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15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2 interferes inducible synthesis of prostaglandins E_2 and $F_{2\alpha}$ that suppress subsequent adipogenesis program in cultured preadipocytes

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ABSTRACT

Cultured preadipocytes enhance the synthesis of prostaglandin (PG) E_2 and $PGF_{2\alpha}$ involving the induction of cyclooxygenase (COX)-2 during the growth phase upon stimulation with a mixture of phorbol 12-myristate 13-acetate, a mitogenic factor, and calcium ionophore A23187. Here, we studied the interactive effect of 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2) on the inducible synthesis of the endogenous PGs in cultured preadipocytes and its implication in adipogenesis program. 15d-PGJ_2 interfered significantly the endogenous synthesis of those PGs in response to cell stimuli by suppressing the induction of COX-2 following the attenuation of NF-κB activation. In contrast, Δ^{12}-PGJ_2 and troglitazone had almost no inhibitory effects, indicating a mechanism independent of the activation of peroxisome proliferator-activated receptor γ for the action of 15-PGJ_2. Pyrrolidinedithiocarbamate (PDTC), an NF-κB inhibitor, effectively inhibited on the inducible synthesis of those PGs in preadipocytes. Endogenous PGs generated by preadipocytes only during the growth phase in response to the cell stimuli autonomously attenuated the subsequent adipogenesis program leading to the differentiation and maturation of adipocytes. These effects were prevented by additional co-incubation of preadipocytes with either 15d-PGJ_2 or PDTC although 15d-PGJ_2 alone has no stimulatory effect. Moreover, 15d-PGJ_2 did not block the inhibitory effects of exogenous PGE_2 and $PGF_{2\alpha}$ on the adipogenesis program in preadipocytes. Taken together, 15d-PGJ_2 can interfere the COX pathway leading to the induced synthesis of endogenous PGs that contribute to negative regulation of adipogenesis program in preadipocytes.

1. Introduction

The adipocytes and the precursor cells can synthesize endogenously several types of prostanoids through the arachidonate cyclooxygenase (COX) pathway as local hormones with opposite effects on adipogenesis at different stages of adipocytes in response to external stimuli [1,2]. The role of prostanoids in the regulation of adipocyte differentiation is complex because of the differences in the type of cultured adipocytes, the culture conditions to induce the differentiation of adipocytes, and the existence of multiple subtypes of receptors for each of prostanoid species.

We have been studying the adipogenesis process in cultured mouse preadipogenic 3T3-L1 cells as a useful model of white adipocytes under the established conditions, including the growth, differentiation, and maturation phases [1–3]. Recently, our laboratory has reported that the gradual synthesis, commonly known as the delayed synthesis, of PGE_2 and $PGF_{2\alpha}$ upon stimulation with a mixture of phorbol 12-myristate 13-acetate (PMA), an active phorbol diester, and a lower concentration of calcium ionophore A23187 occurred in preadipocytes during the growth phase prior to adipocyte differentiation [2]. The delayed synthesis of these prostanoids requires the de novo synthesis of the COX-2 isoform and can provide much higher levels of endogenous prostanoids than the immediate synthesis caused only by the stimulated release

Abbreviations: PG, prostaglandin; PMA, phorbol 12-myristate 13-acetate; 15d-PGJ_2, 15-deoxy-$\Delta^{12,14}$-PGJ_2; PPARγ, peroxisome proliferator-activated receptor γ; COX, cyclooxygenase; PDTC, pyrrolidinedithiocarbamate; NF-κB, nuclear factor-kappa B; L-PGDS, lipocalin-type PGD synthase; DME-HEPES, Dulbecco's modified Eagle's medium with HEPES; BSA, bovine serum albumin; FBS, fetal bovine serum; IBMX, 3-isobutyl-1-methylxanthine; ELISA, enzyme-linked immunosorbent assay; RT, reverse transcriptase; PCR, polymerase chain reaction; GM, growth medium; DM, differentiation medium; MM, maturation medium; mPGES, membrane-bound PGE synthase; cPGES, cytosolic PGE synthase; PGFS, PGF synthase; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PVDF, polyvinylidene difluoride.

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of free arachidonic acid. Similarly, Petersen et al. have described the transient expression of COX-2 upon the induction of the differentiation in the presence of a cAMP-elevating agent in cultured 3T3-L1 cells [4]. PGE_2 and $PGF_{2\alpha}$ have been shown to serve as anti-adipogenic prostanoids that exert their effects through the binding to the specific cell-surface receptors of EP4 [5] and FP [6]. These anti-adipogenic effects have been studied mostly by exposing the cultured cells during the differentiation and maturation phases to exogenous PGE_2 or $PGF_{2\alpha}$. Nevertheless, it remains still unclear regarding the role of these endogenous prostanoids synthesized by preadipocytes only during the growth phase in adipogenesis program leading to the differentiation and maturation of adipocytes.

Peroxisome proliferator-activated receptor γ (PPARγ), a member of nuclear hormone receptor and a ligand-dependent transcription factor, is a master regulator of adipocyte differentiation. PGD_2 can be synthesized by the arachidonate COX pathway and then readily undergo non-enzymatic dehydration to give 15-deoxy-$\Delta^{12,14}$-PGJ_2 (15d-PGJ_2) and Δ^{12}-PGJ_2. These PGJ_2 derivatives are effective to be active ligands for PPARγ and to stimulate adipogenesis during the differentiation and maturation of cultured adipocytes [7,8]. Recently, we have shown the selective gene expression of lipocalin-type PGD synthase (L-PGDS) and the up-regulation of its mRNA levels in cultured 3T3-L1 cells during the maturation phase [1]. In addition, we have reported the increased ability of adipocytes to generate endogenous synthesis of 15d-PGJ_2 during the maturation phase, contributing to adipogenesis in an autocrine manner [3]. Thus, PGD_2 and its dehydration products, 15d-PGJ_2 and Δ^{12}-PGJ_2, are now regarded as pro-adipogenic prostanoids. Alternatively, 15d-PGJ_2 has been described to be a negative regulator of experimental inflammation by the control of transcription factors, such as nuclear factor-kappa B (NF-κB) or the related activator protein [9,10].

The function of adipocytes in animal adipose tissues is always affected by a variety of mitogenic and inflammatory factors in the blood circulation in autocrine and paracrine manners [11]. More generally, COX-2 has been shown to be one of several factors that contribute to inflammation associated with adiposity [12]. By contrast, some of PPARγ agonists are known to serve as a negative regulator of experimental inflammation as described above. Taking these into consideration, we hypothesized that prostanoids generated at different life stages of adipocytes could exert their interactive effects on the arachidonate COX pathway in adipocytes or the precursor cells in adipose tissues. The present study was undertaken to unravel the interacting effects of 15d-PGJ_2 on the inducible biosynthesis of PGE_2 and $PGF_{2\alpha}$ by cultured preadipocytes during the growth phase as a model system. We show that the synthesis of those PGs by preadipocytes is significantly suppressed by the co-incubation with 15d-PGJ_2 due to the reduced induction of COX-2 following the interference of NF-κB pathway. Our study also reveals that 15d-PGJ_2 can rescue the inhibitory effects of endogenous PGs synthesized in preadipocytes during the growth phase on the subsequent adipogenesis program leading to the differentiation and maturation of adipocytes.

2. Materials and methods

2.1. Materials

Dulbecco's modified Eagle's medium with HEPES (DME-HEPES), penicillin G potassium salt, streptomycin sulfate, dexamethasone, ExtrAvidin–peroxidase conjugate, essentially fatty acid-free bovine serum albumin (BSA), phorbol 12-myristate 13-acetate (PMA), calcium ionophore A23187, pyrrolidinedithiocarbamate (PDTC) ammonium salt, and recombinant human insulin were purchased from Sigma (St. Louis, MO, USA). Fetal bovine serum (FBS) was obtained from MP Biomedicals (Solon, OH, USA). L-Ascorbic acid phosphate magnesium salt n-hydrate, 3-isobutyl-1-methylxanthine (IBMX), and Triglyceride E-Test Kit were provided by Wako (Osaka, Japan). Biotin-conjugated rabbit anti-mouse IgG antibody and biotin-conjugated goat anti-rabbit IgG antibody were supplied by Jackson ImmunoResearch Laboratories (West Grove, PA, USA). Rabbit polyclonal antibodies for murine COX-1 and COX-2, authentic PGs, and arachidonic acid, aspirin, and troglitazone were purchased from Cayman Chemical (Ann Arbor, MI, USA). Rabbit polyclonal antibodies for NF-κB p65 (C-20) or IκB-α (C-21) were supplied by Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibodies specific for PGE_2 and $PGF_{2\alpha}$ were prepared in our laboratories and used for the development of enzyme-linked immunosorbent assay (ELISA) specific for each of them as reported earlier [2,13,14]. M-MLV reverse transcriptase (RT) (Ribonuclease H minus, point mutant) and Polymerase Chain Reaction (PCR) MasterMix were purchased from Promega (Madison, WI, USA). Oligonucleotides used for the RT reaction were provided by Sigma Genosys Japan (Ishikari, Japan). 96-Well microplates for ELISA were purchased from BD Falcon (Durham, NC, USA), and other Petri dishes and multiwell plates with the Iwaki brand for tissue culture were from Asahi Glass (Tokyo, Japan). All other chemicals used are of reagent or tissue culture grade.

2.2. Cell culture, stimulation with PMA and A23187, and adipocyte differentiation

Preadipogenic mouse 3T3-L1 cells (JCRB9014) have been extensively characterized for the differentiation and maturation of adipocytes as described earlier [1,2,15,16]. 3T3-L1 cells were plated at 5×10^4 cells/ml in the growth medium (GM) consisting of DME-HEPES, 10% FBS, 100 U/ml penicillin G, 100 μg/ml streptomycin sulfate, and 200 μM ascorbic acid, and cultured until confluence at 37 °C under the humidified atmosphere of 7% CO_2. The confluent monolayer cells were then cultured for 45 h in the differentiation medium (DM) with 1 μM dexamethasone, 0.5 mM IBMX, and 10 μg/ml insulin to allow the cultured cells to enter the differentiation phase. After this phase, the cells were cultured additionally in the maturation medium (MM) with 5 μg/ml insulin for total 6 days by replacing with the fresh MM every 2 days to promote adipogenesis for fat storage.

For the stimulation of the synthesis of PGE_2 and $PGF_{2\alpha}$ as well as the protein expression of isoformic enzymes involved in the COX pathway, the cultured 3T3-L1 preadipocytes at the growth phase were stimulated for 24 h with a mixture of 0.1 μM PMA and 0.1 μM A23187 in the presence or absence of either 15d-PGJ_2 or Δ^{12}-PG_2 at 0.1 μM or 1 μM, 1 μM troglitazone, or 10 μM PDTC following the pretreatment for 30 min with or without either of the inhibitors. Alternatively, to determine the transcript levels of isoformic enzymes in the COX pathway, the confluent cultured preadipocytes were treated for 3 h with a mixture of PMA and A23187 along with either of the compounds to be tested.

Attempts were made to assess the influence of the pretreatment of cultured preadipocytes during the growth phase for 2 days with a mixture of PMA and A23187 in the presence or absence of each of exogenous PPARγ agonists such as 15d-PGJ_2 and triglitazone, either PGE_2 or $PGF_{2\alpha}$ at 1 μM, or PDTC at 5 μM or 10 μM on the adipogenesis program leading to the accumulation of triacylgycerols and cell number of attached cells after 6 days of the maturation phase. For these, cultured preadipocytes were pretreated with the indicated reagents for 2 days only in GM from 80% to 100% confluence, after which the culture medium was replaced with DM for 45 h during the differentiation phase and then changed to MM for total 6 days during the maturation phase under the standard culture conditions without the indicated stimulators or inhibitors as described above. Separately, the cultured preadipocytes only at the growth phase