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Development of enzyme-linked immunosorbent assay for Δ^{12}-prostaglandin J_2 and its application to the measurement of the endogenous product generated by cultured adipocytes during the maturation phase

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ABSTRACT

Peroxisome proliferator-activated receptor (PPAR)γ is a well-known master regulator for the differentiation and maturation of adipocytes. Prostaglandin (PG) D_2 can be produced in adipocytes and dehydrated to J_2 series of PGs including 15-deoxy-$\Delta^{12,14}$-PGJ_2 (15d-PGJ_2) and Δ^{12}-PGJ_2, which serve as pro-adipogenic prostanoids through the activation of PPARγ. However, the quantitative determination of Δ^{12}-PGJ_2 has not been attempted during the life stage of adipocytes. In this study, we developed an enzyme-linked immunosorbent assay using mouse antiserum specific for Δ^{12}-PGJ_2. According to the standard curve, the amount of Δ^{12}-PGJ_2 can be measured from 0.5 pg to 14.4 ng in an assay. Our antiserum did not recognize most other prostanoids including 15d-PGJ_2, while it only showed the cross-reaction of 28% with unstable PGJ_2. This immunological assay was applied to the determination of the endogenous formation of Δ^{12}-PGJ_2 in cultured 3T3-L1 adipocytes during the maturation phase. The ability of cultured adipocytes to form endogenous Δ^{12}-PGJ_2 increased gradually at an earlier stage of the maturation phase and detectable at higher levels than 15d-PGJ_2. Treatment of cultured cells with either aspirin or indomethacin, a general cyclooxygenase inhibitor, significantly reduced the production of endogenous Δ^{12}-PGJ_2 in the maturation medium as expected. Furthermore, we evaluated individually the exogenous effects of PGJ_2 series at various doses on adipogenesis during the maturation phase. Although Δ^{12}-PGJ_2 was slightly less potent than 15d-PGJ_2, each of these PGJ_2 series rescued effectively both the accumulation of fats and the gene expression of typical adipocyte-markers that were attenuated in the presence of aspirin. Taken together, our findings indicate that endogenous Δ^{12}-PGJ_2 contributes substantially to the up-regulation of adipogenesis program through the activation of PPARγ together with 15d-PGJ_2 during the maturation phase of cultured adipocytes.

1. Introduction

Obesity is characterized by an increase in the number or size of adipocytes, or both [1]. Adipocytes serve as a depot for storage and mobilization of fats, and also contribute to the regulation of food intake to maintain energy homeostasis through the production of adipocytokines like leptin [2]. Moreover, the pathological changes in the quality of adipocytes are associated with the development of insulin resistance following adipocyte inflammation and the related other life-style diseases [3,4]. One of the earliest events in the adipocyte differentiation is the activation of the peroxisome proliferator-activated receptor (PPAR)γ as a master regulator of adipogenesis [5,6]. PPARγ is a member of the nuclear hormone receptor superfamily, involving the related receptors for the steroid, thyroid and retinoid hormones [7]. Since PPARγ is a ligand-activated transcription factor, endogenous ligands should be provided for the activation of PPARγ in adipose tissues. Several studies have described that certain fatty acids and some eicosanoids including prostaglandins

Abbreviations: PPARγ, peroxisome proliferator-activated receptor γ; PG, prostaglandin; 15d-PGJ_2, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2; COX, cyclooxygenase; L-PGDS, lipocalin-type prostaglandin D synthase; ELISA, enzyme-linked immunosorbent assay; DME-HEPES, Dulbecco's modified Eagle medium with 25 mM HEPES; FBS, fetal bovine serum; RT, reverse transcriptase; PCR, polymerase chain reaction; IBMX, 3-isobutyl-1-methylxanthine; RP-HPLC, reverse-phase high-performance liquid chromatography; GM, growth medium; DM, differentiation medium; MM, maturation medium; aP2, adipocyte protein 2; LPL, lipoprotein lipase.

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(PGs) of J_2 series serve as natural ligands to activate PPARγ [8–10].

Adipocytes can produce different types of endogenous prostanoids with opposite effects on adipogenesis. To study the regulation of the arachidonate cascade in adipocytes, we have been making use of cultured mouse preadipogenic 3T3-L1 cells that are a useful system for monitoring the life cycle of adipocytes under the established culture conditions [11–13]. We have reported the stimulation of the endogenous synthesis of anti-adipogenic prostanoids including PGE_2 and $PGF_{2\alpha}$ upon stimulation of preadipocytes with external stimuli [12]. On the other hand, A PGI_2-related compound has been shown to serve as a pro-adipogenic prostanoid in Ob1771 preadipose cells [14]. Moreover, PGD_2 generated through the arachidonate cyclooxygenase (COX) pathway undergoes non-enzymatic dehydration to produce biologically active PGJ_2 derivatives including 15-deoxy-$\Delta^{12,14}$-PGJ_2 (15-PGJ_2) and Δ^{12}-PGJ_2 through the formation of PGJ_2 as an unstable intermediate [15,16]. Earlier, we [11] and other groups [17,18] have revealed the specific gene expression of lipocalin-type PGD synthase (L-PGDS) in cultured 3T3-L1 cells. More recently, we demonstrated the endogenous synthesis of 15-PGJ_2 in 3T3-L1 adipocytes and its contribution to the up-regulation of fat storage in adipocytes during the maturation phase [13]. However, until now no attempts have been described to determine quantitatively the endogenous synthesis of Δ^{12}-PGJ_2, another PGJ_2 series generated from the spontaneous dehydration of PGD_2, in life stages of cultured adipocytes. Moreover, it remains elusive about the relative contribution of endogenous Δ^{12}-PGJ_2 and 15d-PGJ_2 to adipogenesis in the terminal differentiation during the maturation phase.

In this study, we prepared a useful antiserum specific for Δ^{12}-PGJ_2 and successfully developed a sensitive enzyme-linked immunosorbent assay (ELISA) to quantify endogenous Δ^{12}-PGJ_2 produced by cultured 3T3-L1 adipocytes. Here, we describe the evidence for the generation of endogenous Δ^{12}-PGJ_2 during the maturation phase of adipocytes. Furthermore, we assessed the ability of endogenous Δ^{12}-PGJ_2 to contribute to the promotion of adipogenesis in an autocrine manner by comparing with that of 15d-PGJ_2.

2. Materials and methods

2.1. Materials

Dulbecco's modified Eagle medium with 25 mM HEPES (DME-HEPES), penicillin G potassium salt, streptomycin sulfate, dexamethasone, recombinant human insulin, and ExtrAvidin-peroxidase conjugate were purchased from Sigma (St. Louis, MO, USA). Biotin-conjugated rabbit anti-mouse IgG antibody was supplied by Jackson ImmunoResearch Laboratories (West Grove, PA, USA). Fetal bovine serum (FBS) was supplied by MP Biomedicals (Solon, OH, USA). Authentic PGs, aspirin, and troglitazone were obtained from Cayman Chemical (Ann Arbor, MI, USA). M-MLV reverse transcriptase (RT) (Ribonuclease H minus, point mutant) and polymerase chain reaction (PCR) MasterMix were purchased from Promega (Madison, WI, USA). Triglyceride E-Test Kit, and 3-isobutyl-1-methylxanthine (IBMX) were obtained from Wako (Osaka, Japan). Oligonucleotides used for the PCR 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). L-column ODS (4.6 mm × 250 mm) for reverse-phase high-performance liquid chromatography (RP-HPLC) was supplied by Chemical Evaluation and Research Institute (Tokyo, Japan). All other chemicals used are of reagent or tissue culture grade. Other materials used for RT-PCR and ELISA were obtained as described previously [11,12,19].

2.2. Cell culture and adipocyte differentiation

The preadipogenic mouse 3T3-L1 cells (JCRB9014) were plated at 5×10^4 cells/ml in the growth medium (GM) containing DME-HEPES, 10% FBS, 100 U/ml penicillin G, 100 μg/ml streptomycin sulfate, and 200 μM ascorbic acid, and cultured at 37 °C under 7% CO_2. After the cells become confluent, the monolayer cells were exposed to the differentiation medium (DM) corresponding to GM supplemented with 1 μM dexamethasone, 0.5 mM IBMX, and 10 μg/ml insulin for 45 h. Then, the culture medium was replaced with maturation medium (MM) consisting of GM containing 5 μg/ml of insulin, and refed every 2 days to promote adipogenesis of mature adipocytes as described earlier [11–13,20,21]. The compounds to be tested were dissolved in ethanol as a vehicle and added to the culture medium. The volume of ethanol was adjusted to 0.2%.

2.3. ELISA for Δ^{12}-PGJ_2 and 15d-PGJ_2

For generating the antiserum specific for Δ^{12}-PGJ_2, BALB/c mice were immunized with a conjugate of Δ^{12}-PGJ_2 and bovine serum albumin according to our earlier protocols [11,12,19,22,23]. For the ELISA of Δ^{12}-PGJ_2, the conjugate of Δ^{12}-PGJ_2 and γ-globulin was prepared and coated in a 96-well ELISA plate as an immobilized antigen. The resulting immunocomplex was allowed to react competitively with a mouse antiserum specific for Δ^{12}-PGJ_2 in the presence or absence of various amounts of the standard Δ^{12}-PGJ_2 or samples to be measured. The resulting immunocomplex was detected spectrophotometrically by monitoring the peroxidase reaction using *o*-phenylenediamine as a substrate after binding to biotin-conjugated rabbit anti-mouse IgG antibody and the subsequent ExtrAvidin-peroxidase conjugate as described previously [11–13,23]. Standard curve of Δ^{12}-PGJ_2 was generated by diluting authentic compounds serially using an equal mixture of fresh MM and the ELISA buffer containing phosphate-buffered saline without Ca^{2+} and Mg^{2+} ions, 0.5% bovine serum albumin and 0.02% NaN_3. For the analysis of the samples, MM during the maturation phase was collected at the indicated period and diluted serially by every 2-fold with an equal mixture of fresh MM and the ELISA buffer as above. The resulting solutions at three different dilutions in duplicate for each sample were applied to the determination of the amount of Δ^{12}-PGJ_2 series by the specific ELISA. To obtain the final concentration of Δ^{12}-PGJ_2 in the original MM from unknown samples, the values within the range of 10–90% of the binding from the calibration curve were used for the calculation of the average vales of each sample after multiplying the dilution folds. Moreover, ELISA specific for 15d-PGJ_2 was performed for the determination of the endogenous product synthesized by adipocytes during the maturation phase as we reported previously [13].

2.4. RP-HPLC of PG extracts for the immunological detection of Δ^{12}-PGJ_2

The MM of 3T3-L1 adipocytes after 10 days of maturation phase was collected and used for extraction of PGs. The resulting MM (1 ml) was acidified with 1 ml of 1 M sodium acetate (pH 3.5), and then PGs were extracted with 2 ml of ethyl acetate. The extracts were analyzed by RP-HPLC using a Shimadzu LC-9A HPLC system equipped with a SPD-6AV UV-VIS detector and L-column ODS (4.6 mm × 250 mm). The HPLC column was eluted with acetonitrile/17 mM phosphoric acid (50:50, v/v) as a mobile phase at a flow rate of 1 ml/min. The peaks of authentic Δ^{12}-PGJ_2, PGJ_2, and 15d-PGJ_2 were detected by monitoring the absorbance at 230 nm.