Focus Article

Cannabinoid activation of peroxisome proliferator-activated receptors: an update and review of the physiological relevance

Saoirse Elizabeth O’Sullivan

Since 2002, evidence has been building that cannabinoids, including endocannabinoids and endocannabinoid-like compounds, phytocannabinoids and synthetic cannabinoid ligands, bind to and activate the different isoforms of the nuclear receptors, peroxisome proliferator-activated receptors (PPARs; \( \alpha \), \( \beta \), and \( \gamma \)). This has been shown through the use of reporter gene assays, binding studies, the use of antagonists and knockout animals. Increasing use of tools to assess a potential role for PPAR activation in underpinning the physiological effects of cannabinoids means that a picture is emerging of the relevance of PPAR activation by cannabinoids. There is now evidence that activation of PPAR\( \alpha \) and \( \gamma \) mediate some of the anti-inflammatory, analgesic, neuroprotective, and cardiovascular effects of cannabinoids, sometimes in combination with activation of the more traditional target sites of action such as CB\(_1\), CB\(_2\), and TRPV1. There is also a role for PPAR\( \alpha \) activation by cannabinoids in some of their central effects including memory acquisition, reward processing, food intake and body weight regulation. Activation of PPAR\( \gamma \) plays a role in the apoptotic effects of cannabinoids. However, much further work is required to fully establish the profile of cannabinoid compounds at all isoforms of the PPAR family and the relevance of this in normal physiology and pathological situations. © 2013 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

INTRODUCTION

Peroxisome proliferator-activated receptors (PPARs, three isoforms: \( \alpha \), \( \delta \), and \( \gamma \)) are a family of nuclear receptors which heterodimerise with the retinoid X receptor (RXR), and bind to DNA sequences called PPAR response elements (PPRE), leading to changes in the transcription of target genes. Ligand binding to PPARs causes the recruitment of regulator proteins that bind to a third site on PPARs, and these are thought to modulate transactivation. PPAR target genes are primarily involved in the regulation of metabolism and energy homeostasis, cell differentiation and inflammation. The extensive research on PPARs, including their role in disease modulation, has been reviewed elsewhere.\(^1\)\(^-\)\(^4\)

PPARs have large ligand binding domains and are relatively promiscuous receptors, capable of being activated by a large number of natural and synthetic ligands of different chemical structure. Endogenous activators of PPARs include the unsaturated fatty acids linolenic acid, linoleic acid, petroselenic acid and arachidonic acid, with EC\(_{50}\) values in the 2–20 \( \mu M \) range.\(^5\) The eicosanoids 15-deoxy-A\(_{12,14}\)-prostaglandin J\(_2\) (15d-PGJ\(_2\)) and 8S-HETE interact with PPARs with an EC\(_{50}\) of around 500 nM.\(^5\) By contrast, most synthetic ligands of PPARs have EC\(_{50}\)sin the low nanomolar range.\(^6\)

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As endocannabinoids are fatty acid derivatives, it is not surprising that an increasing body of evidence has shown that endocannabinoids and other cannabinoid compounds also activate PPARs. This review will present the literature implicating a role for cannabinoid activation of PPARs and discuss the physiological effects of cannabinoids which might be mediated through PPAR activation.

REVIEW OF THE EVIDENCE OF PPAR ACTIVATION BY CANNABINOIDS

A summary of the current data supporting the activation of PPAR nuclear receptors by cannabionoids is provided in Table 1 (PPARα) and Table 2 (PPARγ). These tables do not include studies where a role for endocannabinoid activation of PPARs has been implicated after administration of fatty acid amide hydrolase (FAAH) inhibitors\(^7\text{–}\text{10}\) or endocannabinoid uptake inhibitors\(^11,12\) to increase local endocannabinoids levels, but where the activating ligand has not been specifically identified.

### PHYTOCANNABINOIDS

The phytocannabinoids \(\Delta^9\text{-tetrahydrocannabinol (THC)},\) cannabidiol (CBD) and ajulemic acid (a synthetic analogue of a tetrahydrocannabinol metabolite, AJA) can all bind to, increase the transcriptional activity of, and have effects that are inhibited by an antagonist of PPAR\(\gamma\) (see Table 2). By contrast, two studies investigating the potential activity of phytocannabinoids at PPAR\(\alpha\) found that THC does not bind to PPAR\(\alpha\);\(^13\) and that AJA does not bind to or activate PPAR\(\alpha\).\(^31\) Few studies have investigated the effects of other novel phytocannabinoids on PPAR activity, but we showed that tetrahydrocannabinol (THCV) does not increase the transcriptional activity of PPAR\(\gamma\).\(^33\) Thus activation of PPAR\(\gamma\) is not universal to all phytocannabinoids, and as yet, there is no evidence that phytocannabinoids activate PPAR\(\alpha\).

### ENDOCANNABINOIDS

There is now strong evidence that the endocannabinoid-like compounds oleoylethanolamine (OEA)
### TABLE 2 | Evidence for CB Activation of PPARγ

<table>
<thead>
<tr>
<th>Phyto- and Endocannabinoids</th>
<th>Binding Studies</th>
<th>Reporter Gene Assay</th>
<th>Use of Antagonists</th>
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<tbody>
<tr>
<td><strong>Phytocannabinoids</strong></td>
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<tr>
<td>THC</td>
<td>nd</td>
<td>O’Sullivan et al.32</td>
<td>O’Sullivan et al.32,33 and Carroll et al.34</td>
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<tr>
<td>CBD</td>
<td>O’Sullivan et al.35</td>
<td>O’Sullivan et al.35</td>
<td>O’Sullivan et al.35, Esposito et al.36 and De Filippis et al.37</td>
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<tr>
<td>AYA</td>
<td>Liu et al.31 and Ambrosio et al.38</td>
<td>Liu et al.31</td>
<td>Gonzalez et al.39</td>
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<tr>
<td><strong>Endocannabinoids</strong></td>
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<tr>
<td>AEA</td>
<td>Bouaboula et al.40</td>
<td>Bouaboula et al.40</td>
<td>Rockwell and Kaminski.41, Bouaboula et al.40 and O’Sullivan et al.42</td>
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<tr>
<td>Methanandamide</td>
<td>nd</td>
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<td>Eichele et al.43</td>
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<td>2-AG</td>
<td>nd</td>
<td>Rockwell et al.44</td>
<td>Rockwell et al.44 and Du et al.45</td>
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<tr>
<td>NADA</td>
<td>nd</td>
<td>O’Sullivan et al.46 (A)</td>
<td>O’Sullivan et al.42</td>
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<tr>
<td>OEA</td>
<td>nd</td>
<td>O’Sullivan et al.46 (B)</td>
<td>nd</td>
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<tr>
<td>PEA</td>
<td>nd</td>
<td>O’Sullivan et al.46 (C)</td>
<td>Costa et al.47</td>
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<tr>
<td><strong>Synthetic compounds</strong></td>
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<tr>
<td>WIN55,212</td>
<td>nd</td>
<td>O’Sullivan et al.46</td>
<td>Mestre et al.48 and Giuliano et al.49</td>
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<td>CP55,950</td>
<td>nd</td>
<td>O’Sullivan et al.46</td>
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</table>

A, Kozak et al.15 did not find 2-AG to activate the transcriptional activity of PPARγ; B, Fu et al.16 showed OEA does not to bind to or activate PPARγ; C, Lo Verme et al.42 showed PEA does not increase the transcriptional activity of PPARγ; nd, no known data.

and palmitoylethanolamide (PEA) activate PPARα, as shown through binding studies, reporter gene assays, the use of antagonists and also the absence of responses to these compounds in PPARα knockout mice (see Table 1). Recently, it has been shown that OEA is transported to the nucleus and PPARα by fatty acid binding proteins.\(^{50}\) 2AG, noladin ether and virodhamine have also been implicated in PPARα activation, and some, but not all studies, have shown that anandamide (AEA) activates PPARα, (see Table 1). This suggests, thus far, that most endocannabinoids are activators of this isoform of the PPAR family.

Despite the fact that AEA binds to both PPARα\(^{13}\) and PPARγ,\(^{40}\) few studies have probed this as a mechanism of action for the physiological effects of AEA, which is also true for 2-AG. Lesser known endocannabinoids such as N-arachidonyl-dopamine (NADA), virodhamine and noladin ether might also have activity at PPARs,\(^{13,42}\) but again, this requires further investigation to establish how much of the effects of endocannabinoids other than OEA and PEA are mediated by PPARs.

Some studies have shown that metabolites of 2-AG are PPAR activators. Raman and colleagues\(^{51}\) showed that 15-deoxy-\(\Delta^{12,31}\)-PGJ(2)-glycerol ester activates PPARγ in a reporter gene assay, and Kozak and colleagues\(^{15}\) showed that 15-hydroxyeicosatetraenoic acid glyceryl ester increases the transcriptional activity of PPARα. Whether the same is true of metabolites of other endocannabinoids remains to be established.

It is worth pointing out that a study by Lenman & Fowler\(^{52}\) showed that PPARγ ligands inhibited FAAH activity in rat brain homogenates and glioma cells. Thus part of the physiological effects of PPAR agonists may be through upregulation of local endocannabinoid levels by inhibiting their degradation.

### SYNTHETIC CANNABINOIDS

Few studies have investigated the potential for synthetic cannabinoid compounds to activate PPARs. WIN55212 has been shown to bind to and activate the transcriptional activity of both PPARα and PPARγ, and to have effects that are antagonized by PPARγ antagonists (see Tables 1 and 2). CP55,950 also increases the transcriptional activity of PPARγ.\(^{33}\)
PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS δ

PPARδ is the least investigated of the three PPAR isoforms and there is little information on the effects of cannabinoids at PPARδ. Fu et al. showed that OEA activates the transcriptional activity of PPARδ, but no further studies have examined this. AJA, 2-AG metabolites, and PEA do not activate PPARδ. However, there may be some interactions between the endocannabinoid system and PPARδ, as Yan et al. showed that silencing PPARδ significantly increases CB1 receptor expression, and that over expression of PPARδ significantly reduces CB1 receptor expression.

PHYSIOLOGICAL RESPONSES TO CANNABINOIDS MEDIATED BY PPARs

Since the first indication that cannabinoids can interact with PPARs, an important task has been to establish how much of the physiological effects of cannabinoids are mediated through activation of nuclear receptors, particularly since their affinity for PPARs tends to be in the micromolar range (although this is not dissimilar to the affinity of other endogenous ligands, see Ref 5). Fortunately, many cannabinoid-based studies have now included the use of tools to assess a role for PPAR activation. Below is a summary of the evidence for PPAR activation as a mechanism of action for cannabinoids in some of the more commonly recognized physiological effects of cannabinoids; anti-inflammatory actions, neuroprotection, analgesia, and effects on memory reward, the cardiovascular system and metabolism.

ANTI-INFLAMMATORY EFFECTS

One study has shown that the anti-inflammatory effects of CBD on intestinal inflammation in lipopolysaccharide (LPS)-treated mice are PPARγ-mediated. The anti-inflammatory effects of AJA are also suggested to be a result of PPARγ activation since AJA inhibits the promoter activity of the pro-inflammatory cytokine, interleukin (IL)-8, in a PPARγ-dependent manner. However, other studies report that the anti-inflammatory effects of AJA are not PPARγ mediated. The effects of AJA could be due to increased production of lipoxin A4, the endogenous pro-resolving and anti-inflammatory eicosanoid. More recently, AJA was found to inhibit skin fibrosis in mice overexpressing transforming growth factor β, which was sensitive to PPARγ antagonism.

ROCKWELL & KAMINSKI found that AEA inhibits the secretion of the pro-inflammatory cytokine, IL-2, in a CB1/CB2 receptor-independent manner that could also be prevented by a PPARγ antagonist. 2-AG also inhibits IL-2 secretion through the suppression of pro-inflammatory transcription factors, sensitive to PPARγ antagonism. More recently, 2-AG was found to decrease the expression of COX-2 in response to IL1β or LPS, which were sensitive to PPARγ antagonism. The 2-AG metabolite 15-deoxy-Δ12,31-PGJ(2)-glycerol ester also has anti-inflammatory actions mediated by PPARγ, so the effects of 2-AG on PPARγ may be due to both activation by 2-AG itself and/or by its metabolites.

Both OEA and PEA have anti-inflammatory actions in 12-O-tetradecanoylphorbol-13-acetate-induced and carrageenan-induced edema that were absent in PPARα knock-out mice. Similarly, PEA decreases intestinal inflammation induced by ischemia/reperfusion injury that was reduced in PPARα knock-out mice. However, PEA has also been shown to have a protective role in a model of contact dermatitis that was mediated by TRPV1 but not PPARα.

Upregulation of local endocannabinoid levels by either FAAH inhibition (URB597) or inhibition of the AEA transporter (AM404) significantly potentiated the circulating cytokine response to LPS in rats, and this effect was sensitive to antagonism of CB1, CB2, TRPV1 and PPARγ.

Thus activation of both PPARγ (AJA, CBD, AEA, and 2-AG) and PPARα (OEA and PEA) underpins some of the anti-inflammatory effects of cannabinoids.

NEUROPROTECTION

OEA reduces infarct volume after cerebral artery occlusion in mice, which is absent in PPARα knock-out mice. Similarly, Zhou et al. showed that OEA improves neurological dysfunction, reduces infarct size and brain edema after cerebral artery occlusion, which is inhibited by PPARα antagonists. Importantly, this effect could be observed when OEA was administered within 1 h of reperfusion. Several studies have now shown that PEA also has neuroprotective effects that are mediated by PPARα; PEA protects against excitotoxicity in hippocampal cultures which is blocked by a PPARα antagonist, but not PPARγ antagonist, blunts the expression of pro-inflammatory molecules in response to β-amyloid in astrocytes in a PPARα-dependent, PPARγ-independent manner, and decreases infiltrating astrocytes in hippocampal...
slices treated with β-amyloid, sensitive to PPARα, but not PPARγ, antagonism.25

CBD also protects against β-amyloid neurotoxicity in rats, but this is through activation of PPARγ.36 THC similarly has neuroprotective effects in a cell culture model of Parkinson’s disease that was not inhibited by CB1, but was inhibited by a PPARγ antagonist.34 In a model of multiple sclerosis, increasing local levels of endocannabinoids by inhibiting their uptake using UCM707, had neuroprotective effects against excitotoxicity which could be inhibited by CB1, CB2, and PPARγ antagonism.12

Thus activation of both PPARγ (CBD, THC, and endocannabinoid upregulation) and PPARα (OEA and PEA) underpins some of the neuroprotective effects of cannabinoids, in combination with activation of more traditional sites such as CB1 and CB2. Several studies have shown that the neuroprotective effects of PEA are not mediated by PPARγ.

**ANALGESIA**

PEA has analgesic effects in vivo in several different models of pain behavior, which are absent in PPARα knock-out mice.22,30 A PEA analog, palmitoylallylamide, reduces hypersensitivity in neuropathic pain that was inhibited by CB1, CB2, and PPARα antagonists.58 By contrast, Costa et al.47 found that the analgesic effects of PEA, also in a model of neuropathic pain, involved CB1,TRPV1, and PPARγ (but not PPARα or CB2), and de Novellis et al.26 found that the analgesic effects of centrally administered PEA are mediated by CB1,TRPV1 and PPARγ (PPARγ not examined).

Upregulation of local endocannabinoid levels by inhibition of FAAH with URB597 induces analgesia in an inflammatory pain model, and this was inhibited by a PPARα antagonist but not a CB1 antagonist or a PPARγ antagonist.8 In the Jhaveri study, URB597 increased local levels of AEA and 2-AG, so either ligand could be activating PPARα, although there is possibly more evidence to suggest that AEA is the ligand responsible (see Table 1). Interestingly, Jhaveri and colleagues also showed that COX2 inhibition increased local PEA levels and caused analgesia that was inhibited by a PPARα antagonist. Another FAAH inhibitor, ST4070, reduces neuropathy, increases AEA and 2-AG levels, and is sensitive to CB1,TRPV1, and PPARα antagonism.59

In summary, activation of both PPARγ (PEA) and PPARα (PEA, upregulation of local endocannabinoids) underpins some of the analgesic effects of cannabinoids, sometimes in addition to activation of CB1, CB2, and TRPV1.

**APOPTOSIS**

In many different cancer cell lines, WIN55212 (hepatoma HepG2 cells49) and methanandamide (cervical carcinoma cells (HeLa and C33A) and lung carcinoma cells (A549)45) induce apoptosis which can be inhibited with a PPARγ antagonist or by silencing of PPARγ. However, the anti-proliferative effect of the endocannabinoid noladin ether on prostate carcinoma cells was not found to be PPARγ-mediated.60

**MEMORY**

Mazzola et al.9 showed that memory acquisition in rats is enhanced by the FAAH inhibitor URB597, which was sensitive to PPARα antagonism. Campologno et al.20 showed that OEA administration also has a central memory enhancing effect which was absent in PPARα-null mice. More recently, chronic PEA administration was found to protect against the memory deficits induced by β-amyloid in an Alzheimer’s disease model, which was absent in PPARα-null mice.29 Together, these studies suggest that OEA, PEA and elevated endogenous levels of AEA have positive effects on memory through PPARα activation.

**REWARD**

Upregulation of local endocannabinoids by URB597, or administration of OEA and PEA, inhibits neuronal responses in the reward area of the brain to nicotine (but not cocaine or morphine61), which was sensitive to both CB1 and PPARα antagonism.17,61 Interestingly, some of the effects of OEA and PEA mediated by activation of PPARα were through non-genomic stimulation of tyrosine kinases.17 A similar effect on nicotine reward mediated by PPARα was seen by the same authors in response to methyl OEA, a long-lasting form of OEA, or to PPARα agonists.62

**CARDIOVASCULAR SYSTEM**

We have shown that THC causes time-dependent, PPARγ-dependent vasorelaxation in rat isolated arteries (the aorta and superior mesenteric artery) that is dependent on nitric oxide (NO) and hydrogen peroxide (H2O2) production, and superoxide dismutase (SOD) activity.32 Furthermore, in vitro THC enhances vasodilator responses in isolated arteries, which was also inhibited by a PPARγ antagonist.46 A similar time-dependent and PPARγ sensitive vasorelaxant response was seen in response to CBD35 and the endocannabinoids AEA and NADA, but not PEA.63
Interestingly, Romano and Lograno recently showed a similar time-dependent vasorelaxant response to AEA and PEA in the bovine ophthalmic artery that could be inhibited by a PPARα, but not PPARγ, antagonist.

In a viral model of multiple sclerosis, WIN55212 suppressed the increase in intercellular adhesion molecule (ICAM) and vascular cell adhesion molecule (VCAM) in brain endothelium, sensitive to PPARγ antagonism, but not CB1 or CB2 antagonism. In a similar study, an analogue of OEA (OPA) decreased the expression of VCAM and ICAM and monocyte adhesion in response to inflammation in human umbilical endothelial cells, and this appeared to be mediated by PPARα.

In summary, activation of PPARγ (CBD, THC, AEA, NADA, WIN55212) and PPARα (OPA, AEA, PEA) in vascular cells partly mediates the positive effects of cannabinoids in the vasculature.

METABOLISM

Fu et al. showed that the appetite-suppressing and weight-reducing effects of OEA were absent in PPARα knock-out mice, and that daily treatment with OEA reduced serum cholesterol levels in rat and mouse models of obesity. Guzman et al. also showed that the stimulatory effect of OEA on lipolysis in vivo was absent in PPARα knock-out mice. A number of structural analogues of OEA with a high affinity for PPARα cause similar reductions in food intake. More recent work has shown that the anorexic effects of OEA are mediated centrally by oxytocin signaling, which was absent in PPARα knock-out mice. Surprisingly, no studies have examined whether the metabolic effects of cannabinoids other than OEA are mediated by activation of PPARs.

NO ROLE FOR PPAR-ACTIVATION

There are several studies which have not found a role for PPAR-activation to underpin the effects of cannabinoids in their experimental model. For example, we have not found a role for either PPARα or PPARγ in mediating the effects of THC, CBD, AEA, 2-AG on intestinal permeability. Similarly, the effects of OEA in inhibiting upper GI transit or in reducing gastric emptying are not mediated by PPARα. Some behavioral effects of AEA and methanandamide are not mediated by PPARα.

CONCLUSION

This review of the current literature suggests that many of the well recognized physiological responses to cannabinoids are at least partly mediated by the activation of PPARs, but this area still requires further research. There are still many cannabinoids compounds whose activity at PPAR remains unknown. For example, there is little known about the effects, if any, of phytocannabinoids on PPARα, there is still almost no data on any potential role for the activation of PPARδ by either endo- or phytocannabinoids, and the effects of some of the lesser known endocannabinoids at PPARs are not known. The majority of evidence for a role for a physiological response via PPAR activation by cannabinoids comes from studies using OEA and PEA, primarily at PPARα, but further work is required to establish how much of the effects of AEA and 2-AG are mediated by nuclear receptors. From a therapeutic point of view, the beneficial effects of PPAR ligands are well recognized in a range of disorders including type II diabetes, cancer, hyperlipidemia, atherosclerosis, metabolic syndrome, neurodegenerative disorders and nicotine addiction. However, some drugs which target PPARs such as the thiazolidinediones (TZDs), have been associated with severe side effects including heart failure leading to their withdrawal from the market. The excellent tolerability profile of cannabinoids, combined with their known activity at PPARα and γ, warrants further investigation into their potential utility in diseases where PPARs are known to be beneficial.

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