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15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2 impairs the functions of histone acetyltransferases through their insolubilization in cells

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ABSTRACT

The cyclopentenonic prostaglandin 15-deoxy-$\Delta^{12,14}$-PG J_2 (15d-PGJ_2) is a metabolite derived from PGD_2. Although 15d-PGJ_2 has been demonstrated to be a potent ligand for peroxisome proliferator activated receptor γ (PPARγ), the functions are not fully understood. In order to examine the effect of 15d-PGJ_2 on histone acetyltransferases (HATs), several lines of cell including mouse embryonic fibroblast (MEF) cells were exposed to 15d-PGJ_2. Three types of HAT, p300, CREB-binding protein (CBP), and p300/CBP-associated factor (PCAF), selectively disappeared from the soluble fraction in time- and dose-dependent manners. Inversely, HATs in the insoluble fraction increased, suggesting their conformational changes. The decrease in the soluble form of HATs resulted in the attenuation of NF-κB-, p53-, and heat shock factor-dependent reporter gene expressions, implying that the insoluble HATs are inactive. The resultant insoluble PCAF and p300 seemed to be digested by proteasome, because proteasome inhibitors caused the accumulation of insoluble HATs. Taken together, these results indicate that 15d-PGJ_2 attenuates some gene expressions that require HATs. This inhibitory action of 15d-PGJ_2 on the function of HATs was independent of PPARγ, because PPARγ agonists could not mimick 15d-PGJ_2 and PPARγ antagonists did not inhibit 15d-PGJ_2.

Introduction

Cyclopentenone types of prostaglandin J (PGJs) are bioactive molecules that exist in various tissues and cells, and are generated from a sole precursor, PGD_2, by a series of reactions *in vivo* (see reviews in Refs. [1–3]). The non-enzymic dehydration of the cyclopentanone ring of PGD_2 converts it to PGJ_2 with a cyclopentenone ring [4]. PGJ_2 is further metabolized sequentially to Δ^{12}-PGJ_2 and then 15d-PGJ_2 by isomerization and dehydration [2,4]. Δ^{12}-PGJ_2 and 15d-PGJ_2 were detected in human urine and cells, suggesting their natural occurrence in human body [1,2]. 15d-PGJ_2 possesses diverse biological activities including anti-neoplastic-, anti-inflammatory-, anti-viral-, and growth-regulatory activities in various types of cell [1,2]. A part of the bioactivities of 15d-PGJ_2 is mediated by a nuclear receptor, PPARγ [1–3], because 15d-PGJ_2 is also a potent ligand of the nuclear receptor [5,6]. 15d-PGJ_2 also plays a novel role in the regulation of transcription factors including NF-κB and AP-1, which are inhibited by covalent modification due to 15d-PGJ_2 and/or non-covalent interaction with 15d-PGJ_2 [3]. Thus, 15d-PGJ_2 regulates the expression of various genes in a PPARγ-dependent and -independent manners [1,3].

CBP, p300, and PCAF are histone acetyltransferases (HATs), which acetylate various nuclear proteins including histones and transcription factors such as p53 and NF-κB (see reviews in Refs. [7–9]). As transcriptional co-activators, they make up a large transcriptional complex with transcription factors, and are recruited to chromatin-remodeling complexes on enhancers of various gene promoters in response to growth factors. Together, these facts suggest that HATs play crucial roles in the expression of genes involved in cell cycle progression, differentiation, and apoptosis, and that the functional impairment of HATs causes several diseases [10,11]. Nevertheless, the mechanism by which HAT activity is regulated in response to internal and external stimuli remains to be

Abbreviations: ALLM, *N*-acetyl-Leu-Leu-methioninal; BADGE, bisphenol A diglycidil ether; CBP, CREB (cAMP response element-binding protein) binding protein; 15d-PGJ_2, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HAT, histone acetyltransferase; HSE, heat shock element; HSF, heat shock factor; MEF, mouse embryonic fibroblast; MG115, benzyloxycarbonyl-Leu-Leu-norvalinal; MG132, benzyloxycarbonyl-Leu-Leu-Leucinal; NF-κB, nuclear factor κB; PARP, poly(ADP- ribose)polymerase; PCAF, p300/CBP-associated factor; PG, prostaglandin; PPAR, peroxisome proliferator activated receptor; PSI, benzyloxycarbonyl-Ile-[(2S)-2-amino-4-(*t*-butyloxycarbonyl)butanoyl]-)-Ala-Leucinal; SDS, sodium dodecyl sulfate; TLCK, *p*-toluenesulfonyl-Lys-chloromethylketone; TNFα, tumor necrosis factor α; z-VAD-fmk, benzyloxycarbonyl-Val-Ala-[(2S)-2-amino-3-(methoxy-carbonyl)propionyl]fluoromethane.

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elucidated. In this report, we demonstrated that 15d-PGJ_2 caused the conversion of native HATs into insoluble forms, and, thereby, gene expressions that require HATs were down-regulated in a PPARγ-independent fashion. Further analysis using protease inhibitors suggested that the insoluble forms of HATs are removed by proteasome.

Materials and methods

Reagents. 15d-PGJ_2, Δ^{12}-PGJ_2, PGJ_2, PGD_2, PGA_1, troglitazone, ciglitazone, indomethacin, Wy14643, and GW9662 were purchased from Cayman Chemical (MI, USA), and BADGE was from Tocris Biosci. (UK). PSI, MG132, MG115, z-VAD-fmk, leupeptin, phosphoramidon, E64, lactacystin, and Ac-VEID-CHO were purchased from Peptide Institute Inc. (Osaka, Japan). Protease inhibitor cocktail, TLCK, and ALLM were obtained from Nacalai Tesque, Inc. (Kyoto, Japan).

Cells. MEF, SH-SY5Y, HeLa, 294T, H1299, and N1E cells were grown and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS) in a humidified CO_2-incubator.

Treatment of cells with prostaglandins. Cells (1×10^5 cells) were seeded into 6-well plates. Twenty-four hours after seeding, the culture medium was changed to OPTI-MEM1 medium (Gibco, Canada) and treated with 20 μM of prostaglandin at 37 °C for the indicated times. After treatment, the cells were washed once with phosphate-buffered saline (PBS), harvested by trypsin–EDTA, and then collected by low speed centrifugation. The cells were lysed in 50 μl of a lysis buffer (10 mM Hepes–KOH, pH 7.5, 0.5 mM DTT, 0.5 mM EDTA, 0.5 mM EGTA, 0.42 M NaCl, and 1/100 vol. of protease inhibitor cocktail) containing a 0.1% concentration of a nonionic detergent, Nonidet P-40 (NP-40), by mixing with a vortex-mixer. The total lysate was centrifuged at 15,000*g* for 20 min, and the resultant supernatant was defined as a "soluble fraction". On the other hand, the residual precipitate was mixed with an SDS-containing buffer (62.5 mM Tris–HCl, pH 6.8, 2% SDS, 10% glycerol, and 5% β-mercaptoethanol), sonicated by a sonicator, and then re-centrifuged under the above conditions. The resultant supernatant was defined as an "insoluble fraction". The protein concentration of the extracts obtained was measured using the Bio-Rad protein assay kit.

Immuno-blotting. The extracts (corresponding to 20 μg of protein) were separated by SDS–PAGE and transferred to a polyvinylidene fluoride (PVDF) membrane. Then, the transferred proteins were immuno-stained using a combination of appropriate antibodies, as described previously [12]. The primary antibodies used were anti-PCAF (E-8), anti-p300 (N-15), anti-CBP (C-20) (Santa Cruz Biotechnology), anti-glyceraldehyde-3-phosphate dehydrogenase (G3PDH) (Ambion), anti-poly(ADP-ribose)polymerase-1 (PARP-1) (R&D systems), anti-p53 (Stressgen), and anti-Hsp90 antibodies (Transduction Lab.).

Analysis of the half-lives of HATs in MEF cells. For the determination of the half-lives of HATs, MEF cells were treated with 100 μg/ml of cycloheximide and harvested at the indicated time as described in the legends of figures. Equal amounts of protein were analyzed by SDS–PAGE followed by immuno-blotting.

Reporter luciferase assay. Fifty ng of reporter plasmids, pNF-κB-luciferase, pp53-TA-luciferase, or pHSE-luciferase (Clontech) were co-transfected with 50 ng of pRSV-β-galactosidase (Promega) into MEF cells (1×10^5 cells/well of a 24-well plate) by Lipofectamine 2000 (Invitrogen), as described in our previous report [13]. Twenty-four hours after, the cells were pretreated with 20 μM of either PGJ_2, Δ^{12}-PGJ_2, or 15d-PGJ_2 in OPTI-MEM1 culture media for the indicated times. In some experiments, after the pretreatment with 15d-PGJ_2 for 2 h, the cells carrying pNF-κB-luciferase were further incubated in a fresh DMEM medium supplemented with FBS in the presence or absence of 10 ng/ml of TNFα for 3 h. Similarly, the cells carrying pHSE-luciferase were heated at 42 °C for 20 min, and then cultured at 37 °C for 2 h. The luciferase and β-galactosidase activities in cell lysate were assayed as described previously [14]. The transfection efficiency was standardized against β-galactosidase activity [14], and the luciferase activity of control cells was set at 100%.

Results

15d-PGJ_2 decreases soluble forms of PCAF, p300, and CBP in MEF cells

In order to examine the effect of 15d-PGJ_2 on MEF cells, proteins in the soluble fractions were subjected to Western-blot analyses (Fig. 1A). 15d-PGJ_2 significantly decreased PCAF, p300, and CBP in a time- and dose-dependent manner. Among these HATs, PCAF was most rapidly decreased to about 40% at 1 h, and had almost completely disappeared at 3 h after treatment with 20 μM 15d-PGJ_2 (Fig. 1B, left panel). The decay curves indicated that the half-lives of PCAF, p300, and CBP were approximately 40 min, 3 h, and 4 h, respectively (Fig. 1B, left panel). These half-lives were clearly shorter than those of HATs in cells in which *de novo* protein synthesis was inhibited by cycloheximide (CHX), because the half-lives of HATs in the cells were over 8 h (Fig. 1C). These results suggest that the 15d-PGJ_2-induced decrease in soluble HATs is not only due to the inhibition of *de novo* protein synthesis.

15d-PGJ_2-induced reduction in PCAF and p300 are not cell type-specific events

As shown in Fig. 1D, 15d-PGJ_2 reduced the soluble forms of PCAF and p300, in all of 6 and 3 cell lines tested, respectively. Like p300, CBP was decreased in the three cell lines (data not shown). These results indicate that the effect of 15d-PGJ_2 is not cell type-specific.

15d-PGJ_2 causes a decrease in soluble HATs in a PPARγ-independent manner

Since 15d-PGJ_2 is a high-affinity natural ligand for PPARγ [5,6], we examined whether PPARγ is involved in the 15d-PGJ_2-induced reduction of soluble HATs.

Firstly, we attempted to block the 15d-PGJ_2-mediated reduction of soluble HATs by PPARγ-specific antagonists, GW9662 or BADGE [15,6,16] (Fig. 2A). As shown in this figure, neither antagonist prevented the 15d-PGJ_2-mediated reduction of soluble HATs, though 40 μM of BADGE slightly increased soluble PCAF in the absence of 15d-PGJ_2, (Fig. 2A). Next, the effect of PPARγ-specific agonists such as troglitazone, indomethacin, and ciglitazone were examined [16] (Fig. 2B). Even at a concentration sufficient to activate PPARγ [1], none of theses agonists mimicked 15d-PGJ_2 to reduce soluble PCAF and p300 (Fig. 2B). Together, these results show that 15d-PGJ_2 causes the reduction of HATs in a PPARγ-independent manner.

In addition, although 15d-PGJ_2 is a weak agonist of PPARα [6], a potent PPARα ligand, Wy14643 [6], however, failed to cause a reduction of PCAF and p300 (Fig. 2B).

Precursors of 15d-PGJ_2 are weak inducers of the reduction of soluble HATs

In vivo, 15d-PGJ_2 is synthesized by a pathway in which PGD_2 is converted sequentially to PGJ_2, Δ^{12}-PGJ_2, and finally 15d-PGJ_2 [1,2]. Therefore, we compared the effect of these PGs with that of 15d-