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Signal transduction system for interleukin-6 and interleukin-11 synthesis stimulated by epinephrine in human osteoblasts and human osteogenic sarcoma cells

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Abstract

Epinephrine increased gene- and protein-expression of interleukin-6 (IL-6) and interleukin-11 (IL-11), which are capable of stimulating the development of osteoclasts from their hematopoietic precursors, in human osteoblast (SaM-1) and human osteosarcoma (SaOS-2, HOS, and MG-63) cell lines. An increase in IL-6 and IL-11 synthesis in response to epinephrine appeared to be a common feature in osteoblastic cells, but the magnitude of expression was different in these cell lines. In HOS cells treated with epinephrine, increases of IL-6 and IL-11 synthesis were inhibited by timolol (a β-blocker), H-89 (*N*-[2-((*p*-bromocinnamyl)amino)ethyl]-5-isoquinolinesulfonamide; an inhibitor of protein kinase A (PKA)) and SB203580 [4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)1*H*-imidazole; an inhibitor of p38 mitogen-activated protein kinase (MAPK)], but not by phentolamine (an α-blocker), calphostin C [an inhibitor of protein kinase C (PKC)], or PD98059 (2′-amino-3′-methoxyflavone; an inhibitor of classic MAPK), suggesting a common pathway mediated by β-adrenergic receptors in the PKA and p38 systems involved in the signal transduction of IL-6 and IL-11. Furthermore, expression of both genes was inhibited by curcumin [an inhibitor of activating protein-1 (AP-1) activation], but not by pyrrolidine dithiocarbamate (PDTC) [an inhibitor of nuclear factor (NF)-κB]. The pharmacological study suggested that coinduction of the two genes in response to epinephrine occurred via activation of AP-1. The findings of the present study suggest that coinduction of IL-6 and IL-11 in response to epinephrine probably occurs via the PKA and p38 MAPK systems, leading to the transcriptional activation of AP-1 in human osteoblastic cells.

Keywords: Epinephrine; Osteoblast; Interleukin-6; Interleukin-11; MAPK; Transcriptional factor

1. Introduction

It is well known that osteoblasts and marrow stromal cells are capable of producing a wide array of factors that can potentially act as autocrine and paracrine regulators of bone cell function. Several cytokines that can be produced by stromal cells and osteoblasts, such as colony-stimulating factors, IL-6, and IL-11, may affect osteoclast recruitment rather than the function of mature cells [1]. The synthesis of IL-6 and IL-11, which are capable of stimulating the differentiation of osteoclasts from their hematopoietic precursors [2,3], is stimulated by various systemic and local factors, such as parathyroid hormone (PTH), parathyroid hormone-related peptide (PTHrP), 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), tumor necrosis factor (TNF), transforming growth factor (TGF), and IL-1 [4–6].

The signal transduction systems involved in these hormonal actions on IL-6 and IL-11 synthesis have been drawing increasing attention to aid in understanding the intracellular network of proteins that transduce extracellular signals to intracellular responses in osteoblasts. Recent studies reported that the PKA pathway is involved in prostaglandin E_2 (PGE_2)-induced IL-6 synthesis [7], and in PTH-induced IL-6 and IL-11 synthesis [6]; the PKC pathway is involved in basic fibroblast growth factor- and $PGF_{2\alpha}$-induced IL-6

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Abbreviations: IL, interleukin; PKA, protein kinase A; PKC, protein kinase C; MAPK, mitogen-activated protein kinase; cAMP, cyclic AMP; FBS, fetal bovine serum; α-MEM, α-modified minimum essential medium; RT–PCR, reverse transcription–polymerase chain reaction; NF-κB, nuclear factor-κB; AP-1, activating protein-1; PDTC, pyrrolidine dithiocarbamate; and G3PDH, glyceraldehyde 3-phosphate dehydrogenase.

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synthesis [8,9] and IL-1-induced IL-6 and IL-11 synthesis [6]. In addition, MAPKs are important mediators of intracellular signaling; an inhibitor for MAPKs has been reported to inhibit sphingosine 1-phosphate- and IL-1β-induced IL-6 synthesis [10,11].

Osteoblasts have been demonstrated to express β-adrenergic and cytokine receptors [12]. Stimulation of bone resorption by adrenergic agonists and an anabolic action on bone by the β-adrenergic antagonist have been demonstrated. In neonatal mouse calvaria, an adrenergic receptor agonist increased cAMP production and stimulated bone resorption [13]. Propranolol inhibited cAMP formation induced by β-adrenergic receptor agonists in bone organ culture [14] and increased bone strength and the rates of endochondral bone formation in rats [15]. Stimulation of β-adrenergic receptors in rat osteoblast-like osteosarcoma cells (UMR106–01) has been demonstrated to enhance the cAMP signaling pathway, which regulates IL-6 synthesis [16], leading to increased osteoclastic bone resorption.

To date, the signal transduction systems involved in stimulating IL-6 and IL-11 synthesis by epinephrine have not been studied in human osteoblasts. In the present study, we characterized the signal transduction systems involved in the stimulation of IL-6 and IL-11 production in human osteoblasts and in human osteosarcoma cell lines, using RT–PCR and the ELISA method. In addition, we investigated the transcriptional mechanisms regulating the expression of IL-6 and IL-11, using an inhibitor of NF-κB and AP-1 activation.

2. Materials and methods

2.1. Materials

SaM-1 cells were prepared from an explant of ulnar periosteum from a 20-year-old male patient undergoing curative surgery, with informed consent. These cells have a mitotic life span of 34 population doubling levels (PDLs) [17]. SaM-1 cells were used at 23–24 PDLs in our experiments. SaOS-2 cells were obtained from the RIKEN Cell Bank, and HOS and MG-63 cells were obtained from the American Type Culture Collection. α-MEM was purchased from Gibco BRL, and FBS from Cell Culture Laboratories and Irvine Scientific. Epinephrine, calphostin C, PDTC, and curcumin were obtained from the Sigma Chemical Co. The human IL-6 ELISA kit was purchased from Endogen, and the human IL-11 ELISA kit from R&D Systems. PD98059 (2′-amino-3′-methoxyflavone) was obtained from Biomol. SB203580 [4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)1*H*-imidazole] and H-89 (*N*-[2-((*p*-bromocinnamyl)amino)ethyl]-5-isoquinolinesulfonamide) were from the Calbiochem-Novabiochem Corp. PD98059, SB203580, and calphostin C were dissolved in DMSO (Sigma). All other chemicals used were of reagent grade.

2.2. Cell cultures of SaM-1, SaOS-2, HOS, and MG-63 cells

SaM-1 cells were maintained in α-MEM containing 10% FBS and 60 μg/mL of kanamycin at 37° in a humidified atmosphere containing 5% CO_2 in air. Osteosarcoma cells were cultured in α-MEM containing 10% FBS, 100 U/mL of penicillin, and 100 μg/mL of streptomycin. For the experiments examining gene-expression, cells were grown to confluence for extraction of total RNA. For ELISA analysis, each cell line was cultured in 6-well plates until the cells were almost confluent.

2.3. Analysis of mRNA levels by RT-PCR

RNA was extracted from SaM-1 and osteosarcoma cells in 10-cm dishes (Falcon Plastics) by the guanidinium-thiocyanate method [18]. Total RNA was solubilized in 500 μL of guanidinium-thiocyanate buffer/2 dishes, and then phenol extracted and treated with DNase I (Boehringer Mannheim). cDNA was synthesized using random primers and Moloney murine leukemia virus reverse transcriptase (Gibco-BRL). This was followed by PCR amplification using synthetic gene primers specific for human IL-6, IL-11, and G3PDH produced from the reported cDNA sequences, respectively [19–21]. The oligonucleotide primers were prepared, using a DNA synthesizer (Expedite model 8909; PerSeptiv Biosystem), and were purified on a polypropylene filter (Oligo Prep kit; Pharmacia Biotech). G3PDH primers (forward primer 5′-ACCACAGTCCATGCCATCAC-3′, reverse primer 5′-TCCACCACCCTGTTGCTGTA-3′) were used to amplify a 452 bp DNA fragment. IL-6 primers (forward primer 5′-CATCCTCGACGGCATCTCAGC-3′, reverse primer 5′-TTGGGTCAGGGGTGGTTATTG-3′) were used to amplify a 332 bp DNA fragment. IL-11 primers (forward primer 5′-AGCCACCACCGTCCTTCCAAA-3′, reverse primer 5′-CCTCCGTCCCCACCCCAACAT-3′) were used to amplify a 351 bp DNA fragment. PCR amplification was performed, using the GeneAmp PCR System (Perkin Elmer/Cetus), as follows: denaturation at 95° for 15 sec, annealing at 55° for 30 sec, and elongation at 72° for 30 sec for the appropriate cycles. PCR products were electrophoresed on a 2% NuSieve GTG agarose gel (FMC BioProducts), stained with ethidium bromide, and detected on a fluoroimage analyzer (FluorImager 575; Molecular Dynamics).

2.4. Measurement of IL-6 and IL-11 proteins

IL-6 and IL-11 in conditioned medium were quantified using an ELISA kit. Data were presented as the means ± SEM of three cultures. Differences between the control and the experimental values were determined using Student's *t*-test.

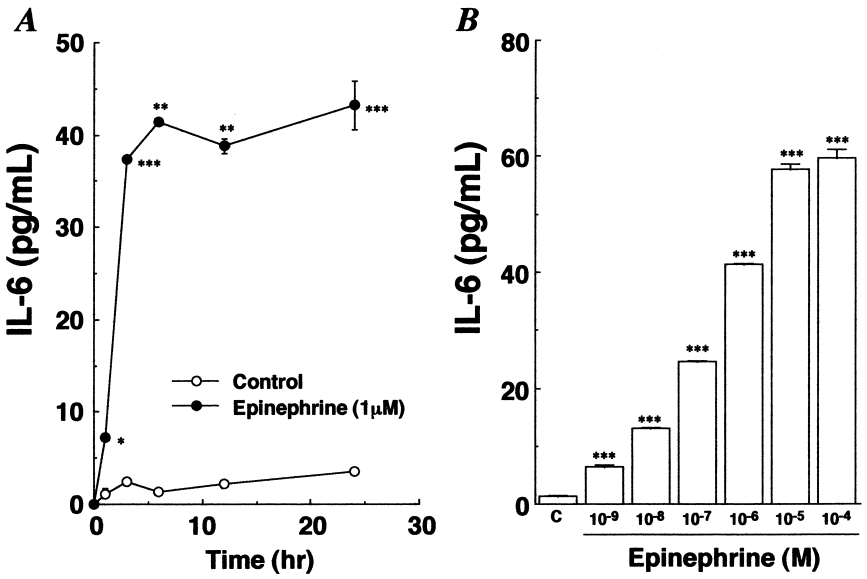

Fig. 1. Effect of epinephrine on IL-6 production in SaM-1 cells. (A) Time course of the effect of epinephrine on IL-6 production in SaM-1 cells. Cells were incubated for 1, 3, 6, 12, and 24 hr with 1 μM epinephrine in the presence of 1% FBS. Conditioned medium was used for IL-6 production analysis, using an ELISA system. Values are means ± SEM (N = 3). Key: (*) $P < 0.05$, (**) $P < 0.01$, and (***) $P < 0.005$ vs control. (B) Concentration-dependency for the effect of epinephrine on IL-6 production. SaM-1 cells were incubated for 6 hr with the indicated concentration of epinephrine in the presence of 1% FBS. Conditioned medium was used for analysis of IL-6 production, using an ELISA system. Values are means ± SEM (N = 3). Key: (***) $P < 0.005$ vs control (C).

3. Results

3.1. Stimulation by epinephrine of the production of IL-6 in osteoblasts

Figure 1 shows the effect of epinephrine on IL-6 production in SaM-1 cells. Cells were treated with the indicated concentration of epinephrine for the indicated periods, and the conditioned medium was analyzed for IL-6 production using an ELISA system. IL-6 production was increased by epinephrine in a time- and concentration-dependent manner in the conditioned medium of SaM-1 cells (Fig. 1). The induction of IL-6 production was rapid (as early as 1 hr after treatment), and the effect of epinephrine was concentration-dependent in the range between 1 nM and 100 μM. The maximum level of IL-6 production was over 40 pg/mL after 6 hr (Fig. 1A). The maximum level of IL-6 production was obtained at 10–100 μM epinephrine (Fig. 1B).

3.2. Effects of epinephrine on the induction of IL-6 and IL-11 mRNA and the synthesis of IL-6 and IL-11 in osteoblasts

Figure 2 shows the effects of epinephrine on the expression of IL-6 and IL-11 mRNA and the synthesis of IL-6 and IL-11 in osteoblasts or osteosarcoma cells. Cells were treated with 1 μM epinephrine for the indicated periods and subjected to RT–PCR analysis. Under normal conditions, SaM-1 cells expressed an undetectable level of IL-6 mRNA. As shown in Fig. 2A, epinephrine enhanced the steady-state level of IL-6 mRNA in a time-dependent manner, and the maximum effect of epinephrine on IL-6 mRNA in SaM-1 cells was observed 0.5 to 1 hr after stimulation. Epinephrine increased the expression of IL-6 mRNA in SaM-1, SaOS-2, and HOS cells. MG-63 cells expressed very weak levels of IL-6 mRNA at 50 cycles (Fig. 2B). However, epinephrine elevated the expression of IL-11 mRNA in all cell lines (Fig. 2B). Although epinephrine elevated the expression of IL-6 and IL-11 mRNA, the magnitude of expression was different in these cell lines. Treatment with epinephrine for 1 hr induced a 3.5-, 6.9-, and 3.3-fold increase in IL-6 mRNA in SaM-1, SaOS-2, and HOS cells, respectively, but in MG-63 cells epinephrine did not increase the expression of IL-6 mRNA significantly. In HOS cells, epinephrine induced a 3.2-fold increase in the expression of IL-11 mRNA. As shown in Table 1, epinephrine increased IL-6 and IL-11 production in all cell lines. Since epinephrine stimulated IL-6 and IL-11 synthesis by about the same extent (4.0- and 4.8-fold, respectively) in HOS cells, we used HOS cells to investigate signaling transduction of epinephrine-induced IL-6 and IL-11.

3.3. Effect of α- or β-blockers on epinephrine-induced IL-6 and IL-11 production

HOS cells were treated with 1 μM timolol, used as a β-blocker [22], or with 1 μM phentolamine, used as an α-blocker [23], for 30 min before stimulation with epinephrine. Timolol inhibited epinephrine-induced IL-6 and IL-11 production but phentolamine did not (Fig. 3). Therefore, we

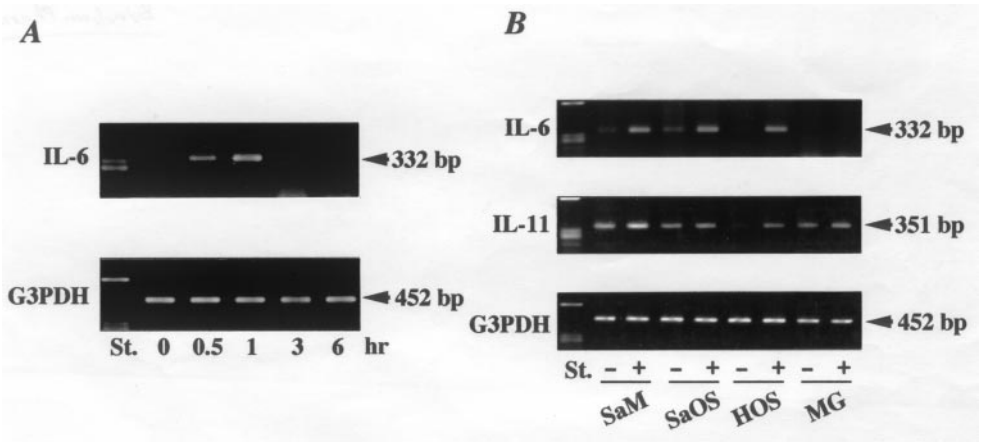

Fig. 2. Effects of epinephrine on the expression of IL-6 and IL-11 mRNA in osteoblastic cells. (A) Effect of epinephrine on the expression of IL-6 mRNA in SaM-1 cells. Cells were incubated for 0.5, 1, 3, and 6 hr with 1 μM epinephrine. Total RNA was extracted and subjected to RT–PCR analysis. (B) Effects of epinephrine on the expression of IL-6 and IL-11 mRNA in SaM-1 (SaM), SaOS-2 (SaOS), HOS, and MG-63 (MG) cells. Cells were incubated for 1 hr with (+) or without (−) 1 μM epinephrine. Then total RNA was extracted and subjected to RT–PCR analysis. DNA size markers (ϕX 174/*Hae*III digest) are shown in the left lanes (St.). Arrowheads indicate the predicted size of PCR production. Data shown are representative of three similar experiments.

investigated whether cAMP was involved in epinephrine-induced IL-6 production. In this respect, SaM-1 cells were grown to confluence and treated for 6 hr with the adenylate cyclase agonist forskolin (1 μM). Forskolin (1 μM), like epinephrine, induced IL-6 production, and epinephrine stimulated intracellular cAMP production (data not shown). Further, forskolin (1 μM) increased the expression of IL-6 and IL-11 mRNA after treatment for 1 hr, and thereafter increased IL-6 production in HOS cells after treatment for 6 hr (0.05 $\pm$ 0.05 ng/mL for control vs 39.10 $\pm$ 1.68 ng/mL for forskolin-treated cells).

3.4. Effects of PKA, PKC, and MAPK inhibitors on epinephrine-induced IL-6 and IL-11 synthesis

As shown in Fig. 4A, in HOS cells epinephrine-induced IL-6 and IL-11 production was prevented by the PKA inhibitor H-89 [24], in a concentration-dependent manner in the range between 1 and 100 μM [25,26]. The PKC inhibitors calphostin C (0.01 to 1 μM) [27–29] and staurosporine (1 to 100 nM) [8, 9] had no effect on epinephrine-induced IL-6 and IL-11 production in these cells (data not shown).

We also examined the effects of the MEK-1 inhibitor PD98059 [30,31] and the p38 MAP kinase inhibitor SB203580 [29,31,32] on the production of IL-6 and IL-11 in HOS cells. The epinephrine-induced increase in IL-6 and IL-11 synthesis was prevented by the p38 MAP kinase inhibitor SB203580 in a concentration-dependent manner in the range between 0.1 and 1 μM in HOS cells (Fig. 4B). In contrast, the MEK-1 inhibitor PD98059 (0.01 to 100 μM) did not inhibit the epinephrine-induced increase in IL-6 and IL-11 synthesis in these cells (data not shown).

3.5. Effect of curcumin or PDTC on gene- and protein-expression of epinephrine-induced IL-6 and IL-11

Figure 5 shows the effects of curcumin (an inhibitor of AP-1 activation) [33] and PDTC (an inhibitor of NF-κB activation) [34] on the transcription and synthesis of IL-6 or IL-11 in HOS cells. Curcumin and PDTC were used at

Table 1
Effect of epinephrine on IL-6 and IL-11 production in osteoblastic cells

	Epinephrine (1 μM)	IL-6 (pg/mL)	IL-11 (pg/mL)
SaM-1	−	9.27 $\pm$ 0.11	0.29 $\pm$ 0.19
	+	31.36 $\pm$ 0.35*	9.64 $\pm$ 1.58**
SaOS-2	−	9.22 $\pm$ 1.18	506.98 $\pm$ 7.33
	+	27.67 $\pm$ 0.91*	869.79 $\pm$ 12.23*
HOS	−	6.57 $\pm$ 0.86	16.33 $\pm$ 3.24
	+	26.60 $\pm$ 0.62*	76.08 $\pm$ 1.33*
MG-63	−	7.04 $\pm$ 0.16	1.04 $\pm$ 0.56
	+	9.16 $\pm$ 0.39*	25.208 $\pm$ 0.61*

At confluence, cells were incubated with 1 μM epinephrine in the presence of 0.1 or 1% FBS for 6 h. Conditioned medium was used for analysis of IL-6 and IL-11 production, using an ELISA system. Values are means $\pm$ SEM (N = 3).

*,** Significantly different from without epinephrine (−): * $P < 0.005$, and ** $P < 0.05$.

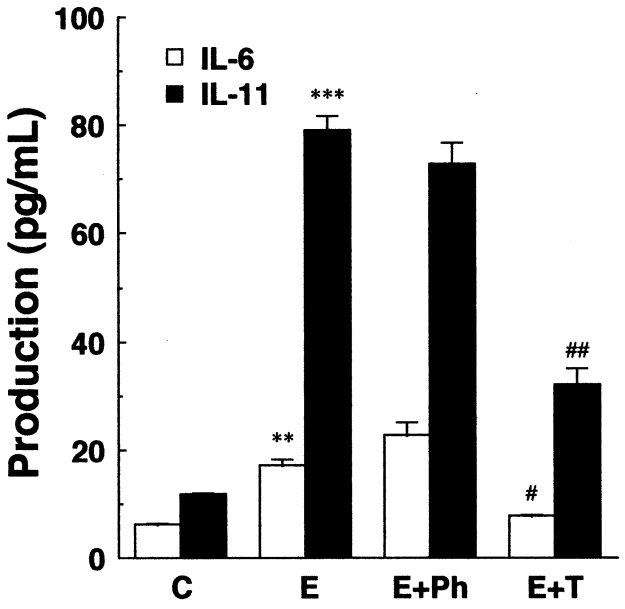

Fig. 3. Effects of timolol and phentolamine on epinephrine-induced IL-6 or IL-11 production in HOS cells. Cells were incubated with 1 μM timolol (T) or 1 μM phentolamine (Ph) in the presence of 0.1% FBS for 30 min before the addition of 1 μM epinephrine (E). After treatment with epinephrine for 6 hr, conditioned medium was collected and used for analysis of IL-6 and IL-11 production. Values are means ± SEM (N = 3). Key: (**) $P < 0.01$, and (***) $P < 0.005$ vs control (C); (#) $P < 0.05$, and (##) $P < 0.01$ vs treatment with epinephrine (E).

concentrations that we have described previously [35]. Curcumin (30 μM) inhibited epinephrine-induced transcription of IL-6 and IL-11 mRNA, but PDTC (50 μM) did not (Fig. 5A). In addition, curcumin inhibited epinephrine-induced IL-6 and IL-11 production but PDTC did not (Fig. 5B).

4. Discussion

In the present study, we demonstrated the coinduction of IL-6 and IL-11 in human osteoblasts (SaM-1) and human osteosarcoma cells (SaOS-2, HOS, and MG-63). The increase in IL-6 and IL-11 synthesis in response to epinephrine appears to be a common feature in osteoblastic cells, but the magnitude of expression was different in these cell lines. IL-6 is a potent regulator for osteoclast differentiation and elicits bone resorption in *in vivo* and *in vitro* models that contain early osteoclast precursors [36]. IL-11 is an IL-6-type cytokine, which inhibits adipogenesis and activates osteoclasts. Thus, both IL-6 and IL-11, synthesized in osteoblasts, are important stimulators of osteoclast development [2,3] and physiologically regulate bone metabolism [37,38]. These findings suggest that bone resorption elicited by epinephrine involves increased synthesis of IL-6 and IL-11. Even though there are some differences between the roles of IL-6 and IL-11 in bone resorption [39,40], IL-11 shares several properties with IL-6. Using pharmacological inhibitors, we examined the signal transduction systems for IL-6 and IL-11 synthesis mediated by epinephrine. In HOS

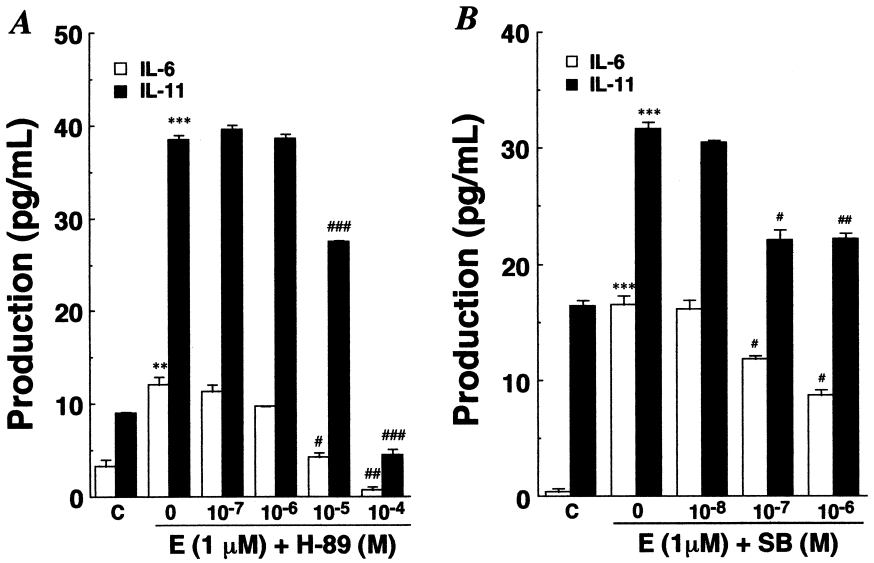

Fig. 4. Effects of H-89 and SB203580 on epinephrine-induced IL-6 and IL-11 synthesis in HOS cells. (A) Effect of H-89 on epinephrine-induced IL-6 and IL-11 production in HOS cells. Cells were incubated with H-89 in the presence of 0.1% FBS for 30 min before the addition of 1 μM epinephrine (E). Control (C) cells were incubated with vehicle. After treatment with epinephrine, conditioned medium was collected and assayed, by ELISA, for IL-6 and IL-11. Values are means ± SEM (N = 3). Key: (**) $P < 0.01$, and (***) $P < 0.005$ vs control (C); (#) $P < 0.05$, (##) $P < 0.01$, and (###) $P < 0.005$ vs treated with epinephrine (E). (B) Effects of SB203580 on epinephrine-induced IL-6 and IL-11 production in HOS cells. Cells were incubated with SB203580 (SB) in the presence of 0.1% FBS for 30 min before the addition of 1 μM epinephrine (E). Control (C) cells were incubated with vehicle. After treatment with epinephrine for 6 hr, conditioned medium was collected and assayed, by ELISA, for IL-6 and IL-11. Values are means ± SEM (N = 3). Key: (***) $P < 0.005$ vs control (C); (#) $P < 0.05$, and (##) $P < 0.01$ vs treatment with epinephrine (E).

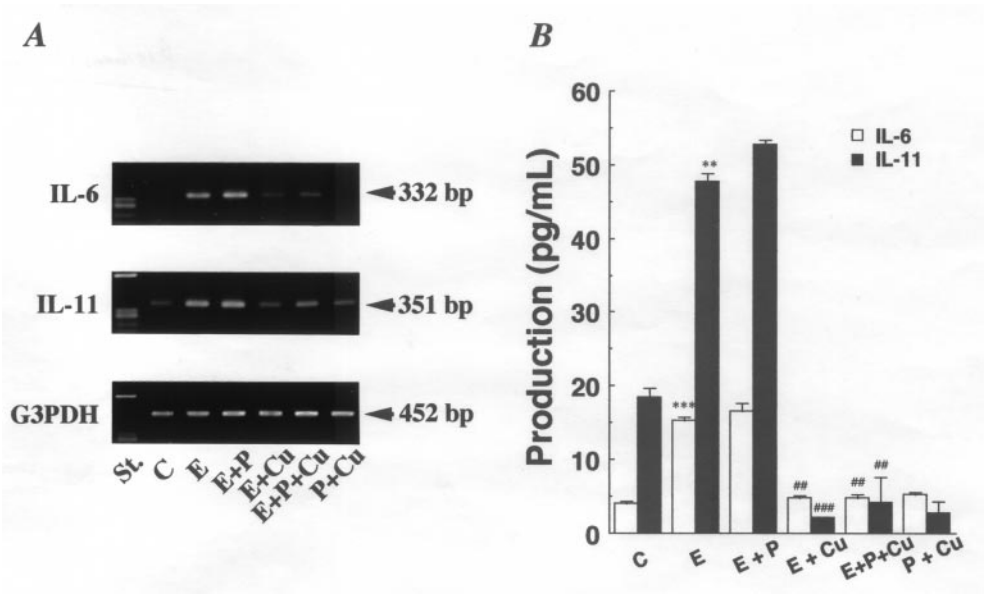

Fig. 5. Effects of PDTC and curcumin on epinephrine-induced IL-6 and IL-11 gene- and protein-expression in HOS cells. (A) Effects of PDTC (P) and curcumin (Cu) on the expression of epinephrine-induced IL-6 or IL-11 mRNA in HOS cells. Cells were incubated with 50 μM PDTC and/or 30 μM curcumin in the presence of 0.1% FBS for 1 hr before the addition of 1 μM epinephrine (E). Then total RNA was extracted and subjected to RT–PCR analysis. DNA size markers (ϕX 174/*Hae*III digest) are shown in the left lanes (St.). Arrowheads indicate the predicted size of PCR production. Data shown are representative of three identical experiments. (B) Effects of PDTC and curcumin on epinephrine-induced IL-6 and/or IL-11 production in HOS cells. Cells were incubated with 50 μM PDTC and/or 30 μM curcumin in the presence of 0.1% FBS for 1 hr before treatment with 1 μM epinephrine (E). After treatment with epinephrine for 6 hr, conditioned medium was collected and assayed, by ELISA, for IL-6 and IL-11. Values are means $\pm$ SEM (N = 3). Key: (**) $P <$ 0.01, and (***) $P < 0.005$ vs control (C); (##) $P < 0.01$, and (###) $P < 0.005$ vs treatment with epinephrine (E).

cells treated with epinephrine, increases in IL-6 and IL-11 synthesis were inhibited by timolol or H-89, but not by phentolamine or calphostin C. This indicates that the same signal transduction system is involved in the synthesis of both IL-6 and IL-11. This signaling pathway is activated through the stimulation of the β-adrenergic receptor (β-AR) in human osteoblastic cells. Both syntheses were stimulated via the PKA, but not the PKC pathway.

Recently, signaling through G protein-coupled receptors has been demonstrated and implicates several MAPKs, including the extracellular signal-regulated kinase (ERK), c-Jun amino-terminal protein kinase, and p38 MAPK [41], which could be involved in the regulation of the differentiation of some cell types. The role of these different MAPKs in IL-6 and IL-11 synthesis in human osteoblastic cells is poorly understood. In the present study, a specific inhibitor of p38 MAPK (SB203580) partly inhibited the coinduction of IL-6 and IL-11 by epinephrine in HOS cells, suggesting the involvement of not only the p38 MAPK signaling pathway but also other signaling pathways. However, the MEK inhibitor PD98059 had no effect on the coinduction of IL-6 and IL-11. This may indicate that epinephrine-induced IL-6 and IL-11 synthesis could be regulated in part by the p38 MAPK system, without involving the MEK system. This observation, taken together with the finding of the inhibitory effect of SB203580 on epinephrine-induced alkaline phosphatase activity in MC3T3-E1 cells [31], suggests a potential role of p38 MAPK in controlling osteoblastic function in resorption in response to epinephrine. However, at this time, the relationship between the PKA and the p38 MAPK systems in epinephrine-induced IL-6 and IL-11 synthesis remains to be established.

Stimulation of β-AR is well known to induce immediate early gene, c-*fos* gene-expression via increases in cAMP, which, in turn, activate the cAMP/PKA pathway in osteoblastic cells [25]. Then, c-Fos forms heterodimers with Jun proteins, which regulate transcription of AP-1 responsive genes [42]. Recently, platelet-derived growth factor has been demonstrated to induce IL-6 transcription in murine osteoblasts by regulating nuclear proteins of the AP-1 complex and activating transcription factor-2 [43]. These findings suggest that AP-1 is involved in coinduction of IL-6 and IL-11 by β-AR stimulation. However TNF-α has been reported to induce IL-6 and intracellular adhesion molecule-1 in osteoblast-like Ros17/2.8 cells through activation of NF-κB, leading to the promotion of bone resorption and inflammation [44]. In the present study, using pharmacological inhibitors, we examined the involvement of AP-1 and/or NF-κB in the induction of IL-6 and IL-11. Although there are several reported targets for curcumin, e.g. NF-κB, cyclooxygenase, and PKC isozymes [45–47], it also has been reported that curcumin is a relatively specific inhibitor for transcriptional regulation factor, AP-1, in mouse osteoblastic cells [35]. Since PDTC (NF-κB inhibitor, Fig. 5B), calphostin C (PKC inhibitor), staurosporine (PKC inhibitor), and NS398 (cyclooxygenase inhibitor) had no effect on epinephrine-induced IL-6 mRNA levels in osteoblastic cells (data not shown), we utilized curcumin as an inhibitor for

AP-1 in the cells. Figure 5 shows that both gene- and protein-expression of IL-6 and IL-11 were inhibited by treatment with an inhibitor of c-Jun/AP-1, curcumin, but not with an inhibitor of NF-κB, PDTC. These findings reveal that the induction of c-Jun/AP-1 is essential for the expression of IL-6 and IL-11 by epinephrine. In human fibroblast-like synoviocytes, Miyazawa *et al.* [11] observed that SB203580 significantly reduces the stability of IL-6 mRNA without affecting the rate of IL-6 gene transcription, and they concluded that p38 MAPK, which is activated in response to IL-1β, is involved in IL-6 synthesis by stabilizing IL-6 mRNA. Inhibition of IL-6 and IL-11 synthesis by SB203580 may be based on reducing gene stability, because complete inhibition of epinephrine-induced coinduction of IL-6 and IL-11 at relatively high concentrations of SB203580 was not observed.

In conclusion, the present findings indicate that coinduction of IL-6 and IL-11 by activating β-AR, which appears to be a common feature in osteoblastic cells, is probably mediated via a common signaling pathway involving the PKA and p38 MAPK systems, leading to the transcriptional activation of AP-1 in human osteoblastic cells.

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