Prostaglandins & other Lipid Mediators 123 (2016) 16–27

Contents lists available at ScienceDirect

Prostaglandins and Other Lipid Mediators

Original research article

Pretreatment of cultured preadipocytes with arachidonic acid during the differentiation phase without a cAMP-elevating agent enhances fat storage after the maturation phase

Ferdous Khan [a], Pinky Karim Syeda [a], Michael Nii N. Nartey [a], Mohammad Shahidur Rahman [a], Mohammad Safiqul Islam [a], Kohji Nishimura [b], Mitsuo Jisaka [a], Fumiaki Shono [c], Kazushige Yokota [a,*]

[a] Department of Life Science and Biotechnology, Shimane University, 1060 Nishikawatsu-cho, Matsue, Shimane 690-8504, Japan
[b] Department of Molecular and Functional Genomics, Center for Integrated Research in Science, Shimane University, 1060 Nishikawatsu-cho, Matsue, Shimane 690-8504, Japan
[c] Department of Clinical Pharmacy, Faculty of Pharmaceutical Sciences, Tokushima Bunri University, 180 Yamashiro-cho, Tokushima-shi, Tokushima 7700-8514, Japan

ARTICLE INFO

Article history:
Received 14 December 2015
Received in revised form 4 February 2016
Accepted 24 February 2016
Available online 27 February 2016

Keywords:
Arachidonic acid
Differentiation phase
Preadipocyte
Prostaglandin
Adipogenesis

ABSTRACT

Arachidonic acid (AA) and the related prostanoids exert complex effects on the adipocyte differentiation depending on the culture conditions and life stages. Here, we investigated the effect of the pretreatment of cultured 3T3-L1 preadipocytes with exogenous AA during the differentiation phase without 3-isobutyl-1-methylxanthine (IBMX), a cAMP-elevating agent, on the storage of fats after the maturation phase. This pretreatment with AA stimulated appreciably adipogenesis after the maturation phase as evident with the up-regulated gene expression of adipogenic markers. The stimulatory effect of the pretreatment with AA was attenuated by the co-incubation with each of cyclooxygenase (COX) inhibitors. Among exogenous prostanoids and related compounds, the pretreatment with MRE-269, a selective agonist of the IP receptor for prostaglandin (PG) I_2, strikingly stimulated the storage of fats in adipocytes. The gene expression analysis of arachidonate COX pathway revealed that the transcript levels of inducible COX-2, membrane-bound PGE synthase-1, and PGF synthase declined more greatly in cultured preadipocytes treated with AA. By contrast, the expression levels of COX-1, cytosolic PGE synthase, and PGI synthase remained constitutive. The treatment of cultured preadipocytes with AA resulted in the decreased synthesis of PGE_2 and $PGF_2\alpha$ serving as anti-adipogenic PGs although the biosynthesis of pro-adipogenic PGI_2 was up-regulated during the differentiation phase. Moreover, the gene expression levels of EP4 and FP, the respective prostanoid receptors for PGE_2 and $PGF_{2\alpha}$, were gradually suppressed by the supplementation with AA, whereas that of IP for PGI_2 remained relatively constant. Collectively, these results suggest the predominant role of endogenous PGI_2 in the stimulatory effect of the pretreatment of cultured preadipoccytes with AA during the differentiation phase without IBMX on adipogenesis after the maturation phase.

Abbreviations: AA, arachidonic acid; IBMX, 3-isobutyl-1-methylxanthine; COX, cyclooxygenase; PG, prostaglandin; PPAR, peroxisome proliferator-activated receptor; L-PGDS, lipocalin-type PGD synthase; 15d-PGJ_2, 15-deoxy-$\Delta^{12,14}$-PGJ_2; DMEM-HEPES, Dulbecco's modified Eagle's medium with HEPES; FBS, fetal bovine serum; RT, reverse transcriptase; PCR, polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay; GM, growth medium; DM, differentiation medium; MM, maturation medium; mPGES, membrane-bound PGE synthase; cPGES, cytosolic PGE synthase; PGFS, PGF synthase; PGIS, PGI synthase; C/EBP, CCAAT/enhancer-binding protein; LPL, lipoprotein lipase; GLUT, glucose transporter; aP-2, adipocyte protein 2; PKA, protein kinase A; CRTH2, chemoattractant receptor-homologous molecule expressed on Th2 cells; Dex, dexamethasone; 18:2 (n-6), linoleic acid; 20:4 (n-6), arachidonic acid; 18:3 (n-3), α-linolenic acid; 20:5 (n-3), 5, 8, 11, 14, 17-eicosapentaenoic acid; 22:6 (n-3), 4, 7, 10, 13, 16, 19-docosahexaenoic acid; Indo, indomethacin.

* Corresponding author.
E-mail address: yokotaka@life.shimane-u.ac.jp (K. Yokota).

http://dx.doi.org/10.1016/j.prostaglandins.2016.02.003
1098-8823/

1. Introduction

Adipose tissue functions as the storage site of triacyglycerols, which can be used for the mobilization of free fatty acids depending on nutritional status and hormonal response. The body fat mass is controlled by adipogenesis involving the differentiation of preadipocytes into mature adipocytes. Excess uptake of fuel molecules and lower energy expenditure are well known to generate obesity with an increase in the number or size of white adipocytes. In addition, adipocytes serve as an endocrine organ to secrete various bioactive molecules called adipokines, such as leptin, adiponectin, and resistin, to affect other tissues in vivo [1–3]. Hypertrophic adipocytes from obese tissue show alterations in the profiles of adipokines to secrete pro-inflammatory factors including tumor necrosis factor-α, interleukin-6, and monocyte chemoattractant protein-1, which are associated with adipocyte inflammation and insulin resistance [4]. Under these conditions, free fatty acids are released to impair the oxidation of glucose though the inhibition of glucose uptake in adipose tissue and other organs, contributing to the onset of insulin resistance [5]. However, certain types of fatty acids and their metabolites have been shown to act as endogenous ligands for peroxisome proliferator-activated receptor (PPAR) γ, which serves as a master regulator of adipogenesis and a positive regulator of insulin sensitivity [6]. Different classes of free fatty acids, such as saturated, monounsaturated, n-6 and n-3 polyunsaturated fatty acids, are considered to affect the adipogenic process in different manners. Nevertheless, the cellular mechanisms underlying these opposite effects have not been understood fully.

Arachidonic acid (AA), a member of n-6 polyunsaturated fatty acids, can be converted to several prostanoids with pro-adipogenic or anti-adipogenic effects through the arachidonate cyclooxygenase (COX) pathway with two types of COX isoforms, the rate-limiting enzymes of this pathway [7]. A previous animal study described that heterozygous mice deficient for the COX-2 gene exhibit more increased fat mass as compared with wild-type mice, suggesting the functional coupling of COX-2 with the generation of anti-adipogenic prostanoids [8]. Preadipogenic mouse 3T3-L1 cells have been utilized as a useful model cell culture system for studying different life stages of adipogenesis under the defined culture conditions including growth, differentiation, and maturation phases [9,10]. Recent studies have established that prostaglandin (PG) E_2 and $PGF_{2\alpha}$ are synthesized preferentially in cultured 3T3-L1 preadipocytes and serve as anti-adipogenic prostanoids [11]. PGE_2 has been shown to inhibit the differentiation of cultured 3T3-L1 cells through its specific EP4 receptor, one of the receptor subtypes for PGE_2 [12]. Alternatively, $PGF_{2\alpha}$ can also inhibit the differentiation of 3T3-L1 preadipocytes into the adipocytes by interacting with its FP receptor [13]. On the other hand, previous studies have reported the selective expression of lipocalin-type PGD synthase (L-PGDS) necessary for the biosynthesis of PGD_2 after the maturation phase of cultured 3T3-L1 cells [14,15]. PGD_2 readily undergo the non-enzymatic dehydration to give biologically active PGJ_2 derivatives including 15-deoxy-$\Delta^{12,14}$-PGJ_2 (15d-PGJ_2) and Δ^{12}-PGJ_2 [16,17]. Of these, 15d-PGJ_2 is the most potent natural activator for the nuclear hormone receptor PPARγ [18,19]. We have also shown that cultured adipocytes after the maturation phase have the ability to increasingly produce endogenous PGs of J_2 series and contribute to the up-regulation of adipogenesis [20]. Therefore, PGD_2 and the related PGJ_2 derivatives can be regarded as pro-adipogenic prostanoids. More recently, we have reported that endogenous synthesis of prostacyclin, PGI_2, is also positively regulated after the maturation phase of cultured 3T3-L1 adipocytes [21].

Cultured 3T3-L1 preadipocytes have been usually exposed to the differentiation medium supplemented with 3-isobutyl-1-methylxanthine (IBMX), insulin, and dexamethasone to induce the program to drive the resting cells into adipocytes [11,12,20,21,22–24]. The addition of exogenous AA to the differentiation medium has been shown to suppress the differentiation of cultured 3T3-L1 preadipocytes [22–24]. On the other hand, earlier reports described that exogenous AA in the culture medium without IBMX was effective to induce the differentiation of Ob1771 preadipose cells [25,26] and 3T3-F442A cells [25]. Hence, we hypothesized that the opposite effects of exogenous AA on adipogenesis in different cell lines could be explained by presence or absence of IBMX, a cAMP elevating agent. In this study, we attempted to determine the effect of the pretreatment of cultured 3T3-L1 preadipocytes with AA during the differentiation phase without IBMX on adipogenesis of cultured adipocytes after the maturation phase, and to seek the cellular mechanisms underlying the effect of AA.

2. Materials and methods

2.1. Materials

Dulbecco's modified Eagle's medium with HEPES (DMEM-HEPES), penicillin G potassium salt, streptomycin sulfate, dexamethasone, recombinant human insulin, fatty acid-free bovine serum albumin, and ExtraAvidin-peroxidase conjugate were obtained from Sigma (St. Louis, MO, USA). Fetal bovine serum (FBS) was the product of MP Biochemicals (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 was supplied by Jackson ImmunoResearch Laboratories (West Grove, PA, USA). AA, aspirin, indomethacin, H-89, authentic fatty acids, PGs, MRE-269, CAY10441, and cAMP EIA kit were purchased from Cayman Chemical (Ann Arbor, MI, USA). M-MLV reverse transcriptase (RT) (Ribonuclease H minus, point mutant) and polymerase chain reaction (PCR) MasterMix were obtained from Promega (Madison, WI, USA). Oligonucleotides used for the PCR amplification were provided by Sigma Genosys Japan (Ishikari, Japan). 96-Well microplates for enzyme-linked immunosorbent assay (ELISA) were supplied by BD Falcon (Durham, NC, USA), and other Petri dishes for cull culture were from Asahi Glass (Tokyo, Japan). Antibodies specific for PGE_2, $PGF_2\alpha$, and 6-keto-$PGF_1\alpha$ were prepared in our laboratory and used for the development of ELISA for each of them as described earlier [11,21,27,28]. All other chemicals used here are of reagent or tissue culture grade.

2.2. Cell culture of 3T3-L1 cells and differentiation to adipocytes

The mouse 3T3-L1 preadipogenic cell line (JCRB9014) was obtained from JCRB Cell Bank (Osaka, Japan). The cells were plated at 5×10^4 cells/ml in the growth medium (GM) containing DMEM-HEPES, 10% FBS, 100 units/ml penicillin G, 100 μg/ml streptomycin sulfate, and 200 μM ascorbic acid, and grown until confluence at 37 °C under 7% CO_2. Under the standard culture conditions, the confluent monolayer cells were exposed to the differentiation medium (DM) supplemented with 1 μM dexamethasone, 0.5 mM IBMX, and 10 μg/ml insulin for 2 days to enter the differentiation phase as described earlier [11,21,29]. To promote the storage of fats in adipocytes during the maturation phase, the treated cells were cultured furthermore in the maturation medium (MM) with 5 μg/ml insulin for a total of 10 days by replacing with the fresh MM every 2 days.

2.3. Adipogenesis treatments

To assess the influence of the pretreatment of cultured preadipocytes with AA or other compounds during the