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Aromatase expression in a human osteoblastic cell line increases in response to prostaglandin E_2 in a dexamethasone-dependent fashion

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ABSTRACT

Recent progress supports the importance of local estrogen secretion in human bone tissue to increase and maintain bone-mineral density. In a previous report, we found that forskolin (FSK) synergistically induces aromatase (CYP19: a rate-limiting enzyme for estrogen synthesis) expression in dexamethasone (Dex) dependent manner in a human osteoblastic cell line, SV-HFO [Watanabe M, Ohno S, Nakajin S. Forskolin and dexamethasone synergistically induce aromatase (CYP19) expression in the human osteoblastic cell line SV-HFO. Eur J Endocrinol 2005;152:619–24]. In this report, we investigated whether prostaglandin (PG) E_2 induces estrogen production, in other words, if PGE_2 exerts the same effect as FSK because PGE_2 is the major prostanoid in the bone and is one of the key molecules in the osteoblast. We found PGE_2 up-regulates aromatase activity synergistically, but this up-regulation depends on Dex. *CYP19* gene expression was also increased synergistically by Dex and PGE_2. Promoter I.4 was activated synergistically by PGE_2 and Dex. PGE_2 receptor, EP_1, EP_2 and EP_4 were involved in the up-regulation of aromatase activity in response to PGE_2 in a Dex-dependent manner. The cAMP-PKA pathway and Ca^{2+} signaling pathway were involved in the up-regulation of aromatase activity in response to PGE_2. Furthermore, glucocorticoid response element on promoter I.4 sequence was an essential minimum requirement for its activity and synergism of PGE_2 and Dex. These findings are the first report on osteoblastic cell line which uses predominantly promoter I.4 to drive aromatase expression. These findings also suggest that endogenous PGE_2 produced in bone mainly may synergistically support local estrogen production in osteoblastic cells in the presence of glucocorticoid.

1. Introduction

Prostaglandins (PGs) are produced by bone and have powerful effects on bone metabolism. PGE_2 is the major prostanoid in bone and is one of the key molecules in the osteoblast [1]. PGE_2 is also a well-known up-regulator of aromatase (CYP19: rate-limiting enzyme of estrogen biosynthesis) activity and gene expression in human adipose stromal cells [2], adrenal tumor-derived cell line [3], and endometriosis [4]. The human CYP19 gene has multiple untranslated first exon I and coding regions (exon II to exon X). Multiple first exon I contains at least 10 distinct promoters regulated in a tissue- or signaling pathway-specific manner [5]. Among several tissue-specific promoters, promoter II is activated by PGE_2 in those cases.

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In a previous paper, we found that forskolin (FSK) synergistically induces aromatase expression with dexamethasone (Dex) in SV-HFO cells, a human osteoblastic cell line [6]. Therefore, we postulated that some agents that increase intracellular cAMP levels may be involved in the regulation of aromatase expression in bone tissue. In bone tissue, PGE_2 is secreted from osteoblast lineage [1]. One of the second messengers of PGE_2 is cAMP. Based on this, we postulated that PGE_2 would be one of the regulators of aromatase expression in osteoblasts in vivo. In this study, we examined the effect of PGE_2 upon the expression of aromatase in the human osteoblastic cell line SV-HFO. SV-HFO cells retain the properties of human osteoblasts and are widely used for the investigation of phenomena in human osteoblasts [7–10]. Among several tissue-specific promoters, promoter I.4 is activated even in the presence of FSK [6]. Aromatase expression and promoter I.4 activity were not induced by FSK alone. We examined if PGE_2 exerts the same effect as FSK in SV-HFO.

2. Experimental

2.1. Materials

Dexamethasone was purchased from Wako Pure Chemical Industries (Osaka, Japan), RPMI1640 medium from Sigma (St. Louis, MO), RPMI1640 medium without Phenol Red and penicillin/streptomycin from Invitrogen (Carlsbad, CA), fetal bovine serum from Sanko Junyaku (Tokyo, Japan), and [1β-^{3}H]androstenedione from Perkin-Elmer Corp. (Boston, MA). PGE_2 was obtained from Nacalai Tesque (Kyoto, Japan). Sulprostone, misoprostol, and AH6809 were obtained from Cayman Chemical. ONO-DI-004 and ONO-AE1-259-01 [11,12] were generous gifts provided by Ono Pharmaceutical Co. Ltd. (Osaka, Japan). H-89 was purchased from Seikagaku Corporation (Tokyo, Japan), PD98059 and KN-93 from Cosmo Bio Co. Ltd. (Tokyo, Japan), and bisindolylmaleimide I from Calbiochem (San Diego, CA). These EP agonists and protein kinase inhibitors were dissolved in ethanol or dimethyl sulfoxide (DMSO). The final concentration of each solvent in the medium was 0.1% (v/v). Other chemicals and reagents were of the highest grade commercially available.

2.2. Cells

SV-HFO, a human fetal osteoblastic cell line, was a generous gift from Dr Hideki Chiba (Sapporo Medical University, Hokkaido, Japan). The cells were cultured in RPMI1640 medium supplemented with 10% fetal bovine serum, penicillin, and streptomycin at 37 °C and 5% CO_2.

2.3. Aromatase assay

Cells were seeded onto 24-well plates at a density of 2.0×10^5 cells/mL and 500 μL/well. After 2 days of culturing, the cells were treated with the RPMI1640 medium without Phenol Red for 24 h (serum starvation). After serum starvation, they were treated with the RPMI1640 medium without Phenol Red but including Dex and/or PGE_2 for 24 h. After treatment, aromatase activity was measured as described previously [3].

2.4. Reverse transcriptase (RT)-PCR

Total RNA of the cells was extracted using ISOGEN (Nippongene, Toyama, Japan). First-strand cDNA was prepared using avian myeloblastosis virus RT (Promega, Madison, WI, USA). The amplification of human *CYP19* gene transcript using the primers derived from exon II and exon III, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were performed as described previously [3]. The RT-PCR of the gene transcripts of prostaglandin receptors (EP_1, EP_2, EP_3 and EP_4) was based on published data [3,13–16].

2.5. Transfection and luciferase assay

Cells were seeded at a density of 2.0×10^5 cells/mL and 500 μL/well. After 24 h culturing, firefly luciferase reporter vector and sea-pansy luciferase internal control vector (Promega) were transiently transfected using Fugene6 transfection reagent (Roche, Mannheim, Germany). After 16 h transfection, the cells were serum starved for 4 h and treated with various agents for 4 h. After treatment, the firefly luciferase and sea-pansy luciferase activities were measured using a Dual luciferase reporter assay kit (Promega) and a Sirius luminometer (Berthord, Pforzheim, Germany). Promoter activity was calculated by dividing firefly luciferase reporter activity by sea-pansy luciferase internal control activity.

2.6. Statistical analysis

Statistically significant differences between the experimental groups were determined by one-way ANOVA followed by Fisher's protected least-significant difference multiple comparison test (Fisher's PLSD multiple comparison) or Dunnett's multiple comparison test. Statistical analysis was performed using a StatView 5.0 software program (SAS Institute, Cary, NC, USA) or Prism4 for Windows (GraphPad Software, San Diego, CA, USA). The point of a minimal statistically significant difference was set at $P \leq 0.05$.

3. Results

3.1. PGE_2 up-regulates aromatase activity in SV-HFO in the presence of Dex

To determine if PGE_2 up-regulates aromatase activity in SV-HFO synergistically, we measured aromatase activity in the presence and absence of Dex and PGE_2. As shown in Fig. 1, PGE_2 significantly and dose-dependently up-regulated aromatase activity (columns C–F) whereas PGE_2 alone could not up-regulate aromatase activity (column G). This result suggests that PGE_2 up-regulates aromatase activity synergistically, but that this up-regulation depends on Dex.

3.2. PGE_2 up-regulates CYP19 gene transcript in SV-HFO in the presence of Dex

To ascertain whether the aromatase expression with PGE_2 + Dex was up-regulated at the level of gene transcription, we performed RT-PCR of *CYP19* gene transcript in SV-HFO treated