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Parathyroid Hormone (1-34)–Mediated Interleukin-6 Induction

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Abstract Parathyroid hormone (PTH) functions in part by regulating osteoblast cytokine expression. We recently demonstrated that PTH induced a rapid and transient increase in interleukin-6 (IL-6) mRNA expression in rat bones in vivo. To determine the molecular basis of this effect, we analyzed the human IL-6 promoter fused (−1,179 to +9) with the chloramphenicol acetyltransferase (CAT) reporter gene in stable transfections into human osteoblast-like osteosarcoma SaOS-2 cells. We compared the effects of PTH on IL-6 expression with adenylate cyclase activator forskolin, PKC activator phorbol 12-myristate 13-acetate (PMA), calcium ionophore A23187, interleukin-1α (IL-1α), prostaglandin E-2 (PGE-2), RS-66271 (a parathyroid hormone–related peptide analog), and platelet-derived growth factor-BB (PDGF-BB). Analyses of cell clones showed that IL-6 promoter expression was extremely low in the unstimulated state. Exposure to PTH (0.001–100 nM) for 12 h stimulated CAT expression in a dose-dependent manner (200–500% of control). Treatment with IL-1α was more potent than PTH in inducing transcription of the IL-6 promoter (900–1,000%). Activation of the cAMP-PKA pathway by treatment with forskolin induced a comparable level of induction with PTH. Together, the effects of PTH and forskolin were additive. RS-66271, previously shown to have PTH-like effects, induced a comparable level of IL-6 promoter expression. When examined together, PTH + RS-66271 effects were comparable to PTH effects alone. Exposure to PGE-2, PMA, PDGF-BB, or A23187 for 12 h did not significantly alter IL-6 promoter expression. These results demonstrate PTH, forskolin, the PTHrP analog RS-66271, and IL-1α stimulate IL-6 expression by stimulating gene transcription. The response to forskolin suggests that the messenger system mediated by PKA is sufficient to induce IL-6 expression. J. Cell. Biochem. 67:265–274, 1997.

Key words: hPTH 1-34; IL-6 promoter; CAT expression; transfection; osteoblast; in vitro

Interleukin-6 (IL-6) is a multifunctional cytokine which mediates pleiotropic functions in various types of cells. It was originally described as a factor which induces immunoglobin synthesis in B cells, promotes the growth of B-cell hybridomas and plasmacytomas, stimulates acute-phase protein synthesis in hepatocytes, and plays a role in hematopoiesis and cytotoxic T-cell differentiation [for reviews see Jones, 1994; Van Snick, 1990; Akira et al., 1993; Kishimoto, 1989]. IL-6 has also been implicated in the process of inflammation [Hirano, 1992], viral infection [Akira and Kishimoto, 1992], autoimmunity [Kishimoto, 1992], development [Lee, 1992], malignant cell growth [Akira et al., 1993], and bone metabolism [Hughes and Howells, 1993; Jones, 1994; Poli et al., 1994]. The functional significance of upregulated IL-6 production and its potential role in the pathogenesis in several human diseases are currently a subject of intense investigation [Brach and Herrmann, 1992].

IL-6 has been implicated in multiple activities in the bone microenvironment, in osteoporosis, in Paget's disease, and in bone lesions associated with multiple myeloma [Wallach et al., 1993; Poli et al., 1994; Hughes and Howells, 1993; Jones, 1994; Van Snick, 1990; Akira et al., 1993; Kishimoto, 1989; Reddy et al., 1994; Hiraga et al., 1995; Kitamura et al., 1995; De La Mata et al., 1995; Riancho and Mundy, 1995; Votta and Bertolini, 1994; Ohsaki et al., 1992; Klein et al., 1991; Roodman et al., 1992; Klein et al., 1990; Manolagas and Jilka, 1995; Kotake et al., 1994]. Previous studies demonstrated IL-6 knockout mice have a normal amount of

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trabecular bone but higher rates of bone turnover (than control littermates) while constitutive expression of IL-6 suppressed bone turnover [Poli et al., 1994; Kitamura et al., 1995]. These studies demonstrate that IL-6 is an important regulator of bone remodeling.

IL-6 is produced locally in nanomolar quantities by stromal cells and osteoblastic cells in response to stimulation by systemic hormones such as parathyroid hormone (PTH) as well as many other osteotrophic agents [Greenfield et al., 1993; Passeri et al., 1993; Bertolini et al., 1994; Manolagas and Jilka, 1995]. We and others have demonstrated that PTH stimulates IL-6 mRNA, protein synthesis, and secretion in a variety of experimental systems both in vitro and in vivo [Onyia et al., 1995a; Greenfield et al., 1993, 1995, 1996; Feyen et al., 1989; Holt et al., 1994b; Bertolini et al., 1994; Sakagami et al., 1993; Lowik et al., 1989; Pollock et al., 1996]. This response is of interest since IL-6 plays a role in bone remodeling and may mediate downstream effects of PTH [Votta and Bertolini, 1994; Greenfield et al., 1993, 1995, 1996; Riancho and Mundy, 1995; De La Mata et al., 1995]. The molecular mechanisms by which PTH upregulates IL-6 expression to elicit an effect in bone is not understood. However, recent evidence indicates that PTH effects may be mediated at least in part through changes in gene transcription associated with a specific signal transduction pathway in the osteoblasts [Greenfield et al., 1996].

In the studies presented here, we investigated the effect of PTH on IL-6 gene transcription. We analyzed the human IL-6 promoter fused (−1,179 to +9) with the chloramphenicol acetyltransferase (CAT) reporter gene in stable transfections into human osteoblast-like osteosarcoma SaOS-2 cells. We compared the effects of PTH on IL-6 expression with adenylate cyclase activator forskolin, protein kinase C (PKC) activator phorbol 12-myristate 13-acetate (PMA), calcium ionophore A23187, interleukin-1α (IL-1α), prostaglandin E-2 (PGE-2), RS-66271 (a parathyroid hormone related peptide analog), and platelet-derived growth factor-BB (PDGF-BB).

MATERIALS AND METHODS

Materials

Synthetic hPTH (1-34) (Bachem, Torrance, CA) was prepared in a vehicle containing 1 mg/ml bovine serum albumin (BSA), 0.15 M sodium chloride, and 0.001 N HCL [Onyia et al., 1995a]. Interleukin-1α and PDGF-BB (R&D Systems, Minneapolis, MN) were prepared in 0.1% BSA. Prostaglandin E-2, PMA, and A23187 (Sigma, St. Louis, MO) were dissolved in absolute alcohol and diluted in phosphate buffered saline (PBS). Forskolin (Calbiochem, San Diego, CA) was made in 100% dimethyl sulfoxide (DMSO) and used at a final concentration of less than 0.1% DMSO. RSS-66271 was generously provided by Dr. C. Frolik (Eli Lilly and Co., Indianapolis, IN). All other reagents were purchased from Sigma.

Cell Culture

The human osteosarcoma cells SaOS-2 were maintained in DMEM/Ham's F-12 (3:1) containing 10% fetal bovine serum plus glutamine. All cultures were maintained at 37°C in 95% humidity with 5% CO_2. Experiments were initiated following withdrawal of serum for 8–12 h, when cells were approximately 70% confluent.

Plasmid Construction and Transfection

A Bam HI-XhoI fragment of the human IL-6 gene, containing sequences from −1,179 to +9 relative to the transcriptional start site [Ray et al., 1988], was cloned into the SmaI site of pUC-CAT [Gilman et al., 1986], as previously described [Libermann and Baltimore, 1990; Dendorfer et al., 1994] (Fig. 1). The IL-6 promoter construct, positive control plasmid RSVCAT [Gorman et al., 1982], or negative control promoterless plasmid pUC-CAT was stably transfected into SaOS-2 cells by the lipofectamine method (BRL, Grand Island, NY). A second plasmid, pRc/CMV, (Invitrogen, San Diego, CA) encoding the neomycine gene was cotransfected for selection. Forty-eight hours after transfection in T25 flasks (Corning Incorporated, Corning, NY), cells were reseeded (1:10) into T75 flasks (Corning) and selected in media containing 1 mg/ml G418 for 10 days. Regular media changes were made at 3–4 days intervals. On the tenth day, randomly selected G418-resistant colonies were picked (colonies contained more than 50 cells) and expanded in fresh media containing 1 mg/ml G418. The rest of the colonies (>20) were trypsinized and pooled and maintained in the G418-containing media. For analysis of gene expression, selected colonies were plated in T75 flasks, and experiments were initiated following serum withdrawal for 8–12 h. Cells were stimulated with

PTH or other agents, as indicated, for 12 h. Cells were then harvested by trypsin digestion, and extracts (100–200 ul) were prepared by three freeze-thaw cycles in TE (5 mm EDTA and 250 mM Tris, pH 7.8). After a 5 min centrifugation at 10,000*g*, supernatants were heated at 65°C for 10 min, centrifuged at 10,000*g* for 10 min, and frozen at −80°C [Onyia et al., 1995b]. The protein content in each extract was measured by the BCA protein assay reagent (Pierce Chemical Co., Rockford, IL), as described by the manufacturer.

CAT Assays

Cell extracts were assayed for CAT activity by the two-phase partition assay [Neumann et al., 1987; Martin, 1990; Onyia et al., 1995b]. In this method, an aqueous reaction is carried out under an immiscible scintillation fluid. [^{3}H] acetyl coenzyme-A and chloramphenicol are the substrates, but only the product, [^{3}H] acetyl chloramphenicol, diffuses into the fluor for detection. A fixed amount of protein (50 ug) from each extract in 50 ul TE was transferred to miniscintilation vials, with 200 ul reaction mix, containing 125 mM Tris (pH 7.8), 1.25 mM chloramphenicol, and 0.1 uCi [^{3}H] acetyl coenzyme-A. A negative control, containing 50 ul TE without the enzyme, and a positive control, containing 2 mU CAT enzyme in 50 ul TE, were included in each assay. The reaction mixture was gently overlaid with 5 ml cold nonaqueous scintillation fluid (OCS; Amersham Corp., Arlington Heights, IL) and incubated at 37°C. The amount of [^{3}H] acetyl chloramphenicol that partitioned into the scintillation fluid was determined by liquid scintillation counting. Sets of vials were counted at 30 min intervals for up to 4 h. All results reported are those at 2 h of incubation, when the reactions are still linear with time. Values obtained were transformed into picomolar concentrations of acetate bound to chloramphenicol per 50 ug cellular protein/hour by use of the least square fit linear regression. For all results reported, the coefficients of correlation by linear regression ranged from 0.970–0.999. The CAT assay results were analyzed using Student's *t*-test. Probability (*P*) values of less than 0.05 were considered significant.

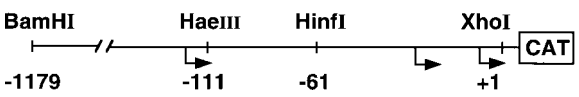

Fig. 1. Schematic diagram of human IL-6 promoter subcloned into the promoterless CAT expression vector, pUC-CAT. A BamHI-XhoI fragment of the human IL-6 gene extending from −1,179 to +9 relative to the major transcription start sites (*arrows*) was cloned into the SmaI site of pUC-CAT as described [Libermann and Baltimore, 1990]. Confirmation of insert identity with the known sequences of the promoter was obtained by restriction mapping and sequencing.

RESULTS

Expression of IL-6 Promoter in Different Clones of Stable Transfectants

We first tested if the sequences upstream of the IL-6 promoter were sufficient to drive expression of IL-6 in SaOS-2 cells. The IL-6 promoter region (−1,179 to +9) linked to the CAT gene was constructed (Fig. 1) and stably transfected in SaOS-2 cells as described in Materials and Methods. After transfection and selection in G418, we analyzed the basal (unstimulated) CAT expression in three randomly picked individual G418-resistant clones (clones 11, 15, and 19) and a polyclonal mixture of over 20 G418-resistant clones (clone M). The activity of IL-6 promoter was extremely low and was 10–150-fold lower when compared with positive control plasmid RSVCAT, which contains the Rous sarcoma virus (RSV) promoter (Fig. 2). Expression levels were highest in clone 19 and M but tenfold lower in clones 11 and 15. CAT expression from the negative control promoterless parental plasmid pUC-CAT was indistinguishable from the test background (data not shown).

Effects of PTH on IL-6 Promoter Expression

To determine if sequences upstream of IL-6 promoter were sufficient to confer PTH induction, we analyzed CAT expression in the cell clones in the presence or absence of PTH (10 nM). Stimulation of cells with PTH for 12 h induced CAT expression significantly higher than control (unstimulated) levels in all clones (Fig. 3). CAT expression was induced by four- to fivefold in clone 11, by 1.7–2-fold in clone 15, and by 2.5-fold in clones 19 and M. Expression of the control plasmid RSVCAT was not significantly affected by PTH.

As clone 11 had low but above background expression and high PTH responsiveness, we selected this clone for subsequent investigations. The time course and dose dependency of CAT expression was determined. There was an approximately linear increase in CAT expres-

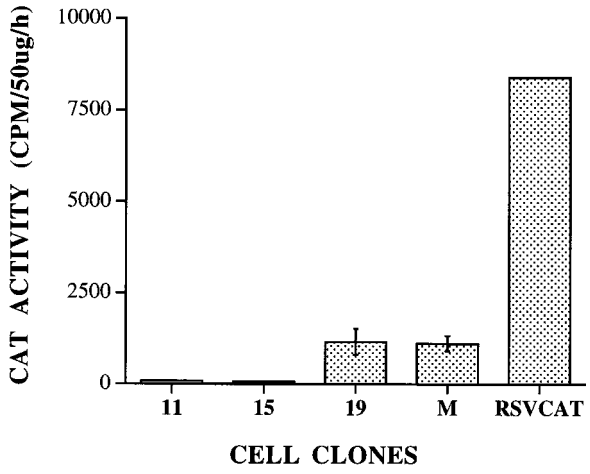

Fig. 2. Expression of stably transfected IL-6/CAT fusion gene in SaOS-2 cells. Cells were stably transfected with IL-6 promoter/CAT construct as described in Materials and Methods, and a few of the G418-resistant colonies (clones 11, 15, 19) were isolated and expanded for analysis of CAT expression. For comparison, cells from a polyclonal mixture of over 20 G418-resistant clones (clone M) were also analyzed. CAT enzyme activity was assayed in a fixed amount of cell extract (50 ug) from unstimulated cell clones, and the amount of [^{3}H] acetate in CPM (counts per minute) bound to chloramphenicol per 50 ug cellular protein/hour was determined. CAT expression from cells transfected with a plasmid RSV CAT was used as positive control. The data shown represents the mean ± SEM of three to four separate experiments.

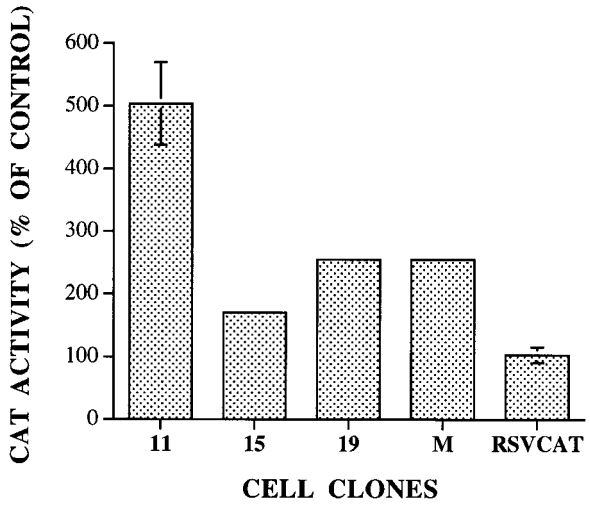

Fig. 3. Stimulation of cells with hPTH (1-34) for 12 h induced CAT expression in all the clones analyzed. Cells were plated in T75 flasks and were treated with or without PTH (10 nM) after serum was withdrawn for 8–12 h. CAT enzyme activity was determined and expressed as the percent increase over control activity (no PTH addition). The results represent the mean ± SEM of three to six separate experiments.

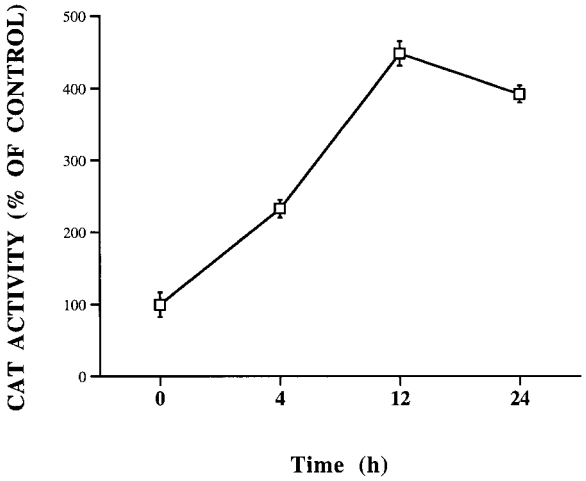

Fig. 4. Time course of the effect of hPTH (1-34) on IL-6 promoter/CAT expression. SaOS-2 cells from clone 11 were serum-deprived for 8–12 h and treated with or without PTH (10 nM) for the indicated times. CAT enzyme activity was assayed and expressed as the percent increase over CAT activity of the control cells (no PTH addition). The results represent the mean ± SEM of three separate experiments.

sion up to 12 h (Fig. 4). Exposure to PTH (0.01–100 nM) stimulated CAT expression in a dose-dependent manner (2.5–5-fold of control) (Fig. 5). The optimal increase in CAT expression occurred at doses ranging from 0.1–10 nM PTH. At these doses, a significant increase in CAT expression (four- to fivefold higher than that exhibited by control cultures) was detected. These results indicate a normal response to PTH. We next compared the effect of acute PTH (one exposure) to intermittent (multiple exposures) PTH (10 nM) on IL-6 promoter expression (Fig. 6). PTH was administered once a day for 1–3 days, and CAT expression was analyzed 12 h following the last treatment. IL-6 promoter expression was increased by four- to fivefold above control following either acute or intermittent exposure.

Effects of Interleukin-1, Prostaglandins, Forskolin, Phorbol Esters, Calcium Ionophore, PTH-Related Peptide Analog, and Platelet-Derived Growth Factor on IL-6 Promoter Expression

We compared the effects of PTH on IL-6 expression with adenylate cyclase activator forskolin, PKC activator PMA, calcium ionophore A23187, IL-1α, PGE-2, RS-66271 (a parathyroid hormone–related peptide analog), and PDGF-BB. As shown in Figure 7, treatment with IL-1α induced the transcription of the IL-6

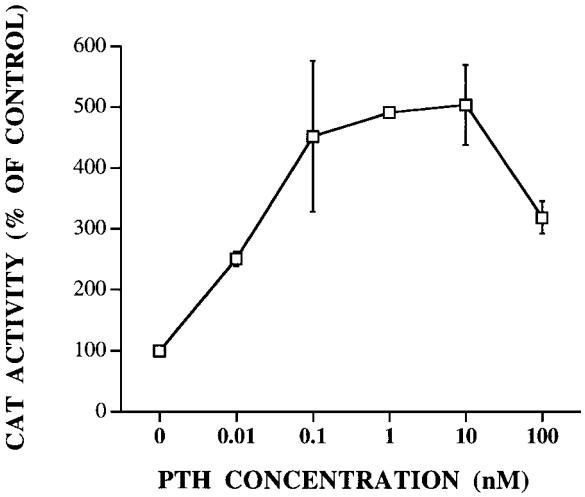

Fig. 5. Effect of increasing concentrations of hPTH (1-34) on IL-6 promoter/CAT expression. SaOS-2 cells from clone 11 were plated and grown in T75 flasks until cells were approximately 70% confluent. Cells were then serum-deprived for 8–12 h and treated with increasing concentrations of PTH for 12 h, as indicated. Control cells were incubated in serum-free media without PTH for 12 h. CAT enzyme activity was assayed and expressed as the percent increase over the activity of control cells (no PTH addition). The results represent the mean ± SEM of three to six separate experiments.

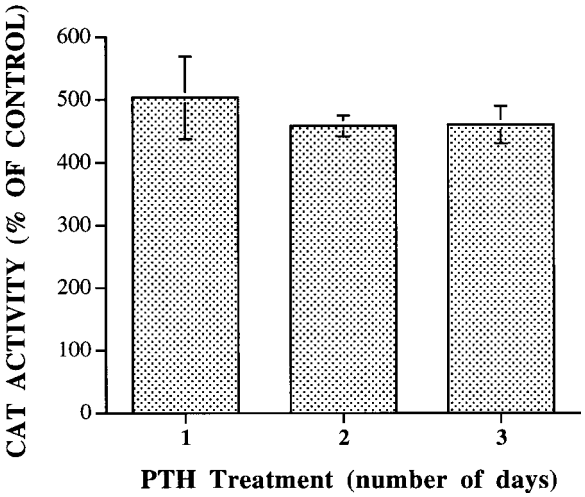

Fig. 6. Effect of single and multiple exposures to hPTH (1-34) on IL-6 promoter/CAT expression. After 8–12 h of serum starvation, SaOS-2 cells from clone 11 were given once daily treatment of PTH or vehicle (10 nM) for 1, 2, and 3 days, and CAT activity was determined 12 h after the last treatment. Cells were plated and grown in T75 flasks until cultures were approximately 70% confluent. Cells were then serum-deprived for 8–12 h and treated with or without PTH, as indicated. CAT enzyme activity was assayed and expressed as the percent increase over the activity of control cells (no PTH addition). The results represent the mean ± SEM of two to three separate experiments.

promoter to a greater extent than PTH (900–1,000%). Activation of the cAMP-PKA pathway by forskolin induced a comparable level of induction to that of PTH. RS-66271 induced a comparable level of IL-6 promoter expression to that of PTH. In contrast, 12 h exposure to PGE-2, PMA, A23187, and PDGF-BB did not significantly alter IL-6 promoter expression. Exposure to lower concentrations of PGE-2, PMA, A23187, and PDGF-BB also did not regulate IL-6 promoter expression (data not shown). These results suggest that signal transduction pathways involving PKA are likely to be critical for PTH-induced IL-6 gene transcription. Although the magnitude of the stimulatory effects of PTH, RS-66271, and forskolin on IL-6 expression were comparable, these agents may exert their effects through different mechanisms. To examine this possibility we determined the effect of combined treatment of PTH + forskolin as well as the effect of PTH + RS-66271 (Fig. 8). Untreated cells or cells exposed to the optimal concentration of PTH (10 nM) were also treated with or without forskolin or RS-66271. PTH and forskolin in combination were additive. The fold induction by PTH alone or in combination with RS-66271 was comparable (Fig. 8).

DISCUSSION

Previously, we and others have reported that PTH induced a rapid and transient increase in IL-6 mRNA expression in bone in vivo and in cultured osteoblast-like cells [Onyia et al., 1995a; Pollock et al., 1996; Greenfield et al., 1993, 1995, 1996; Li et al., 1991]. While the role of IL-6 in PTH action remains controversial in bone, it has been hypothesized that IL-6 may act as a PTH signal transducer between accessory cells and osteoclasts [Riancho and Mundy, 1995; Greenfield et al., 1993, 1995, 1996; De La Mata et al., 1995; Lowik et al., 1989]. To further understand the mechanism by which PTH stimulates IL-6 expression, we investigated the ability of PTH and other agents to regulate gene transcription from a human IL-6 promoter fused (−1179 to +9) with a chloramphenicol acetyltransferase (CAT) reporter gene after stable transfection into human osteoblast-like osteosarcoma SaOS-2 cells. Our results suggest that PTH mediates its effect on IL-6 by direct stimulation of IL-6 transcription, as evidenced by increased CAT expression. PTH may exert its effects on transcriptional initiation, as the

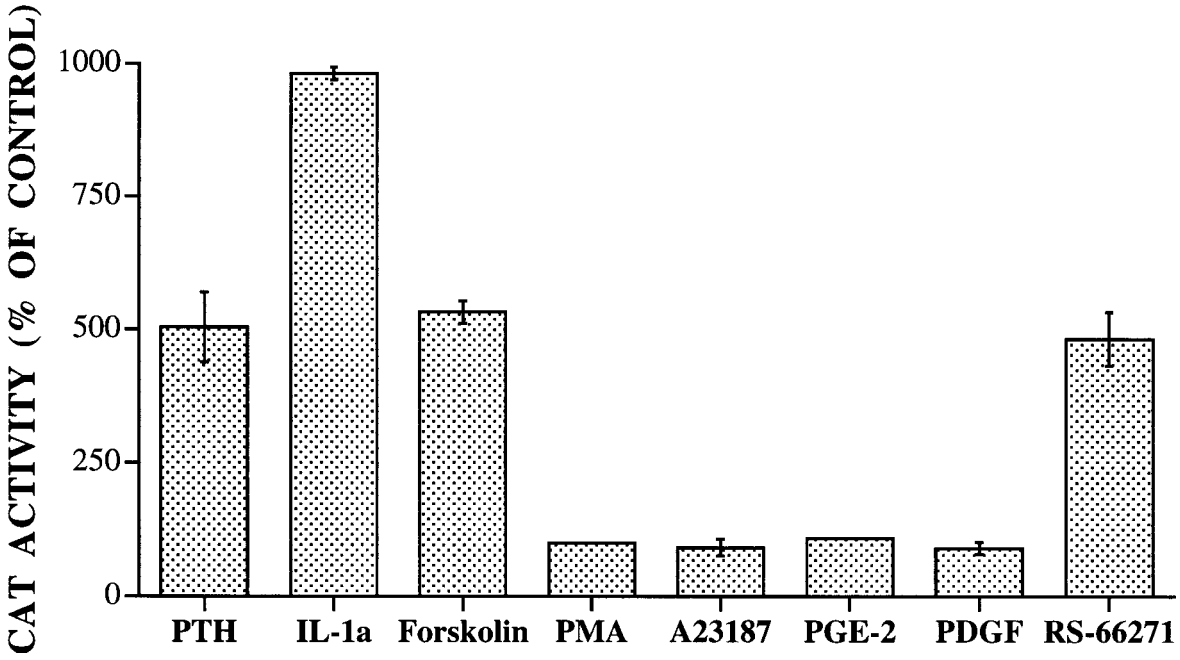

Fig. 7. Effect of IL-1α, PGE-2, forskolin, PMA, and PTH-related peptide analog on IL-6 promoter/CAT expression. SaOS-2 cells from clone 11 grown in T75 flasks to approximately 70% confluency were serum-deprived for 8–12 h. Individual plates of cells were either left untreated or treated with either IL-1α (50 ng/ml), PGE-2 (1 uM), forskolin (10 uM), PMA (1 uM), PTH-related peptide analog (10 nM), or PTH (10 nM) for 12 h. CAT enzyme activity was assayed and expressed as the percent increase over the activity of control cells (unstimulated). The results represent the mean ± SEM of three to six separate experiments.

IL-6 promoter was sufficient to confer PTH-induced expression of a reporter gene. A recent study has also reported that the stimulation of IL-6 mRNA expression by PTH is inhibited by the transcription blocker actinomycin D in MC3T3-E1 cells [Greenfield et al., 1996].

In unstimulated cells, IL-6 promoter activity is apparently very low to silent, as activity of the IL-6 promoter was close to or indistinguishable from the low test background in two of the three independent clones examined. The level of expression in the clone with the highest CAT expression level was still ten- to fifty-fold less when compared to expression from an RSV promoter. In addition, promoter activity was lower than that of other promoters that we tested, including SV40 early region and β-actin (unpublished data). The ability to detect this low level of expression was due to the sensitivity of the radioactive CAT assays [Neumann et al., 1987]. These low levels of reporter expression correlate with the virtual absence of endogenous IL-6 gene expression prior to stimulation. The induction of CAT expression by PTH was rapid but not transient and persisted up to 24 h examined. This later observation is not unexpected because the kinetics of induced CAT activity are dependent upon CAT transcript processing, CAT translation, and CAT mRNA and protein stability. The differences in the magnitude of induction by PTH on different cell clones may arise from differences in stable integration into the chromosome or differences in basal expression in individual clones. Multiple treatments of cells with PTH did not increase CAT expression and suggests the need for tightly controlled regulation.

The SaOS-2 cells are known to possess a PTH-responsive dual signal transduction system (cAMP-dependent protein kinase (PKA) and calcium-protein kinase C [Ca-PKC]). We found that forskolin, a direct activator of PKA, mimicked the stimulatory effect of PTH on IL-6 expression in SaOS-2 cells. This result is in agreement with previous studies and suggests that the messenger system mediated by PKA is sufficient to induce IL-6 expression [Greenfield et al., 1996]. The additive effect of PTH and forskolin may reflect the ability of forskolin to constitutively induce adenylate cyclase in a PTH receptor–independent manner [Fukayama et al., 1992a,b]. Similar potentiation of PTH acti-

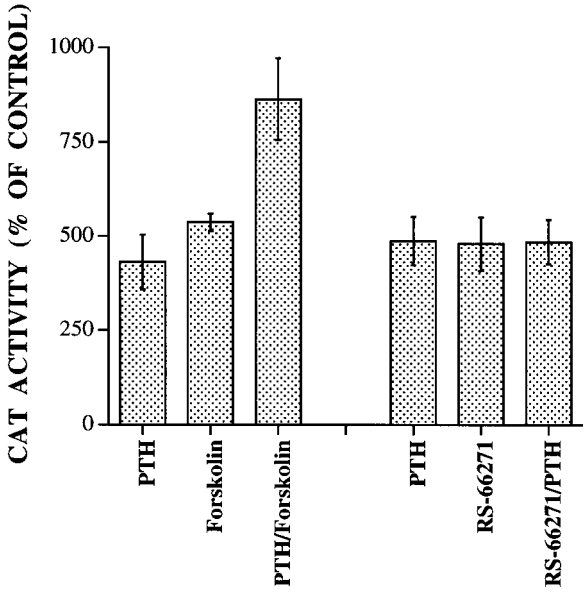

Fig. 8. Effect of combined treatment of hPTH (1-34) and forskolin or RS-66271 on IL-6 promoter/CAT expression. SaOS-2 cells from clone 11 were plated and grown in T75 flasks until cells were approximately 70% confluent. Cells were then serum-deprived for 8–12 h and were either left untreated or treated with an optimal concentration of PTH (10 nM) with or with out forskolin (1 uM) or RS-66271 (10 nM). CAT enzyme activity was assayed and expressed as the percent increase compared to the activity of the control cells (unstimulated). The results represent the mean ± SEM of three to six separate experiments.

vation by forskolin in SaOS-2 cells has been previously reported [Fukayama et al., 1992b; Minor et al., 1996; Yu and Chandrasekhar, in press]. As activation of Ca-PKC by PMA or A23187 had no effect on IL-6 expression, IL-6 activation in these cells is independent of PKC and calcium.

It has been suggested that PTH and other agents that stimulate bone resorption, such as IL-1, PGE-2, PTHrP, and PDGF, mediate their resorptive effects by modulating osteoblast IL-6 [Manolagas and Jilka, 1995; Riancho and Mundy, 1995; De La Mata et al., 1995; Holt et al., 1994a; Franchimont and Canalis, 1995; Linkhart et al., 1991]. This study confirms previous findings that IL-1 induces IL-6 expression and release in bone [Bertolini et al., 1994; Manolagas and Jilka, 1995; Linkhart et al., 1991]. Our data suggest that this effect on IL-6 is probably mediated at a transcriptional level. In contrast to previous findings demonstrating PGE-2– and PDGF-induced IL-6 production, we failed to detect reproducible changes in CAT expression following treatment with either PGE-2 or PDGF-BB. The few reports on PGE-2 effects on IL-6 in bone have measured IL-6 release from fetal or neonatal bone organ cultures [Holt et al., 1994a,b]. One study showed that PGE-2 effects on IL-6 are indirect and dependent on new protein synthesis [Holt et al., 1994a]. Our inability to detect significant changes in promoter expression suggest that PGE-2–induced IL-6 production may be post-transcriptional. Alternatively, PGE-2 effects on IL-6 production may be mediated by cells other than osteoblasts. However, in vitro studies which have investigated responsiveness of osteoblast-like cells to PTH have suggested osteoblasts may be a source of IL-6 production [Onyia et al., 1995a; Greenfield et al., 1993, 1995, 1996; Feyen et al., 1989; Holt et al., 1994b; Bertolini et al., 1994; Sakagami et al., 1993; Lowik et al., 1989]. In parietal bones from neonatal mice, Holt et al. [1994a] showed that the endocranial and ectocranial membranes associated with bone may be a more important source of PGE-2–induced IL-6 than osteoblasts per se.

As expected, RS-66271 had a comparable effect to PTH [Martin and Suva, 1988; Fukayama et al., 1988]. RS-66271 has been previously shown to have PTH-like effects both in vivo and in vitro and function by binding and signaling through the same receptor [Vickery et al., 1993; Avnur et al., 1993]. The finding that the effect of PTH and RS-66271 in combination was comparable to that of either agents alone indicates that both agents induce IL-6 transcription via the same mechanism.

The 5′ flanking region of the IL-6 gene contains a number of putative *cis*-acting elements which might be modulated by PTH. Some of these regulatory elements have been demonstrated to be required for inducible and tissue-specific transcriptional regulation in other systems [Sehgal, 1992]. They include multiple initiation sites, a region of homology to the c-fos serum response element encompassing a multiple response element (MRE), an NF-IL6 binding site, a potential recognition sequence for members of the *ets* family of transcription factors, two glucocorticoid response element (GRE) homologies, an AP-1 consensus site, a potential GATA-helix-loop-helix, and an NF-kB site [Akira et al., 1990; Libermann and Baltimore, 1990; Orkin, 1992; Ray et al., 1989; Seth et al., 1992; Shimizu et al., 1990; Tanabe et al., 1988; Zhang et al., 1990; Yasukawa et al., 1987]. The exact element(s) through which PTH affects IL-6 transcription has not been delineated. In

our studies, we used a large fragment of IL-6 upstream sequences containing the regulatory elements and multiple initiation sites so far described. This was to ensure a comprehensive PTH effect on gene transcription. Future studies directed at a systematic analysis of PTH-responsive element(s) is needed and would enhance our understanding of PTH-induced IL-6 expression.

In summary, our results demonstrate that, in cultured osteoblast-like osteosarcoma cells SaOS-2, PTH-regulated IL-6 expression is partly mediated by alterations in IL-6 transcription. The IL-6 promoter was sufficient to confer PTH and forskolin inducibility. Additional studies will be necessary to determine which cis elements in the IL-6 promoter mediate PTH action. The identification of these *cis* elements and interacting *trans* factors will facilitate our understanding of the molecular actions of PTH. We conclude that IL-6 is a target of PTH action and may be involved in the downstream effects of PTH in bone.

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