliferation, all three of the well known MAPK family members (ERK, p38, and JNK) are known to phosphorylate PPARs leading to changes in transcriptional activity. In particular, ERK- and p38-dependent PPAR α phosphorylation resulted in increased transcriptional activity (Juge-Aubry et al., 1999; Barger et al., 2001), whereas PPAR γ phosphorylation by MAPKs decreased transcriptional activity (Hu et al., 1996; Camp et al., 1999). These data are intriguing in that they suggest that PPAR agonists not only directly activate PPARs via ligand binding but also stimulate kinases that indirectly modulate receptor activity. This may be a universal feature for a number of nuclear receptors; extensive work has shown that in addition to the ability of estrogen to bind and activate the estrogen receptor, activation of MAPKs or coactivators occurs, thereby amplifying the transcriptional activity of the estrogen receptor (Coleman and Smith, 2001). The identification of genes up- or down-regulated in response to PPAR phosphorylation by MAPKs, as well as how MAPK-dependent changes in receptor activity influence the biological effects of PPAR ligands, requires further study. This presents an exciting new area of research that has been only initially explored in the literature. In light of the current studies, it would be of interest to determine whether the mechanisms of MAPK activation by PPAR agonists described herein also contribute to MAPK-dependent PPAR phosphorylation.

Conclusions

The ability of PPAR ligands to activate MAPK signaling pathways is an attractive hypothesis to explain, at least in part, how these agents induce nongenomic effects. Yet additional studies provide evidence that the nongenomic actions of PPAR ligands are not limited to influencing MAPK activity. Thiazolidinediones inhibit the production of inflammatory mediators in both wild-type and PPAR γ -deficient macrophages (Chawla et al., 2001) and inhibit pancreatic cell invasiveness via mechanisms that involve matrix metalloproteases and plasminogen activator inhibitor-1 as opposed to PPAR γ (Galli et al., 2004). Both troglitazone and ciglitazone and the $\Delta 2$ -derivatives were recently shown to specifically ablate cyclin D1 protein in breast cancer cells (Huang et al., 2005). Likewise, $\Delta 2$ -ciglitazone and $\Delta 2$ -troglitazone induced apoptosis of PPAR γ -expressing (PC-3) and PPAR γ -deficient (LNCaP) prostate cancer cell lines through effects on Bcl-x1 and Bcl-2 (Shiau et al., 2005). Thus, it is becoming clear that PPAR ligands have the potential to induce a variety of different effects in multiple cell types, irrespective of their ability to act as nuclear hormone receptor agonists. An understanding of these additional PPAR-independent signaling pathways is necessary because these mechanisms would

support the use of PPAR ligands not only for treating metabolic disorders but also as therapies for inflammation and cancer.

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