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Anandamide induced PPARγ transcriptional activation and 3T3-L1 preadipocyte differentiation

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Abstract

We investigated the effects of anandamide on peroxisome proliferator-activated receptor γ (PPARγ) activity. In two different transactivation systems using either full-length or only the ligand binding domain of PPARγ, we showed that anandamide, but not palmitoylethanolamide induced transcriptional activation of PPARγ in a dose dependent manner with an EC_{50} of 8 μM. In addition, competition binding experiments showed that anandamide but not palmitoylethanolamide binds directly to PPAR-ligand binding domain. We also found that anandamide treatment induced 3T3-L1 fibroblast differentiation into adipocytes. Indeed, anandamide induced triglyceride droplet accumulation and the expression of PPARγ responsive genes such as CCAAT enhancer binding protein α (C-EBPα), aP2, PerilipinA and Acrp30. Furthermore, the PPARγ antagonist (GW9662) inhibited the anandamide-induced 3T3-L1 differentiation confirming that this is a PPARγ-mediated process. Altogether, these data indicate that anandamide binds PPARγ and induces cellular PPARγ signaling.
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1. Introduction

Anandamide is a polyunsaturated fatty acid amide endocannabinoid (Devane et al., 1992; Di Marzo et al., 1999) which was initially found to act as a mediator in the brain and in peripheral tissues, mainly through the stimulation of brain (cannabinoid CB_1 receptor) and peripheral (cannabinoid CB_2 receptor) cannabinoid receptors. Anandamide is produced through hydrolysis of the phospholipid precursor *N*-arachydonoyl-phosphatidylethanolamide, which is catalyzed by a Ca^{2+}-dependent phospholipase D (Di Marzo et al., 1994). Anandamide signal termination involves cellular reuptake by the anandamide membrane transporter followed by its hydrolysis by fatty acid amide hydrolase, (Di Marzo et al., 1994; Di Marzo et al., 1999), a process that produces arachidonic acid and ethanolamine. In addition to hydrolysis, anandamide can be oxidized by various lipoxygenases and cyclooxygenase-2 (COX-2), leading to ethanolamide analogs of hydroxyeicosatetraenoic acid (HETEs) and prostaglandins (prostamides), respectively (Burstein et al., 2000). Anandamide can also activate vanilloid VR_1 receptor (Zygmunt et al., 1999; Smart et al., 2000), T-type Ca^{2+} or K^+ Task-1 ion channels (Chemin et al., 2001; Maingret et al., 2001).

Over the past few years, it has been shown that anandamide exerts cannabinoid CB_1 receptor- and vanilloid VR_1 receptor-independent biological activities (Di Marzo et al., 1999; Di Marzo et al., 2000a,b; Berdyshev et al., 2001). In particular, anandamide has been reported to induce cell growth inhibition and apoptosis independently of these receptors (Sarker and Maruyama, 2003). The

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involvement of cannabinoid or vanilloid receptors in the anti-inflammatory effect of anandamide is still under debate (Berdyshev et al., 1997). The mechanism used by anandamide to inhibit tumor necrosis factor alpha-induced nuclear factor kappa B (NFκB) activation is still unknown (Sancho et al., 2003).

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor superfamily. They function as heterodimers with the receptor of 9-*cis*-retinoic acid (RXR, NR2B), and bind to specific peroxisome proliferator response elements (PPREs) to regulate transcription of their target genes. Three different PPAR isoforms have been characterized (α or NR1C1, δ or NRC1C2 and γ or NR1C3) (Desvergne and Wahli, 1999). PPARγ was originally shown to play an important role in adipocyte differentiation, glucose homeostasis and insulin resistance, and it is now known to negatively regulate cellular proliferation and apoptosis as well as inflammatory responses (Nencioni et al., 2003; Berger and Moller, 2002). Indeed, PPARγ activators were found to inhibit the activation of inflammatory response genes by negatively interfering with NFκB signaling pathways (Ruan et al., 2003).

An analysis of the crystal structure of the PPAR ligand-binding domain revealed a large hydrophobic cavity for ligand binding (Uppenberg et al., 1998). Thus a wide range of synthetic and naturally occurring substances bind to and activate PPAR. The main natural ligands are fatty acids such as polyunsaturated fatty acids (Kliewer et al., 1997; Yu et al., 1995). PPAR could be activated by arachidonic acid-metabolites generated by the cyclooxygenase and lipoxygenase pathways since 15-HETE was found to be a specific agonist for PPARγ (Huang et al., 1999), while 15-HETE-glyceryl ester (lipoxygenase metabolite of 2-arachidonylglycerol) was found to transactivate PPARα (Kozak et al., 2002). Furthermore, oleylethanolamide, structurally related to anandamide, binds and activates PPARα (Fu et al., 2003; Guzman et al., 2004).

The structural and functional similarities between anandamide and PPAR ligands prompted us to investigate whether anandamide could activate PPARγ. In the present study, we show for the first time that anandamide induces transcriptional activation of PPARγ and differentiation of 3T3-L1 pre-adipocytes.

2. Materials and methods

2.1. Materials

Rosiglitazone, pioglitazone, troglitazone, GW9662, insulin, dexamethasone and Oil-Red O were purchased from Sigma (St. Louis, MO, USA). Anandamide , 2-arachidonylglycerol (2-AG) and palmitoylethanolamide were obtained from Tocris (Bristol, UK). Anti-CCAAT enhancer binding protein α (C-EBPα) antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The anti-Acrp30 antibody was from Chemicon (Temecuta, CA USA). The anti-PerilipinA antibody was from ABR (Golden, CO, USA) and anti-GAPDH was from ABCAM (Cambridge, UK).

2.2. Tissue Culture

3T3-L1, HeLa, Cos and CV1 (monkey kidney) cells were from the American Type Culture Collection. 3T3-L1 cells were maintained as fibroblasts, and differentiated into adipocytes as described previously (Rocchi et al., 2001).

2.3. Transfections and luciferase assays

UAS-TK-Luciferase, PPRE-TK-Luciferase, BD Gal4-PPARγ DE, and cDNAs for β-gal and PPARγ have been described previously (Rocchi et al., 2001). Lipofectamine (Invitrogen, Carlsbad, CA, USA) was used to transfect cells and luciferase and β-galactosidase activity were measured as described (Rocchi et al., 2001). Briefly, cells were exposed to a mix containing 300 ng/ml of either PPRE-TK-Luciferase or UAS-TK-Luciferase, 300 ng/ml of the indicated expression plasmid (PPAR-GAL4 or PPARγ cDNA) and 1.0 ng/ml pCMV-β-Gal in Opti-MEM (Invitrogen). The transfection mix was replaced, after 4–5 h, with 10% charcoal-stripped fetal bovine serum-containing medium supplemented with either 0.1% vehicle or the indicated compound. After 24–36 h, cells were harvested and luciferase activity was measured and normalized to the β-galactosidase activity. Measurements of the protein content indicated that there was no toxicity induced by these treatments.

2.4. Fluorescence polarization assay

Fluorescence polarization was measured using the PolarScreen™ PPAR competitor assay kit (Invitrogen). Briefly, the competitors (anandamide, 2-AG, palmitoylethanolamide, rosiglitazone and GW9662) were diluted in reaction buffer in a black 96-well plate (Thermo Electron Corporation, Vantaa, Finland) and then purified PPARγ-ligand binding domain and fluorescent PPAR ligand (Fluormone™ PPAR Green) were added. The samples were incubated at room temperature for 2 h and fluorescence polarization was measured using Envision™ (Perkin Elmer, Boston, MA, USA) with an excitation wavelength of 485nm and emission wavelength of 535nm.

2.5. 3T3 pre-adipocyte differentiation

3T3-L1 pre-adipocytes were grown to confluence in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum, 100 units/ml of penicillin, and 100 μg/ml of streptomycin, as described previously. Two days after confluence (at a post-confluent stage), adipogenesis was induced by treating cells with various concentrations of anandamide in the presence of 5 μg/ml insulin and 0.5 μM dexamethasone. After 15 days of differentiation, the cells were stained with Oil Red O (Inazawa et al., 2003), an indicator of cell lipid content. In parallel, the cells were harvested for reverse transcriptase-polymerase chain reaction (RT-PCR) or immunoblotting analysis as described in Bouaboula et al., 1999.

2.6. RNA extraction, cDNA synthesis and Real-time RT-PCR analysis

Experiments were carried out as previously described (Winer et al., 1999). Human brain RNA was from Invitrogen. The target

Table 1
Primers and probe sequences

Gene	Forward primer	Reverse primer	FAM-Taqman probe
CB_1	ACCTGCGACACGCTTTCC	TCCCCCATGCTGTTATCCA	CCCTCTTGTGAAGGCACTGCGCA
CB_2	ACAGCCTCTGTGGGTAGCCT	AGAGGACCCACATGATGCC	CTGCTGACCGCCATTGACCGAT
Fatty acid amide hydrolase	ACGAGATCGAGGTGTACCGC	CACCACATCCAGGTCCAGC	AACCGTGATTGCCCAGTGGAGGG
Arachidonate lipoxygenase 12	GGTCCCTAATGCTCCATGCA	CCATCGTCACATCTTCCTTGG	ATGCGGATGCCCCCACCCAC
Arachidonate Lipoxygenase 15	CCACCAGGCTTCTCTCCAGA	CCATAACGGGCTGGCGT	TCCATCACTTGGCAGCTGGGCA
COX2	GAATCATTCACCAGGCAAATTG	TCTGTACTGCGGGTGGAACA	TGGCAGGGTTGCTGGTGGTAGGA
Monoglyceride lipase	CTCAACCTTGTGCTGCCAAA	CCGAGAGAGCACGCTGGA	TTGTCCCTCGGGCCCATCGA
VR_1	GAAGGGAATGACGCCGCT	ATATAGGCCAAGACCCCGATCT	CTCTGGCAGCTGGGACCGGG

transcripts were amplified in ABI PRISM 7700 sequence detector system (Applied Biosystems, Applera, Courtaboeuf, France) by introducing the forward and reverse primers and TaqMan probes designed by Primer Express software (Applied Biosystems) as shown in Table 1. In each sample, the expression of human β2-microglobulin housekeeping gene as well as the genes for Acrp30, aP2, Cannabinoid CB_1 and CB_2 receptors, C-EBPα, COX-2, fatty acid amide hydrolase, lipoprotein lipase, lipoxygenase-12, lipoxygenase-15, monoglyceride lipase and PPARγ were assessed by using TaqMan Gene Expression Assay Reagents containing primer/probe sets purchased from Applied Biosystems.

3. Results

3.1. Anandamide-induced PPARγ transcriptional activation

We used different approaches to evaluate the effect of anandamide on PPARγ activity. In the first approach, a PPAR-responsive reporter gene (PPRE- J3TK-Luciferase) was transiently cotransfected with an expression vector containing the full-length PPARγ protein in CV1 cells. Treatment with 30 μM anandamide resulted in a 3-fold increase in luciferase activity (Fig. 1A), as compared to a 7- fold increase obtained with rosiglitazone, a known PPARγ agonist (Lehmann et al., 1995).

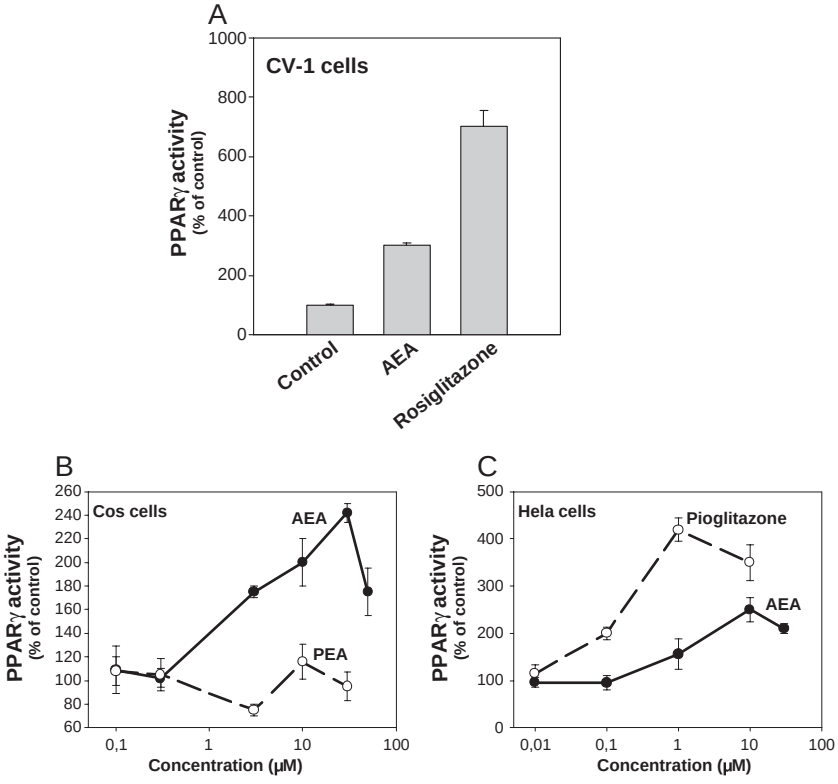

Fig. 1. Transcriptional activation of PPARγ: A. CV-1 cells were transiently cotransfected with PPARγ expression vector and the PPRE-TK-Luciferase reporter. Cells were then treated with vehicle or 30 μM anandamide or 1 μM rosiglitazone for 24 h before harvesting and luciferase assays. B. Cos cells were transiently cotransfected with vector expression for fusion protein of the Gal4 DNA binding domain and ligand binding domain of PPARγ (Gal4-PPARγ) and the Gal4 reporter vector (UAS-TK-Luc). Cells were then treated with vehicle or various anandamide or palmitoylethanolamide concentrations. Luciferase assays were carried out 24 h later. C. HeLa cells stably expressing the Gal4-PPARγ fusion protein and Gal4 reporter (UAS-TK-Luciferase) were treated with vehicle or increasing anandamide concentrations for 24 h before read-out of the luciferase signal. The vehicle was taken as 100%. No toxicity was observed for high anandamide or palmitoylethanolamide concentrations. These results are representative of three independent experiments.

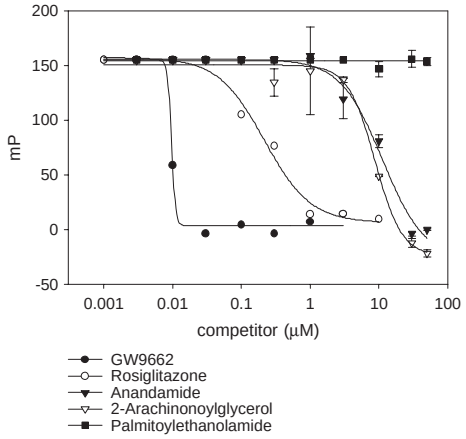

Fig. 2. Direct binding of anandamide to PPAR-ligand binding domain Affinity of PPARγ-ligand binding domain for various ligands was assessed using fluorescence polarization. Purified PPARγ-ligand binding domain and fluorescent PPAR ligand (Fluormone™ PPAR Green) were added to different concentrations of GW9662 (●), rosiglitazone (○), anandamide (▼), 2-AG (△) and palmitoylethanolamide (■). The samples were incubated at room temperature for 2 h and readings were taken with an excitation wavelength of 485 nm and emission wavelength of 535 nm. These results are representative of three independent experiments.

In the second approach, chimeric proteins were used to verify that this response is indeed mediated by the nuclear receptor and that it is specific to PPARγ. The luciferase reporter UAS-TK-Luciferase containing three Gal4 binding sites was cotransfected into cells with a vector coding for the fusion protein of the yeast Gal4 DNA binding domain and the ligand-binding domain of PPARγ. Incubation of transiently transfected Cos cells with various anandamide concentrations (0.1 to 30 μM) resulted in a significant 2–3 fold increase in PPARγ activity (Fig. 1B). Furthermore, anandamide induced PPARγ agonist activity in a dose-dependent manner, with an EC_{50} of 8 μM. Similar results were obtained when stably transfected HeLa cells containing chimeric proteins were used (Fig. 1C). In comparison, when cells were incubated with pioglitazone , a dose dependent increase up to a maximum of 4–5 fold was observed (Fig. 1C). Together, these results indicate that anandamide activates the transcriptional activity of PPARγ.

We also investigated the effect of other endocannabinoids such as palmitoylethanolamide and 2-AG on PPARγ activity. Palmitoylethanolamide, had no significant effect in transactivation experiments under conditions similar to those used with anandamide (Fig. 1B). In addition, 2-AG tested within a range of 0.3–30 μM had a slight non-reproducible effect on PPARγ activity (data not shown). Possibly, the cellular toxicity of 2-AG could mask PPARγ activation.

To determine if the effect of anandamide is due to the direct binding of anandamide to the PPARγ-ligand binding site, we performed a fluorescence polarization assay using the PPARγ-ligand binding domain, PPAR fluorescent ligand (Fluormone™ PPAR Green) and anandamide as a competitor. As shown in Fig. 2, binding of the PPAR fluorescent ligand was inhibited in a concentration-dependent manner by anandamide with an IC_{50} of 10 μM whereas no effect was observed with palmitoylethanolamide. Interestingly, another endocannabinoid, 2-AG, fully

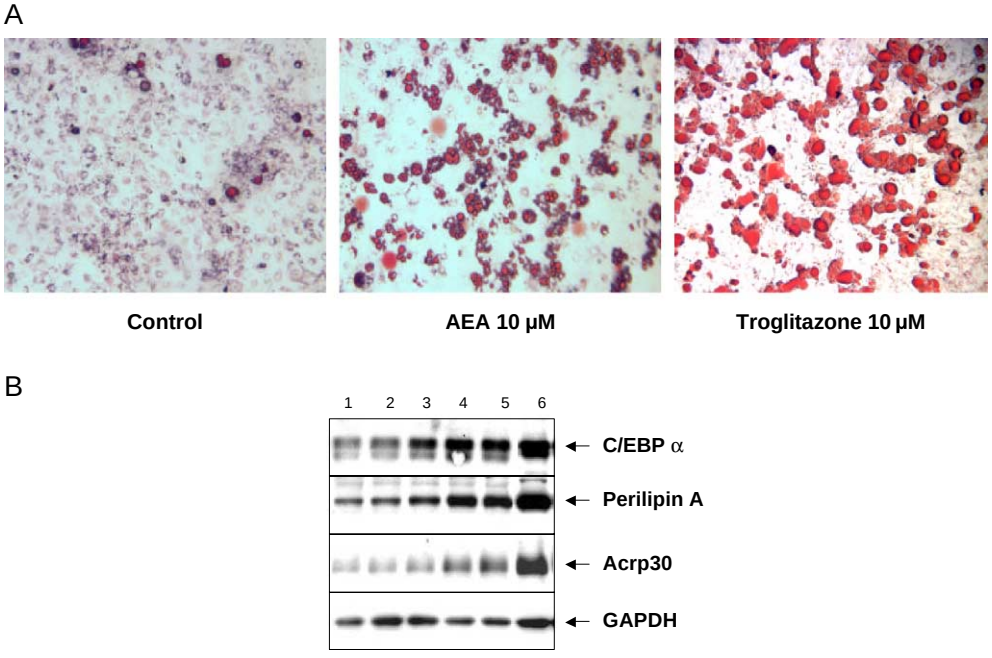

Fig. 3. Anandamide induced 3T3-L1 preadipocyte differentiation 3T3-L1 fibroblasts were cultured in Dulbecco's Modified Eagle's Medium supplemented with 10% calf serum. Two days after confluence, cells were treated with anandamide in the presence of 5 μg/ml insulin and 0.5 μM dexamethazone for 15 days. A. Oil red O staining. After 15 days of 10 μM anandamide or 10 μM troglitazone treatment, the cells were fixed and stained with Oil red O. The red staining indicates lipid droplet accumulation in the cytoplasm. B. Western blot detection. After 15 days of treatment with various anandamide concentrations (lanes 1–5, respectively: 0, 3, 10, 30 and 50 μM) or 10 μM troglitazone (lane 6), cells were harvested for protein analysis of C-EBPα, Perilipin A, Acrp30 or GAPDH expression by immunoblotting.

inhibited PPAR ligand binding. The GW9662 and Rosiglitazone as expected share an IC_{50} of 10 and 250 nM (Leesnitzer et al., 2002).

3.2. Anandamide induced 3T3-L1 pre-adipocyte differentiation

Activation of PPAR by its ligands is a key process for adipocyte differentiation. We assessed the ability of anandamide to induce differentiation of 3T3-L1 fibroblasts into adipocytes in order to obtain biological evidence that anandamide is an activator of PPAR. Pre-adipocyte 3T3-L1 cells were induced to differentiate with increasing doses of anandamide in the presence of insulin plus dexamethasone for 12 days. Lipid accumulation in cells was then assessed by Oil Red O staining. A significant increase in lipid droplet staining in the cytoplasm was observed after 10 μM anandamide treatment (Fig. 3A), suggesting that anandamide induced 3T3-L1 fibroblast differentiation into adipocytes. To confirm that anandamide induces 3T3-L1 cell differentiation into adipocytes, we measured the level of adipocyte-specific gene expression such as *Acrp30*, *aP2*, *C-EBPα*, and *PPARγ*. This set of genes which are PPARγ regulated, are very sensitive hallmarks of adipocyte differentiation (Hamm et al., 2001). Through RT-PCR analysis, we showed that anandamide increases the expression of aP2, C-EBPα, Acrp30, lipoprotein lipase and PPARγ in a dose-dependent manner with a maximum increase of 3–4 fold (Table 2), whereas it decreased CC chemokine ligand 2 (JE, monocyte chemotactic protein-1 [MCP-1]) JE gene expression. As for anandamide, the 3T3-L1 treatment with troglitazone at 10 μM induced lipid droplet accumulation (Fig. 2A) as well as the increase of PPARγ responsive gene expression with a maximum increase of 6–15 fold (Table 2).

Through immunoblotting, we confirmed that anandamide induced C-EBPα and Perilipin A, Acrp30 but not GAPDH protein accumulation after 12 days of treatment in a dose dependent manner with an EC_{50} of 7 μM, and this was closely correlated with PPARγ activation (Fig. 3B). Furthermore the PPARγ antagonist (GW9662) (Leesnitzer et al., 2002) significantly inhibited anandamide induced PPARγ transcriptional activation in Cos cells (Fig. 4A) as well as anandamide induced gene expression (Table 2) and protein accumulation (Fig. 4B) in 3T3-L1. As expected the GW9662 blocked the effects of troglitazone (Fig. 4A). Altogether, these data indicate that anandamide could induce 3T3-L1 fibroblast differentiation into adipocytes through PPARγ-dependent pathway, further supporting that anandamide is an activator of PPARγ transcriptional activity.

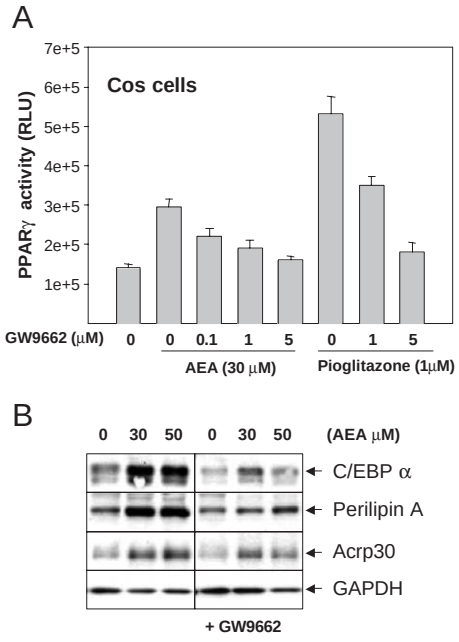

Fig. 4. GW9662 inhibited anandamide induced PPARγ activation and 3T3-L1 preadipocyte differentiation: A. Cos cells were transiently cotransfected with vector expression for fusion protein of the Gal4 DNA binding domain and ligand binding domain of PPARγ(Gal4-PPARγ) and the Gal4 reporter vector (UAS-TK-Luciferase). Cells were pretreated with various concentrations of GW9662 and then 30 μM anandamide was added . Luciferase assays were carried out 24 h later. B. Effect of GW9662 on anandamide induced C-EBPα PerilipinA, Acrp30 and GAPDH protein expression, following 15 days of 3T3-L1 differentiation. These results are representative of three independent experiments.

4. Discussion

Although cannabinoids have already been shown to be PPAR ligands, our research shows that anandamide induces transcriptional activation of PPARγ. In both transactivation experiments using either the PPRE reporter with full-length PPARγ or the Gal responsive reporter with the chimeric

Table 2
RT-PCR analysis

% of β2-microglobulin	Acrp30	Ap2	C/EBPα	JE	Lipoprotein lipase	PPARγ
Control	0.03	2	0.05	6.9	52	0.1
Ins/Dex	5	39	0.35	4.6	59	0.5
Trog 10 μM+Ins/Dex	88	462	7.4	0.3	374	3.8
Anandamide 10 μM+Ins/Dex	12	72	0.94	4	92	1
Anandamide 30 μM+Ins/Dex	37	196	2.3	5.1	190	2.7
Anandamide 50 μM+Ins/Dex	37	197	2.68	2.5	240	2.5
Ins/Dex+GW	10	73	0.66	8.6	80	0.8
Anandamide 10 μM+Ins/Dex + GW	9	74	0.59	10.8	72	0.6
Anandamide 30 μM+Ins/Dex+GW	13	96	0.97	9.4	88	0.8
Anandamide 50 μM+Ins/Dex + GW	22	154	1.7	7.3	113	0.9

RT-PCR analysis of gene expression in 3T3-L1 after anandamide treatment. The data are expressed as percent of housekeeping gene β2-microglobulin.

protein containing the ligand binding domain of PPARγ and yeast gal4, we showed that anandamide induced transcriptional activation of PPARγ in different cell types (CV1, palmitoylethanolamide had no effect. We also showed that anandamide but not palmitoylethanolamide binds PPAR-ligand binding domain directly. We studied the role of anandamide in adipocyte differentiation in order to extend the anandamide-activated PPAR response to a PPAR-dependent biological response. In these experiments, we showed that anandamide induced 3T3-L1 adipocyte differentiation, characterized by triglyceride-droplet accumulation and induction of the expression of PPARγ responsive genes such as *C-EBPα* and *aP2* as well as PerilipinA, Acrp30, lipoprotein lipase, PPARγ, other adipocyte differentiation markers. These effects of anandamide on adipocyte differentiation were dose dependent, strongly correlated with PPARγ transcriptional activation and were reversed by PPARγ antagonist (GW9662).

In these sets of experiments the PPARγ agonists pioglitazone, rosiglitazone or troglitazone induced a maximum increase of 5–8 fold in PPARγ transcriptional activation and 5–15 fold in gene expression of 3T3-L1 differentiation hallmarks. This is in agreement with other studies (Schoonjans et al., 1996; Combs et al., 2002). In most cases, anandamide induced an effect representing 30%–50% of the maximal activity of PPARγ agonists, indicating that anandamide effects are significant and could be physiologically relevant.

The molecular mechanisms by which anandamide induces PPARγ transcriptional activation is *most likely* due to the direct binding of anandamide to PPARγ.

But we cannot rule out that this effect could be indirect through the binding of another cellular target which in turn induces PPARγ activation. Indeed, it has been shown recently that poly-unsaturated fatty acids including arachidonic acid bind and activate RXRα (Lengqvist et al., 2004). Thus, anandamide may also activate RXR that heterodimerize with PPARγ.

Indirect effects through cannabinoid CB_1 or CB_2 receptors or vanilloid VR_1 receptors can be excluded since we did not detect any expression of these receptors in HeLa cells or in Cos cells using RT-PCR techniques (Table 3). It is also possible that anandamide hydrolysis products, such as arachidonic acid, can generate 15-deoxy-delta12,14-prostaglandin J2, which is the natural ligand of PPARγ. This possibility can, however, be ruled out since neither fatty acid amide hydrolase expression (Table 3) nor fatty acid amide hydrolase activity was detected in HeLa cells in agreement with previous results (Deutsch et al., 2001).

Anandamide could also serve as a substrate for other enzymes such as COX-2 (Yu et al., 1997) or lipoxygenase (Burstein et al., 2000), leading to the generation of different substrates that may in turn activate PPARγ. Indeed, in RT-PCR analyses, low levels of lipoxygenase-12 and COX-2 mRNA were detected in HeLa cells (Table 3). However, the effects of anandamide oxidation products such as prostamides (COX-2) or HETE-EA (lipoxygenase) on PPARγ activity have yet to be established. Likewise the involvement of the other cellular targets of anandamide in PPARγ activation, such as T-type Ca^{2+} or K^+ Task-1 ion channels (Chemin et al., 2001; Maingret et al., 2001), requires further investigation.

In this study, anandamide concentrations that were necessary to induce PPARγ activation and 3T3-L1 adipocyte differentiation were fairly high (3–30 μM), but in agreement with the high concentrations reported in other studies (Berdyshev et al., 2001; Sancho et al., 2003). Measurements of protein content or cell viability (MTS) showed that anandamide does not induce cell toxicity at doses up to 30 μM (data not shown).

In our study, we worked in the presence of 10% fetal calf serum, which is known to absorb anandamide and thus shifting the EC_{50} of anandamide to the right. Furthermore, intracellular anandamide concentrations could be higher, particularly under pathological conditions, leading to inflammation and apoptosis, such as during cell injury and tissue damage (Berdyshev et al., 2001; Deutsch et al., 2001; Berdyshev et al., 2000). The cannabinoid CB_1 receptor antagonist SR141716 used at 0.01–0.2 μM (pharmacological concentrations) had no significant effect on anandamide induced lipid droplet accumulation or gene expression in 3T3-L1 cells, indicating that in the 3T3-L1 cells the anandamide effect is CB_1-independent (data not shown).

The effect of anandamide seems to be specific to a class of PPAR receptors since it activates PPARγ but not PPARγ (Fu et al., 2003). However, binding of anandamide to PPARγ remains to be examined.

It has been reported that ajulemic acid, a synthetic cannabinoid with potent anti-inflammatory activity which does not efficiently bind to either cannabinoid CB_1 or CB_2 receptors, activates PPARγ (Liu et al., 2003).

Table 3
RT-PCR analysis

% of β2-microglobulin	CB_1 %	CB_2 %	Fatty acid amide hydrolase %	Monoglyceride lipase %	COX-2 %	Arachidonate lipoxygenase—12 %	Arachidonate lipoxygenase—15 %
Hela	0.05	0.03	0.004	0.4	1.7	0.4	0.09
3T3-L1 Undifferentiated	0.003	0.003	0.014	5.2	0.54	–	–
3T3-L1 Differentiated	0.1	0.006	0.01	65	0.02	–	–
Total brain	30	0.003	3.1	18.6	1.9	0.4	0.06

RT-PCR analysis of gene expression in Hela cells and total brain. The data are expressed as percent of housekeeping gene β2-microglobulin.

While this manuscript was in preparation, one group reported that palmitoylethanolamide activates PPARγ but not PPARγ transcriptional activation which is in agreement with our results (Lo Verme et al., 2004). Another group suggested that anandamide induced the inhibition of interleukin-2 production in phorbol 12-myristate 13-acetate/ionomycin activated splenocytes, could be mediated in part by PPARγ which is also in agreement with our results (Rockwell and Kaminski, 2004).

This suggests that PPAR is a potential convergence point of cannabinoids and poly-unsaturated fatty acid systems.

Together, these data indicate that PPARγ may be a molecular target for anandamide, providing a potential mechanism for the anti-inflammatory and pro-apoptotic activities of anandamide. More importantly, it could open a new avenue for the study of the role of anandamide in a variety of biological processes such as obesity, glucose homeostasis and type 2 diabetes.

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