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Δ⁹-THC modulation of fatty acid 2-hydroxylase (FA2H) gene expression: Possible involvement of induced levels of PPARα in MDA-MB-231 breast cancer cells

Shuso Takeda[a,b], **Eriko Ikeda**[a], **Shengzhong Su**[c], **Mari Harada**[a], **Hiroyuki Okazaki**[d], **Yasushi Yoshioka**[a], **Hajime Nishimura**[a], **Hiroyuki Ishii**[a], **Kazuhiro Kakizoe**[a], **Aya Taniguchi**[a], **Miki Tokuyasu**[a], **Taichi Himeno**[a], **Kazuhito Watanabe**[e], **Curtis J. Omiecinski**[c], and **Hironori Aramaki**[a,d,*]

[a] Department of Molecular Biology, Daiichi University of Pharmacy, 22-1 Tamagawa-cho, Minami-ku, Fukuoka 815-8511, Japan

[b] Laboratory of Xenobiotic Metabolism and Environmental Toxicology, Faculty of Pharmaceutical Sciences, Hiroshima International University (HIU), 5-1-1 Hiro-koshingai, Kure, Hiroshima 737-0112, Japan

[c] Center for Molecular Toxicology and Carcinogenesis, 101 Life Sciences Building, Pennsylvania State University, University Park, PA 16802, United States

[d] Drug Innovation Research Center, Daiichi University of Pharmacy, 22-1 Tamagawa-cho, Minami-ku, Fukuoka 815-8511, Japan

[e] Department of Hygienic Chemistry, Faculty of Pharmaceutical Sciences, Hokuriku University, Ho-3 Kanagawa-machi, Kanazawa 920-1181, Japan

Abstract

We recently reported that Δ^9-tetrahydrocannabinol (Δ^9-THC), a major cannabinoid component in *Cannabis Sativa* (marijuana), significantly stimulated the expression of fatty acid 2 hydroxylase (FA2H) in human breast cancer MDA-MB-231 cells. Peroxisome proliferator-activated receptor α (PPARα) was previously implicated in this induction. However, the mechanisms mediating this induction have not been elucidated in detail. We performed a DNA microarray analysis of Δ^9-THC treated samples and showed the selective up-regulation of the PPARα isoform coupled with the induction of FA2H over the other isoforms (β and γ). Δ-THC itself had no binding/activation potential to/on PPARα, and palmitic acid (PA), a PPARα ligand, exhibited no stimulatory effects on FA2H in MDA MB 231 cells; thus, we hypothesized that the levels of PPARα induced were involved in the Δ^9-THC mediated increase in FA2H. In support of this hypothesis, we herein

* Corresponding author at: Daiichi University of Pharmacy, Department of Molecular Biology, Drug Innovation Research Center, 22-1 Tamagawa-cho, Minamiku, Fukuoka, Fukuoka 815-8511 Japan. Tel.: +81 92 541 0161; fax: +81 92 553 5698. haramaki@daiichi-cps.ac.jp (H. Aramaki)..

Conflicts of interest
No potential conflicts of interest were disclosed.

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demonstrated that; (i) Δ^9 THC activated the basal transcriptional activity of PPARα in a concentration-dependent manner, (ii) the concomitant up-regulation of PPARα/FA2H was caused by Δ^9-THC, (iii) PA could activate PPARα after the PPARα expression plasmid was introduced, and (iv) the Δ^9-THC induced up regulation of FA2H was further stimulated by the co-treatment with L-663,536 (a known PPARα inducer). Taken together, these results support the concept that the induced levels of PPARα may be involved in the Δ^9 THC up-regulation of FA2H in MDA-MB-231 cells.

Keywords

Δ^9-tetrahydrocannabinol; Fatty acid 2-hydroxylase; Peroxisome proliferator-activated; receptorα; Human breast cancer cells; MDA-MB-231 cells

1. Introduction

Δ^9-tetrahydrocannabinol (Δ^9-THC), a major cannabinoid component in *Cannabis Sativa* (marijuana), is known to possess a wide variety of biological effects (Pertwee et al., 2010). Cannabinoids including Δ^9-THC generally exert their biological effects through the engagement of cannabinoid receptors; types CB1 and CB2 (Pertwee et al., 2010). Δ^9-THC has also been suggested to utilize other targets such as nuclear peroxisome proliferator-activated receptors (PPARs), especially the PPARγ isoform, as its ligands (Peters et al., 2012 Vara et al., 2013). PPARs consist of a subfamily of three isoforms; PPARα, PPARβ, and PPARγ, the ligands of which involve a number of natural and synthetic ligands with different structures including fatty acids (FAs), FA-derived compounds, and eicosanoids. PPARs are ligand-activated transcription factors that interact with the PPAR response elements (PPREs) found in various genes involved in lipid metabolism and energy balance. Although PPARγ is known to suppress the proliferation of different types of human cancer cells including breast cancer, Peters's research group reported that PPARβ played a suppressive role in human breast carcinogenesis (Yao et al., 2014).

Fatty acid 2-hydroxylase (FA2H) catalyzes the 2-hydroxylation of FAs (2-OH FAs). The physiological role of FA2H and functional role of 2-OH FAs have not yet been fully established; however, the expression of FA2H has been suggested to increase the efficacy of the anti-cancer agent PM02734 in the human cancer cell lines, HCT116, U937, and HeLa (Herrero et al., 2008). Furthermore, mutations in the FA2H gene were previously reported to be associated with leukodystrophy with spastic paraparesis and dystonia in humans, thereby implicating the role of FA2H in the nervous system (Edvardson et al., 2008). The expression of FA2H varies highly among cell types and can be induced by certain stimuli (Hama, 2010). By utilizing PPAR isoform-selective agonists and antagonists, we showed that Δ^9-THC was able to induce FA2H in human breast cancer MDA MB 231 cells through PPARα, and also by PPARγ to a lesser extent (Takeda et al., 2013a). However, since Δ^9-THC has "no" binding/activation potential to/on the PPARα isoform, unlike the PPARγ (Sun et al., 2006, 2007), the Δ^9-THC-mediated induction mechanism(s) of FA2H currently remain unclear. We herein demonstrated that the induced levels of PPARα, the basal expression of which was the lowest among the three PPAR isoforms in MDA-MB-231 cells, were

involved in the Δ^9-THC induction of FA2H, and that the up-regulated PPARα was activated by serum components, possibly through FAs, resulting in the induction of FA2H.

2. Materials and methods

2.1. Reagents

Δ^9-THC was isolated and purified from drug-type cannabis leaves according to established methods described elsewhere (Aramaki et al., 1968). The purity of Δ^9-THC was found to be at least above 98% by gas chromatography (Takeda et al., 2008; Tachibana et al., 2008). L-663,536 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Palmitic acid (PA) was purchased from Nacalai Tesque, Inc., (Kyoto, Japan). All other reagents were of the highest grade commercially available.

2.2. Cell cultures

Cell culture conditions and methods were basically performed as described previously (Takeda et al., 2011a, 2013a). Briefly, the human breast cancer cell line, MDA-MB-231 (obtained from the American Type Culture Collection, Rockville, MD, USA), was routinely grown in phenol red-containing minimum essential medium α (Invitrogen, Carlsbad, CA, USA), supplemented with 10 mM HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid], 5% fetal bovine serum, 100 U/mL of penicillin, and 100 μg/mL of streptomycin in a humidified incubator, within an atmosphere of 5% CO_2 at 37 °C. Prior to the chemical treatments, the medium was changed to phenol red free minimum essential medium α (MEMα) (Invitrogen) supplemented with 10 mM HEPES, 5% fetal bovine serum, 100 U/mL of penicillin, and 100 μg/mL of streptomycin. Cultures of approximately subconfluence (close to 60% confluence) in a 100-mm Petri dish were used to seed for the experiments on mRNA expression, DNA microarrays, and the transfection analysis (dual-luciferase assay).

2.3. Analysis of reverse transcription polymerase chain reaction (RT-PCR) and real time RT-PCR

Total RNA was prepared from MDA-MB-231 cells using the RNeasy kit (Qiagen, Inc., Hilden, Germany) and purified using RNeasy/QIAamp columns (Qiagen, Inc.). The following syntheses of cDNA, RT, and PCR were performed using the SuperScript One-Step RT-PCR System with Platinum *Taq* polymerase (Invitrogen). The primers used were PPARα (sense) 5′ CTT CGC AAA CTT GGA CCT GA-3′ and PPARα (antisense) 5′ TGA GCA CAT GTA CAA TAC CCT C 3′; PPARγ (sense) 5′ TCT CCG TAA TGG AAG ACC ACT 3′ and PPARγ (antisense) 5′ GCT TTA TCT CCA CAG ACA CGA C 3′. Primers for the PCR of FA2H, PPARβ, and β-actin were taken from previous studies (Hong et al., 2010; Takeda et al., 2013a). PCR was performed under conditions that produced template quantity-dependent amplification. PCR products were separated by 1.5% agarose gel electrophoresis in Tris-acetate EDTA (ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid) buffer and stained with ethidium bromide. When the RT reaction was omitted, no signal was detected in any of the samples. β-Actin was used as an internal control for RT-PCR. In the real-time RT-PCR analysis on PPARα, FA2H, CYP1A1, and β-actin, cDNA was prepared via RT of total RNA using the ReverTra Ace® qPCR RT kit (Toyobo Co. Ltd., Osaka,

Japan). A real-time quantitative RT-PCR assay was performed with FastStart Essential DNA Green Master (Roche Applied Science, Indianapolis, IN, USA) and the LightCycler Nano (Roche Diagnostics, Mannheim, Germany). The primers used were PPARα (sense) 5′ CCT CTG AGC CAG ATA AGC AGA 3′ and PPARα (antisense) 5′ AGA CCG TTG CCA AAG ATG ATG 3′ ; FA2H (sense) 5′ AAC GAG CCT GTA GCC CTT GA 3′ and FA2H (antisense) 5′ ACT GCC ACC GTG TAC TCT GTT 3′; β-actin (sense) 5′ GGC CAC GGG GCT GCT TC 3′ and β-actin (antisense) 5′ GTT GGC GTA CAG GTC TTT GC 3′ . Primers for the PCR of PPARα were taken from a previous study (Villard et al., 2011). The reaction conditions for PPARα, FA2H, CYP1A1, and β-actin were 95 ° C for 10 min, followed by 45 cycles at 95 °C for 10 s, at 56 ° C for 10 s, and 72 ° C for 15 s, while those for CYP1A1 were 95 ° C for 10 min, followed by 45 cycles at 95 ° C for 15 s and at 58 ° C for 1 min. PPARα, FA2H, and CYP1A1 mRNA levels were normalized to the corresponding β-actin mRNA levels.

2.4. DNA microarray analyses

Total RNA was collected from 25 μM Δ^9-THC or vehicle-treated MDA-MB-231 cells 48 h after exposure using the RNeasy kit (Qiagen, Inc.), and was purified using RNeasy/QIAamp columns (Qiagen, Inc.). The specific gene expression pattern in MDA-MB-231 cells was examined by DNA microarray analysis and compared with that in vehicle-controls. Total RNA was extracted from both cell types, and the synthesis of complementary DNA (cDNA) and cRNA labeling were conducted using a low RNA fluorescent linear amplification kit (Agilent, Palo Alto, CA, USA). Overall changes in gene expression were evaluated using a two color microarray-based gene expression analysis (Takeda et al., 2013a). Labeled cRNA (Cy3 to control, Cy5 to Δ^9-THC) was hybridized to human oligo DNA microarray slides (Agilent) that carried spots for human genes. Specific hybridization was analyzed using a Microarray scanner (Agilent) and evaluated as a scatter-plot graph for gene expression. Hokkaido System Science (Sapporo, Japan) provided assistance with the experiments.

2.5. Transfection and luciferase reporter assay

The day before transfection, MDA-MB-231 cells were seeded (5×10^4 cells/well) onto 24-well plates containing Dulbecco's Modified Eagle's medium (Invitrogen). Transfections of each expression plasmid were performed using Lipofectamine® LTX with PLUS™ reagent (Invitrogen) according to the manufacturer's instructions. Maximal transcriptional efficiencies for the use of the each human PPARα/β/γ expression plasmid in combination with the human retinoid × receptor α (RXRα) plasmid were determined as 100 ng and 100 ng, respectively, in the transfections. DNA mixtures of 300 ng of the PPRE-Luc plasmid containing the rat acyl-CoA oxidase PPRE were cotransfected with 20 ng of the *Renilla* luciferase reporter plasmid (pRL-CMV) in 24-well plates. All plasmid concentrations were equalized with the pcDNA 3.1 vector. At 24-h post transfection, cells were washed with phosphate buffered saline and changed to MEMα without phenol red (Invitrogen) supplemented with 0.2 or 5% serum, followed by a treatment with 5 and 25 μM Δ^9-THC or PA for 24 h. After being treated with the chemicals, the cell extracts were prepared with 100 μL of passive lysis buffer (Promega, Madison, WI, USA), and 20 μL of the extracts were used for the firefly luciferase and *Renilla* luciferase assays (Dual-Luciferase Reporter Assay System) by the GloMax-Multi Detection System (Promega). The ratio of firefly luciferase

activity (expressed from reporter plasmids) to Renilla luciferase activity (expressed from pRL-CMV) in each sample served as a measure of normalized luciferase activity.

2.6. Data analysis

Differences were considered significant when the *p* value was calculated as less than 0.05. Significant differences between two groups were calculated by the Student's *t*-test. Other statistical analyses were performed by Scheffe's *F* test, a post hoc test to analyze the results of the ANOVA. Calculations were performed using Statview 5.0 J software (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Selective up-regulation of PPARα by Δ^9-THC

PA, a saturated fatty acid ($C_{16:0}$), was suggested to be an activator of PPARα (Forman et al., 1997; Kliewer et al., 1997; Murakami et al., 1999), and PA was found to be the most abundant FA in human serum (~165 mM) as well as in fetal bovine serum (~140 mM) (Evans and Hardy, 2010). Thus, in the present study, we utilized PA as a natural ligand for PPARα. As shown in Fig. 1A, FA2H was induced by 25 μM Δ^9-THC, but not by 25 μM PA in MDA-MB-231 cells; the concentration of PA (25 μM) was determined based on the potency of the PPARα activation (see Fig. 2A) (Forman et al., 1997; Kliewer et al., 1997; Murakami et al., 1999). Thus, an inconsistency remains in the stimulation of FA2H expression between Δ^9-THC and PA. DNA microarray analyses showed that the expression of the α type of PPAR was the lowest among the three PPAR isoforms, and PPARβ was identified as a major isoform in MDA-MB-231 cells (Fig. 1B). When MDA-MB-231 cells were exposed to Δ^9-THC, PPARα was selectively up-regulated, as clearly evidenced by DNA microarray and RT-PCR analyses (Fig. 1C, left and right panels).

3.2. PA activated PPAR when cells were transfected with the PPARα expression plasmid

We next investigated whether PA could simulate PPAR-driven transactivation after the introduction of a plasmid carrying PPARα cDNA. We utilized a reporter construct containing rat acyl-CoA oxidase PPRE, which is highly sensitive to the PPARα isoform and activated in response to serum (Fig. 2B) (Kane et al., 2006). Although, as shown in Fig. 2A, PA did not exhibit any activation potential for basal PPAR-driven transactivation via the PPAR/PPRE interaction, the stimulation of PPAR driven transactivation was significantly stronger (20-fold) by transfection of the PPARα plasmid into cells (+/−) than the PPRE alone system (−/−). Thus, these results suggested that the endogenous level of PPARα may be a limiting factor for the biological activity of PA (see also Fig. 1A), and also that the ligands available for PPARα were present in cells and/or in culture media supplied with 5% serum. In support of this, basal PPAR driven transcriptional activity was not affected by the serum concentrations examined; 0.2% vs. 5% (Fig. 2C).

3.3. Δ^9-THC activation of PPAR-mediated transcription

Time-course analyses of FA2H, PPARα, PPARβ, PPARγ, and β-actin (used as a housekeeping gene) mRNAs in MDA-MB-231 cells treated with Δ^9-THC for 3, 24, or 48 h showed that the expression of FA2H was coupled with the stimulated expression of PPARα;

however, the others were not essentially affected (Fig. 3), suggesting that the induced levels of PPARα may be engaged in the Δ^9-THC-induced up-regulation of FA2H. The simultaneous expression of PPARα/FA2H was detected, at least, up to 96 h (data not shown). We investigated whether Δ^9-THC could activate the transcriptional activity driven by PPARα in MDA-MB-231 cells. If PPARα up-regulated by Δ^9-THC was functionally active, thereby leading to the induction of FA2H (Figs. 1 and 3), basal PPAR-driven transactivation itself, without introducing the PPARα plasmid to cells, may be stimulated by the cannabinoid. As expected, PPAR activity in the presence of 5% serum was positively enhanced by Δ^9-THC in a concentration dependent manner (Fig. 4).

3.4. Simultaneous up-regulation of FA2H and CYP1A1 by Δ^9-THC

As is clearly shown in Fig. 5 (panels A–C), Δ^9-THC evoked the up-regulation of FA2H and CYP1A1 coupled with the PPARα stimulation as a function of time (3, 24, or 48 h) (see also Fig. 3). Thus, Δ^9-THC may have been able to up regulate PPARα-mediated genes sensitive to serum, as in the case of FA2H and CYP1A1. As further proof of the induction of PPARα by Δ^9-THC, we investigated the effects of L-663,536, a 5-lipoxygenase (5-LOX) inhibitor also known as a PPARα inducer in breast cancer cells (Avis et al., 2001), on the actions of Δ^9-THC. L-663,536 stimulated the expression of PPARα more than the Δ^9-THC alone system, as indicated by a gray bar (indicated as 1.0; Fig. 5, panels D–F) corresponding to the Δ^9-THC incubation at 48 h (Fig. 5, panels A–C). In accordance with this result, the co-treatment with Δ^9-THC and L-663,536 produced a significant increase in two PPARα regulated genes (FA2H and CYP1A1) over the Δ^9-THC alone treatment. Although one possibility may be that the inhibition of 5-LOX was involved in the Δ^9-THC induction of PPARα, it is not applicable in this case because of the failure of Δ^9-THC to inhibit 5-LOX activity, as we demonstrated previously (Takeda et al., 2011b).

4. Discussion

As shown in Figs. 1 and 3, MDA-MB-231 cells expressed higher levels of PPARβ and PPARγ than PPARα. Although the physiological role of PPARα in breast cancer cells has not yet been fully established, previous studies reported that PPARα could protect proximal tubular cells from the toxicity of FAs including PA (Kamijo et al., 2007), and that PPARα played a central role in the regulation of FAs (Tachibana et al., 2008; Vanden Heuvel, 1999). Given that this protective role of PPARα can be applied to the Δ^9-THC induction of FA2H coupled with PPARα activation in MDA-MB-231 cells, Δ^9-THC may activate the cellular defense machinery, thereby protecting against PA lipotoxicity (via 2-OH PA formation). In support of this, 2-OH PA, one of the PA metabolites, was markedly less toxic to MDA-MB-231 cells than PA (Takeda et al., unpublished observations). Δ^9-THC was previously shown to induce PPARγ mRNA in human hepatocellular carcinoma HepG2 cells, with a peak in its expression being observed 1 h after the treatment, followed by a decline within 24 h (Vara et al., 2013). When evaluating the level of PPARγ in Δ^9-THC-exposed MDA-MB-231 cells, PPARγ was still detected at 3 h, and its expression was not affected up to at least 96 h (data not shown). In contrast, a type PPAR was significantly up-regulated 24 h after the treatment with Δ^9-THC, and its expression was detected up to at least 96 h (Figs. 3 and 5) (data not shown). A previous study demonstrated that human PPARα could

stimulate its expression by itself (Pineda Torra et al., 2002); therefore, the prolonged expression of PPARα observed in the present study may have been attributed to PPARα being up-regulated by Δ^9-THC. We searched consensus sequences for response elements to transcriptional factors within the promoter region of the human FA2H gene up to 10 kilobases. Three nucleotide sequences showing homology with possible PPREs for PPARα and one sequence for PPARγ were found within around 6 kilobases of the gene 5′-flanking region. In the present study, we could not definitively confirm the interaction between PPARα and PPREs potentially induced by Δ^9-THC. Studies to obtain this biological evidence are ongoing.

The activation of PPARα was shown to interfere with transcription by abrogating the nuclear factor-κB (NF-κB) and activator protein-1 (AP-1) signaling pathways (Delerive et al., 1999; Peters et al., 2012). Since the expression of cyclooxygenase-2 (COX-2) is known to be positively regulated by both NF-κB and AP-1 (Dong et al., 1997; Kopp and Ghosh, 1994), if PPARα activated by Δ^9-THC is functionally active in MDA-MB-231 cells, different from the case of FA2H, the expression of COX-2 may be down-regulated by the cannabinoid. As expected, real-time PCR analyses revealed that the expression of COX-2 was suppressed (0.58-fold) by Δ^9-THC. Although no binding sites were detected by bioinfor matics analyses for the aryl hydrocarbon receptor (AhR) in the promoter region of the FA2H, at least up to 10 kilobases, Δ^9-THC has been suggested as a potential ligand for AhR due to its ability to induce the expression of CYP1A1 that is also regulated by AhR-mediated signaling in a murine hepatoma cell line (Roth et al., 2011). Based on these findings, we examined the expression levels of AhR-regulated genes, such as CYP1A2, CYP2S1, and UDP-glucuronosyltransferase 1A6 48 h after the exposure of Δ^9-THC to MDA-MB-231 cells expressing functional AhR (Tijet et al., 2006; Beischlag et al., 2008; O'Donnell et al., 2014); however, no observable stimulation was detected, implicating the possible requirement of PPARα, but not AhR, in the induction of CYP1A1 by Δ^9-THC in MDA-MB-231 cells. Since previous studies demonstrated that Δ^9-THC had no binding/activation efficacy toward PPARα (Sun et al., 2006, 2007), the action point(s) of Δ^9-THC on the induction of FA2H and CYP1A1 remain to be determined. Previous studies reported that the expression of CYP1A1 was stimulated by a mechanism underlying the serum-mediated activation of PPARα (Guigal et al., 2000; Villard et al., 2011). We also indicated that the up-regulation of CYP1A1 mRNA was enhanced by transfection of the PPARα plasmid in the presence of serum. In the present study, this positive effect was observed for FA2H (data not shown). Thus, it is suggested that FA2H as well as CYP1A1 are co-regulated by PPARα activated by serum component(s) in MDA-MB-231 cells.

PA exhibits anti-proliferative effects on various cell types, including breast cancer cells, especially highly aggressive human breast cancer MDA-MB-231 cells (Hardy et al., 2003); thus, PA is recognized as a pro-apoptotic FA. Since Δ^9-THC is currently used as a medicine, called dronabinol, a synthetic form of Δ^9-THC, in clinical settings, the cannabinoid may give rise to adverse outcomes whereby FA2H is up-regulating through the activation of PPARα. We observed that MDA-MB-231 cell growth tended to be stimulated by Δ^9-THC in concentrations ranging from 1 to 25 μM (Takeda et al., 2013b; Takeda, 2014). This phenomenon might support the above mentioned adverse outcomes by Δ^9-THC. Although the concentrations of Δ^9-THC (~25 μM) used in this study seem to be high (Ménétrey et al.,

2005; Schwope et al., 2011), judging from its chemical nature with high *n*-octanol/water partition coefficient (i.e., 6000:1 at pH 7) (Mechoulam, 1982), Δ^9-THC might evoke the undesirable effects after its accumulation in fat tissue (Johansson et al., 1989; Azorlosa et al., 1992). Further investigations are needed to confirm these unaddressed questions.

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Abbreviations

9-THC	9-tetrahydrocannabinol
FA2H	fatty acid 2-hydroxylase
PPAR	peroxisome proliferator-activated receptor
PPRE	PPAR response element
CYP1A1	cytochrome P450 1A1

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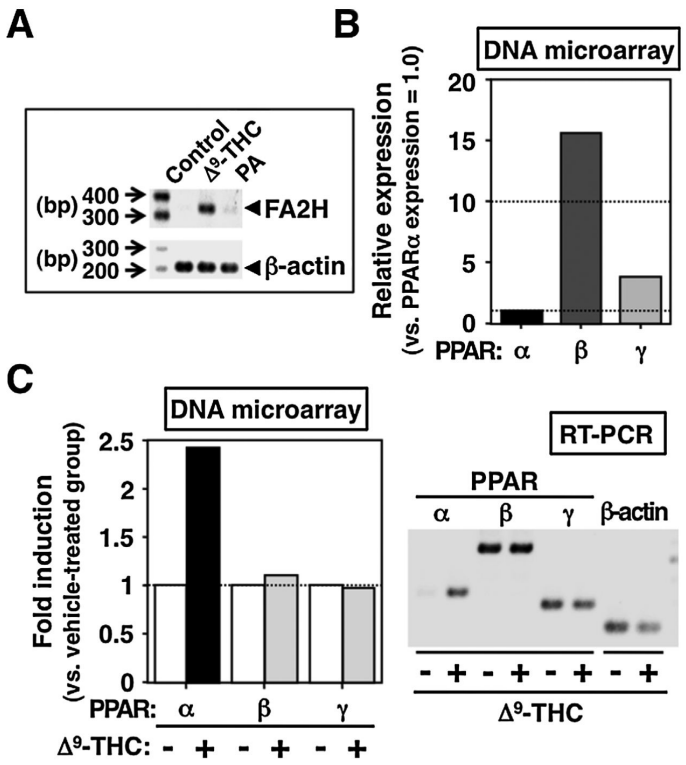

Fig. 1.

Δ^9-THC selectively stimulated the expression of PPARα. (A) RT-PCR analyses of FA2H levels in MDA-MB-231 cells 48 h after the treatment with 25 μM Δ^9-THC or 25 μM PA. Control indicates the vehicle (ethanol)-treated group. *β*-Actin was used as an internal loading control. A 100-bp DNA ladder marker was also loaded. (B) Results of the DNA microarray analysis. Data are expressed as relative expression vs. PPARα (1.0). Basal expression levels of PPARα/β/ξ in MDA-MB-231 cells. (C) (left panel) Results of the DNA microarray analysis. Data are expressed as fold induction vs. vehicle (ethanol)-treated group. MDA-MB-231 cells were treated with vehicle or 25 μM Δ^9-THC for 48 h, followed by the isolation of total RNA. Details of the microarray conditions are described in Section 2. (C) (right panel) RT-PCR analysis of the PPAR isoform a/b/g transcript levels after the treatment with (+) or without (−) 25 μM Δ^9-THC for 48 h. β-Actin was used as an RNA normalization control. A 100-bp DNA ladder marker was also loaded. All data shown are representative of at least three experiments.

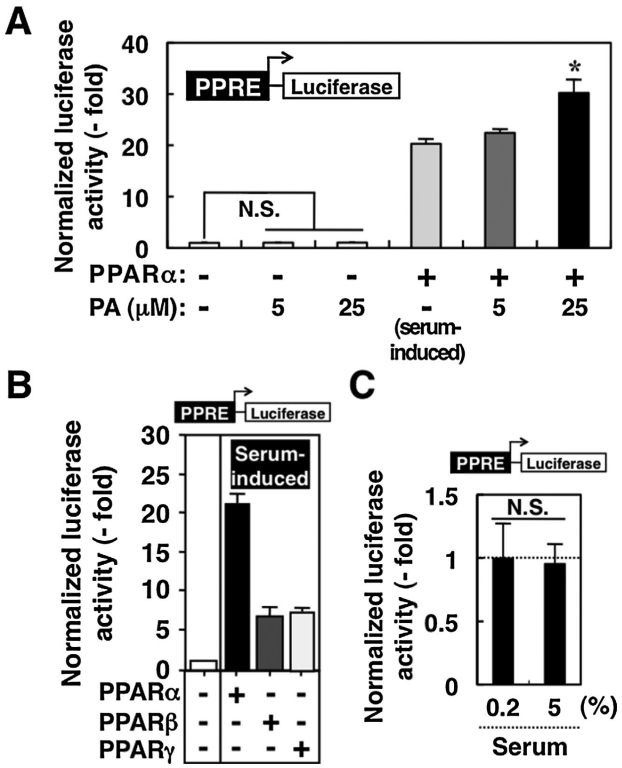

Fig. 2.

Palmitic acid only activated PPAR when cells were transfected with the PPARα expression plasmid. (A) MDA-MB-231 cells were transiently transfected with a PPRE-Luc plasmid containing rat acyl-CoA oxidase PPRE with or without the expression plasmid for human PPARα (+/−). After transfection, cells were treated with vehicle (−/−) or PA (5, 25 μM) in the presence of 5% serum. After 24 h, cells were harvested and assayed for luciferase activity, and all transfections were normalized for efficiency using the internal *Renilla* control plasmid. Data are expressed as the fold-change from the vehicle-treated control (indicated as −/−), as mean ± S.D. ($n = 5$). *Significantly different ($p < 0.05$) from serum-induced activity (indicated as +/−). (B) MDA-MB-231 cells were transiently transfected with a PPRE-Luc plasmid containing rat acyl-CoA oxidase PPRE with or without the expression plasmid for each human PPARα/β/γ. After transfection, cells were incubated in the presence of 5% serum. After 24 h, cells were harvested and assayed for luciferase activity, and all transfections were normalized for efficiency using the internal *Renilla* control plasmid. Data are expressed as the fold-change from the vehicle-treated control (indicated as −/−/-), as mean ± S.D. ($n = 5$). (C) MDA-MB-231 cells were transiently transfected with a PPRE-Luc plasmid containing rat acyl-CoA oxidase PPRE. After transfection, cells were incubated in the presence of 0.2% or 5% serum. After 24 h, cells were harvested and assayed for luciferase activity, and all transfections were normalized for efficiency using the internal *Renilla* control plasmid. Data are expressed as the fold-change from the vehicle-treated control (indicated as −/−/−), as mean ± S.D. ($n = 5$). N.S., not significant.

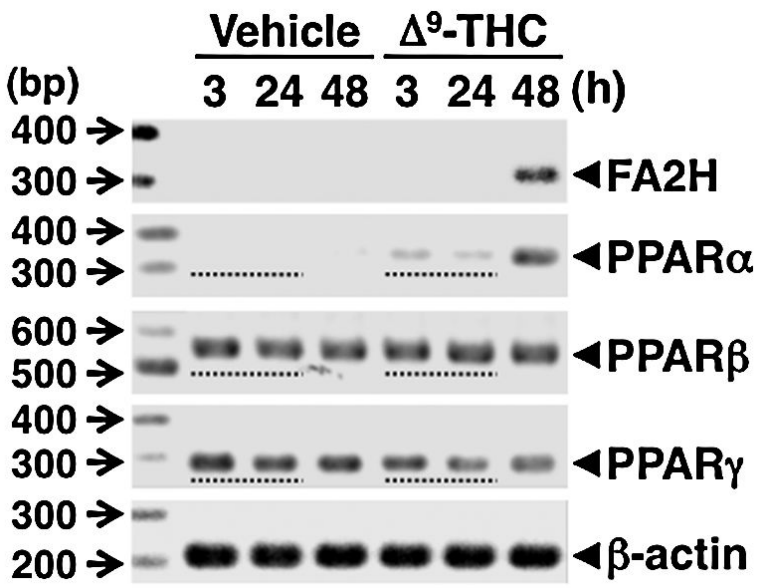

Fig. 3.

Δ^9-THC induced FA2H coupled with the up-regulation of PPARα as a function of time. RT-PCR analyses of FA2H and PPARα/β/γ. Time course analyses (3, 24, or 48 h) of FA2H and PPARα/β/γ transcript levels in MDA-MB-231 cells were performed after the treatment with 25 μM Δ^9-THC or without Δ^9-THC (indicated as vehicle). β-Actin was used as an RNA normalization control. A 100-bp DNA ladder marker was also loaded. Data set shown is representative of three experiments.

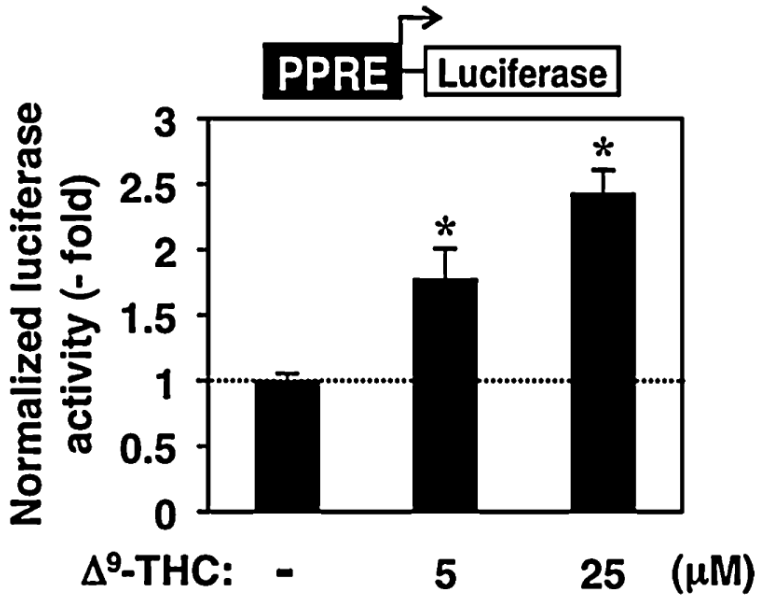

Fig. 4.

Δ^9-THC activated PPAR-mediated transcription. MDA-MB-231 cells were transiently transfected with a PPRE-Luc plasmid containing rat acyl-CoA oxidase PPRE. After transfection, cells were treated with 5 mM and 25 μM Δ^9-THC in the presence of 5% serum. After 24 h, cells were harvested and assayed for luciferase activity, and all transfections were normalized for efficiency using the internal *Renilla* control plasmid. Data are expressed as the fold-change from the vehicle-treated control (indicated as −/−/-), as mean ± S.D of triplicate determinations. *Significantly different ($p < 0.05$) from the vehicle-treated control (indicated as −).

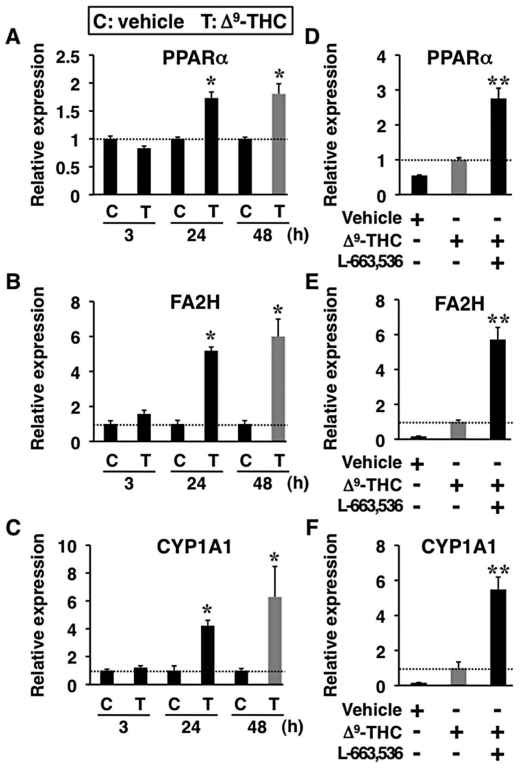

Fig. 5.

Δ^9-THC induced FA2H and CYP1A1 coupled with the up-regulation of PPARα as a function of time. (A–C) Real-time-RT-PCR analyses of PPARα, FA2H, and CYP1A1. Time course analyses (3, 24, or 48 h) of PPARα, FA2H, and CYP1A1 transcript levels in MDA-MB-231 cells were performed after the treatment with 25 μM Δ^9-THC (T) or vehicle (C). Data are expressed as the relative expression of the vehicle-treated group at the respective incubation periods. (D–F) Real-time-RT-PCR analyses of PPARα, FA2H, and CYP1A1 transcript levels in MDA-MB-231 cells 48 h were performed after the treatment with 25 μM Δ^9-THC and/or 5 mM L-663,536. Data are expressed as the relative expression of the Δ^9-THC-treated group. Each relative expression level of PPARα, FA2H, and CYP1A1 after the Δ^9-THC exposure for 48 h (A–C) corresponded to the Δ^9-THC alone group (D–F) (indicated as a gray bar). Data are expressed as the mean ± S.D of triplicate determinations.
*Significantly different ($p < 0.05$) from the vehicle-treated control (indicated as C).
**Significantly different ($p < 0.05$) from the Δ^9-THC-treated group (indicated as −/+/−).