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ORIGINAL ARTICLE

Upregulation of PTH receptor mRNA expression by dexamethasone in UMR-106 osteoblast-like cells

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OBJECTIVES: Glucocorticoids influence receptor interactions of the parathyroid hormone (PTH) that are crucial for osteoblast function. As mechanisms linking receptor mRNA with glucocorticoids are incompletely understood, we investigated regulation of PTH receptor (PTH1R) mRNA expression in rat osteoblast-like UMR-106 cells by using dexamethasone (Dex), a synthetic glucocorticoid.

MATERIALS AND METHODS: UMR-106 cells were exposed to 10^{-8} to 10^{-5} M Dex, while some cells were also exposed to a transcriptional inhibitor (DRB) for 24 h with or without Dex. PTH-stimulated cyclicAMP activities were measured by an enzyme-linked immunosorbent assay. PTH1R mRNA was determined by Northern analysis. Transcriptional activities were measured as heretogeneous nuclear PTH1R RNA and also as luciferase activity in constructs, including the PTH1R gene promoter.

RESULTS: Dexamethasone dose-dependently increased PTH-stimulated adenylyl cyclase activity at 72 h. Dex markedly increased PTH1R mRNA accumulation, but did not change transcriptional activity. PTH1R mRNA stability was significantly increased by Dex in transcriptionally arrested cells.

CONCLUSION: In osteoblast-like cells, Dex induced upregulation of PTH1R mRNA followed by increased functional PTH receptor expression. This was caused by posttranscriptional mechanisms increasing mRNA stability.

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Keywords: glucocorticoids; PTH receptor; mRNA degradation; osteoblasts; osteoporosis

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Introduction

Glucocorticoids are used extensively for treatment of autoimmune and inflammatory disease. A major side effect of glucocorticoid treatment is rapid loss of bone in sites including the jaws, associated with increased risk of fracture (Reid, 1997). Periodontitis is a destructive inflammatory condition of the tissues supporting the teeth, induced by microorganisms colonizing tooth surfaces in close contact with the gingival margin. Certain risk factors for periodontal disease such as bacterial lipopolysaccarides increase glucocorticoid secretion by interfering with negative feedback regulation of glucocorticoid secretion (Page *et al*, 1997). Administration of glucocorticoids was reported to stimulate progression of periodontal disease (Breivik *et al*, 2000a), while treatment with a glucocorticoid receptor antagonist, RU486, reduced periodontitis in rats (Breivik *et al*, 2000b); both findings suggested that glucocorticoids aggravate involvement of periodontal bone and soft tissues in periodontal disease.

Integrity of the skeleton is controlled not only by calcitropic hormones such as the parathyroid hormone (PTH), but is also affected by drugs including glucocorticoids and inhibitors of cholesterol synthesis (statins) (Katagiri and Takahashi, 2002; Horiuchi and Maeda, 2006). Glucocorticoids have marked effects on bone metabolism. Increased circulating concentrations of glucocorticoids alter bone remodeling by decreasing bone formation and increasing bone resorption, resulting in osteoporosis (Patschan *et al*, 2001). However, mechanisms underlying the actions of glucocorticoids upon bone are not yet fully understood. Acting via the glucocorticoid receptor, glucocorticoids promote differentiation of preosteoblasts in the bone marrow stroma into mature osteoblasts with the potential to mineralize. Actions of glucocorticoids upon osteoblasts are complex, and vary with the maturation stage of the osteoblastic population and animal species. For example, glucocorticoids have been shown to increase bone resorption in neonatal mouse calvarium (Conaway *et al*, 1996) and to enhance PTH-stimulated bone formation and resorption by osteoblast-like cells (Kaji *et al*, 1997).

While the osteoclast-activating effects of PTH initially were thought to be mediated by interleukin (IL)-6 and IL-11 secretion by osteoblasts, PTH-stimulated production of IL-6 and IL-11 actually was found to be decreased by dexamethasone (Dex) (Kim *et al*, 1999). Although bone resorption has been suggested to contribute to glucocorticoid-induced osteoporosis (Patschan *et al*, 2001), the mechanism underlying long-term glucocorticoid-induced bone loss now is considered to involve impairment of osteoblast function and bone formation (Epstein *et al*, 2003).

The parathyroid hormone, which maintains calcium ion homeostasis in mammals including humans and rodents, is an 84-amino acid polypeptide hormone secreted by the parathyroid glands in response to changes in serum calcium concentration. A small decrease in serum calcium causes an increase in secretion of PTH, which acts rapidly to raise serum calcium concentrations by direct effects on bone and kidney, and indirect actions on the intestine. Osteoblasts, the target cells for PTH in bone, express the PTH/PTH-related protein (PTHrP) receptor (PTH1R), which has seven transmembrane domains, is coupled to G-proteins, and also binds PTHrP (Juppner *et al*, 1991). Osteoblasts regulate recuitment and activity of osteoclasts by expressing receptor activator of nuclear-κB ligand (RANKL) and osteoprotegerin. RANKL is expressed on the osteoblast/stromal cell surface and binds to its receptor, receptor activator of nuclear-κB, on surfaces of osteoclast precursors; in the presence of macrophage-colony stimulating factor, this binding stimulates osteoclastogenesis (Katagiri and Takahashi, 2002). Upon binding of PTH, osteoblasts decrease their own proliferation rate while increasing surface expression of RANKL, acting to stimulate formation and activity of osteoclasts. Accordingly, continuous exposure to high concentrations of PTH leads to increased bone resorption. On the other hand, intermittent administration of PTH induces anti-apotoctic signaling in osteoblasts (Bellido *et al*, 2003) and stimulates *in vivo* bone formation in vertebra, limb bones, and the mandible (Neer *et al*, 2001; Kawane *et al*, 2002). In sum, PTH is a major regulator of bone metabolism that can stimulate formation and activity of both osteoblasts and osteoclasts (Swarthout *et al*, 2002).

Binding of PTH or PTHrP to PTH1R induces accumulation of several intracellular second messengers including cyclicAMP (cAMP), calcium ion, and diacylglycerol, activating protein kinase (PK) A and PKC pathways. These second messenger systems alter gene transcription in osteoblasts. Many genes have been found to undergo regulation during bone formation, such as those encoding type I collagen, alkaline phosphatase, osteocalcin, and osteopontin, while genes regulated during bone resorption encode collagenase-3 (MMP-13), tissue inhibitors of metalloproteinases, and RANKL (Swarthout *et al*, 2002).

While PTH1R is expressed constitutively, expression ordinarily is downregulated in osteoblasts. Mechanisms involved in the desensitization of cellular responses to PTH have been described in detail in osteoblastic cells; these mainly represent decreases in receptor expression on the cell surface (downregulation). For PTH1R, downregulation involves lower rates of receptor biosynthesis (Gonzalez and Martin, 1996; Jongen *et al*, 1996; Kawane *et al*, 2001), and accelerates clearance of functional receptors from the cell surface via internalization and subsequent proteolysis (Ferrari *et al*, 1999; Tawfeek *et al*, 2002). Prolonged exposure to PTH and other substances such as 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$] lowers PTH1R mRNA expression in osteoblastic cells. Desensitization of the cAMP response to PTH and PTH1R downregulation has been observed in primary cultures of osteoblasts (Jongen *et al*, 1996) and in various osteoblastic cell lines (Gonzalez and Martin, 1996; Kawane *et al*, 2001). Desensitization in these investigations involved elevation of intracellular cAMP concentrations was accompanied by downregulation of receptor numbers. In contrast, glucocorticoids were found to increase PTH1R mRNA concentrations in ROS17/2.8 cells, an osteoblast-like rat osteosarcoma cell line (Urena *et al*, 1994). Although the interrelationship between glucocorticoids and the action of PTH through PTH1R is crucial for osteoblast function, detailed mechanisms of glucocorticoid-induced changes in PTH1R mRNA synthesis are not well documented. The present study was undertaken to regulate PTH1R mRNA expression by Dex, a synthetic glucocorticoid, in UMR-106 cells, another osteoblast-like rat osteosarcoma cell line.

Materials and methods

Cell culture

Osteoblast-like rat osteosarcoma cells (UMR-106; American Type Culture Collection, Rockville, MD, USA) were grown by routine methods in a monolayer culture at 37°C in an atmosphere of 5% CO_2/95% air. Dulbecco's modified Eagle's medium (ICN Pharmaceuticals, Costa Mesa, CA, USA) supplemented with 5% fetal bovine serum (Filtron, Brooklyn, Australia) was used. Cell cultures were passaged once weekly. At 70% confluence the culture medium was replaced for 24 h with serum-free medium containing 0.1% bovine serum albumin (BSA). Cells then were exposed to 10^{-8} to 10^{-5} M Dex (Sigma Chemical Co, St. Louis, MO, USA) for various time periods. Transcriptional arrest was induced in some UMR-106 cells by exposure to 75 μM 5,6-dichlorobenzimidazole riboside (DRB), a RNA polymerase II inhibitor, (Sigma; dissolved in dimethylsulfoxide). DRB was added 24 h after exposure to 10^{-6} M Dex or vehicle (dimetylsulfoxide); cells were then harvested at the times indicated.

Measurement of cAMP accumulation

Confluent UMR-106 cells were washed twice with assay buffer containing 135 mM NaCl, 6 mM KCl, 1 mM, 1 mM $MgCl_2$, 2.8 mM glucose, 1.2 mM $CaCl_2$, and 20 mM HEPES (Sigma) at pH 7.4, and were then incubated in the same buffer containing 0.1% heat-inactivated BSA, 1 mM isobutylmethylxanthine (Sigma), and 10^{-7} M rat PTH(1-34) (Peninsula

Laboratories, Belmont, CA, USA) at 37°C for 15 min. The buffer was then aspirated rapidly, 0.3 M perchloric acid was added to culture dishes, and acid extracts were used for the cAMP assay. Cellular cAMP was measured by using cAMP Biotrak Enzymeimmunoassay (Amersham Biosciences, Piscataway, NJ, USA). Cellular protein was also measured. Results are expressed as picomoles of cAMP per milligram protein over 1 min (Kawane and Horiuchi, 1999).

Northern blot analysis

To assess the effect of Dex on abundance of PTH1R mRNA, Northern blot analysis was performed as described previously (Kawane and Horiuchi, 1999; Kawane *et al*, 2001). Briefly, total RNA was extracted from cells using guanidine thiocyanate. Total RNA was fractionated electrophoretically on a 1.2% agarose gel containing formaldehyde, and transferred to nylon membranes. Membranes were hybridized with PTH1R cDNA labeled with [α-^{32}P]deoxy-CTP. Hybridization was carried out for 2 days at 42°C, followed by washing with 0.1–2x 150 mM NaCl, 10 mM sodium phosphate and 1 mM EDTA, pH 7.4 (SSPE) containing 0.1% SDS at 65°C. For standardization, blots were rehybridized with a cyclophilin cDNA probe. Signal intensity on membranes was quantitated by a Molecular Imager FX (Bio-Rad, Hercules, CA, USA) equipped with Quantity One 4.1.1 image analysis software (Bio-Rad).

Quantification of PTH1R heterogeneous nuclear RNA (hnRNA)

PTH1R hnRNA was determined by reverse transcription-polymerase chain reactions (RT-PCR) using specific primers designed to amplify hnRNA between exons M and T of the PTH1R gene as described previously (Kawane and Horiuchi, 1999). Briefly, the nucleotide sequences of the intron and parts of exon M6/7 and T were determined. A sense primer (5′-CGTCTTTG GGGCATTTGAGT-3′) spanning nucleotides of the intron between exons M7 and T of the PTH1R gene, and an antisense primer (5′-AAACACTGGCTTCTTG GTCC-3′) spanning nucleotides of exon T were synthesized. Total RNA was extracted from UMR-106 cells and treated with ribonuclease-free deoxyribonuclease I (Boehringer, Indianapolis, IN, USA) to remove potentially contaminating DNA. One microgram of RNA was copied into cDNA using reverse transcriptase and random hexanucleotide primers. The newly synthesized cDNA was amplified by PCR through 21 cycles. PCR products were loaded onto a 1.2% agarose gel and transferred to a nylon membrane. Southern blotting was performed using a rat PTH1R cDNA probe radiolabeled with [α-^{32}P]deoxy-CTP. Amplified RNA from rat PTH1R gene was corrected for level of cyclophilin hnRNA by RT-PCR using the same synthesized cDNA. PCR was performed for 18 cycles. Signal intensity was quantified with the Molecular Imager FX.

Transient transfections and reporter assays

Promoter constructs used in transfection assays were assembled as described previously (Kawane *et al*, 2001). Briefly, promoter constructs used in transfection assays were derived from rPRP12BB (a 7-kb clone of PTH1R promoter containing exons U2 and U3). The *Xho*I/*Bam*H1 and *Bgl*II/*Bam*H1 fragments from rPRB12 BB, respectively, were subcloned into the *Xho*I/*Bgl*II site and the *Bgl*II site of the pGL-3 basic vector. Transient transfections were performed with UMR-106 cells grown to 70% confluence in six-well plates. Cells were cotransfected with 0.5 μg of luciferase-reporter plasmid and 0.5 μg of β-galactosidase (β-gal) expression vector (pEFBOS-LacZ) as an internal control, using GenePorter transfection reagent (Gene Therapy Systems, San Diego, CA, USA). Cells were harvested 48 h after transfection. Cell extracts were subjected to assay using the Promega Luciferase Assay System (Madison, WI, USA). All samples were measured in triplicate. Luciferase activity was corrected for transfection efficiency as indicated by β-galactosidase activity.

Statistical analysis

Data are presented as mean ± s.e.m. Differences between treated and untreated groups were assessed by Student's *t*-test. Multiple comparisons were evaluated by analysis of variance (ANOVA) followed by Scheffe's *F*-test. Statistical analysis was performed with the Statview 4.02 software package (Abacus Concepts, Berkeley, CA, USA). $P < 0.05$ was considered as statistically significant.

Results

Increase of PTH-stimulated adenylyl cyclase (AC) activity by Dex

We examined the effect of Dex pretreatment on PTH-stimulated accumulation of cAMP in osteoblast-like UMR-106 cells (Figure 1). The time course of the effect of Dex in UMR-106 cells showed a significant increase in PTH-stimulated AC activity occurred as early as 24 h after initiation of treatment with 10^{-6} M Dex. Time-dependent increase of cAMP accumulation was observed for up to 72 h treatment (Figure 1a). Dex at 10^{-7} M significantly enhanced PTH-stimulated AC activity, while maximal stimulation was obtained at concentrations of 10^{-6} to 10^{-5} M (Figure 1b). These results indicated that Dex augments numbers of functional PTH receptors on the surfaces of UMR-106 cells.

Elevation of PTH1R mRNA accumulation by Dex

We tested whether Dex increases PTH1R mRNA abundance in UMR-106 cells. The time course of the effect of Dex on the amount of PTH1R mRNA was determined in cells treated with 10^{-6} M Dex (Figure 2). A significant increase in PTH1R mRNA was evident 12 h after initiation of treatment with Dex. A time-dependent stimulation was observed for up to 48 h of treatment. In a dose–response experiment, the increase in PTH1R mRNA in UMR-106 cells in response to Dex was significant at a concentration of 10^{-8} M and was maximal at 10^{-7} M (Figure 3). Thus, Dex enhanced PTH1R mRNA accumulation in UMR-106 osteoblast-like cells.

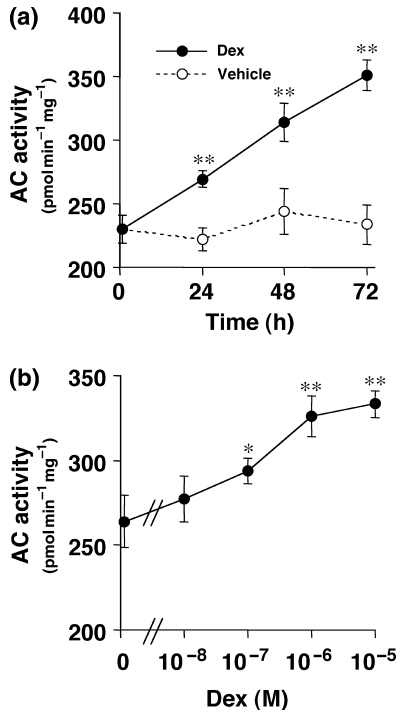

Figure 1 Effect of dexamethasone (Dex) on parathyroid hormone (PTH)-stimulated adenylyl cyclase (AC) activity in UMR-106 cells. Cells were preincubated with vehicle (○) or 10^{-6} M Dex (●) for the time periods indicated (a), and with vehicle or indicated concentrations of Dex for 72 h (b). Cells were then incubated with 10^{-7} M rat PTH(1-34) for 15 min in assay buffer. AC activity is expressed as cellular cAMP production in picomoles per milligram protein per minute. Data are expressed as mean ± s.e.m. of triplicate determinations. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with vehicle-treated control

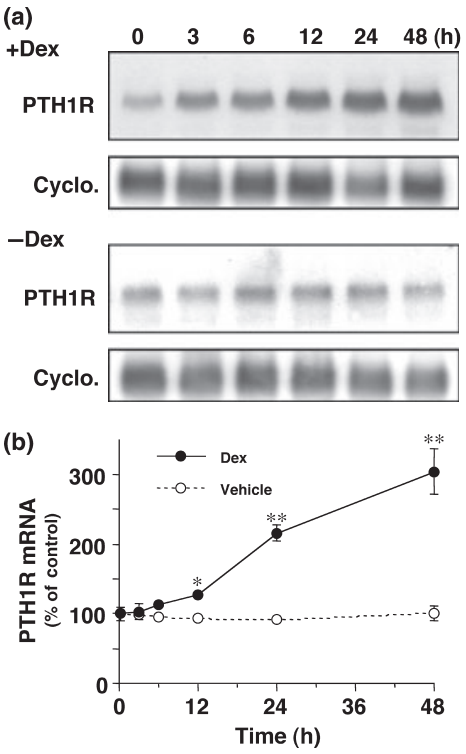

Figure 2 Time course of the increase of PTH1R mRNA expression by dexamethasone (Dex) in UMR-106 cells. Cells were harvested after time periods indicated for vehicle or 10^{-6} M Dex in serum-free Dulbecco's modified Eagle's medium containing 0.1% bovine serum albumin. (a) Total RNA was extracted and subjected to Northern blot analysis for mRNAs encoding PTH1R and cyclophilin (Cyclo.). (b) Determination of PTH1R mRNA abundance normalized by cyclophilin mRNA concentrations. Cells were treated with vehicle (○) or 10^{-6} M Dex (●). Data are expressed as mean ± s.e.m. of triplicate determinations. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with vehicle-treated control at each time point

Transcriptional regulation of the PTH1R gene by Dex

To determine whether Dex stimulates transcription of the PTH1R gene, a primary transcript of the PTH1R gene, hnRNA, was measured in Dex-treated cells (Figure 4). Because transcriptional regulation frequently leads to changes in hnRNA synthesis, concentrations of hnRNA were assessed in the presence or absence of Dex. Addition of vehicle or Dex at 10^{-6} M for 12–48 h did not show any change in concentrations of PTH1R hnRNA in UMR-106 cells. We next examined whether Dex affected activity of a key rat PTH1R gene transcription promoter using firefly luciferase as a reporter. A construct containing the PTH1R promoter and the luciferase gene was used. The upstream construct (from −3365 to +103) contained the exon U3 promoter, a major promoter of the gene in osteoblasts (Figure 5). Several deletion mutants of the rat PTH1R gene promoter driving expression of the luciferase gene were transiently transfected into UMR-106 cells in the absence or presence of 10^{-6} M Dex, which did not affect promoter activity of any construct containing the U3 promoter region. Even the shortest construct (−128/+103) was not influenced by Dex treatment. The presence of a putative glucocorticoid response element (GRE) was considered at the location (−1820 to −1806) of the 5′-flanking sequence of exon U3. However, the putative GRE would not account for PTH1R mRNA accumulation in response to Dex because luciferase activity of two constructs, −1946/+103 and −1005/+103, was not changed by treatment with Dex. These results indicated that Dex did not affect transcription of the PTH1R gene in UMR-106 cells.

Alternatively, we considered whether Dex altered the stability of PTH1R. UMR-106 cells were exposed to vehicle or Dex at 10^{-6} M for 24 h, after which 75 μM DRB, a RNA polymerase II inhibitor, was added to cultures (Figure 6). Decay of PTH1R mRNA was strikingly slowed in Dex-treated cells compared with vehicle-treated cells. The half-life of PTH1R mRNA was approximately 2.5 h in vehicle-treated cells, but was 7 h in Dex-treated cells. These results indicated that Dex increased the stability of PTH1R mRNA in UMR-106 cells.

Discussion

Anti-inflammatory and immunosuppressive properties of glucocorticoids such as Dex have promoted their extensive clinical use. These agents are prescribed widely for treatment of asthma, rheumatoid arthritis, inflammatory bowel disease, autoimmune disease, and

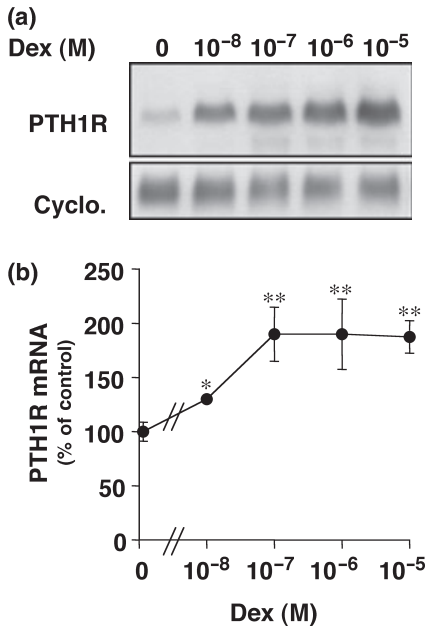

Figure 3 Effect of dexamethasone (Dex) on PTH1R mRNA expression in UMR-106 cells. (a) Cells were treated with the indicated concentrations of Dex for 24 h. Total RNA was subjected to Northern blot analysis. (b) Quantitative analysis of PTH1R mRNA abundance in Dex-treated cells. PTH1R mRNA abundance was normalized to cyclophilin mRNA concentrations. Data are given as mean ± s.e.m. of triplicate determinations. $*P < 0.05$ and $**P < 0.01$ compared with vehicle-treated control

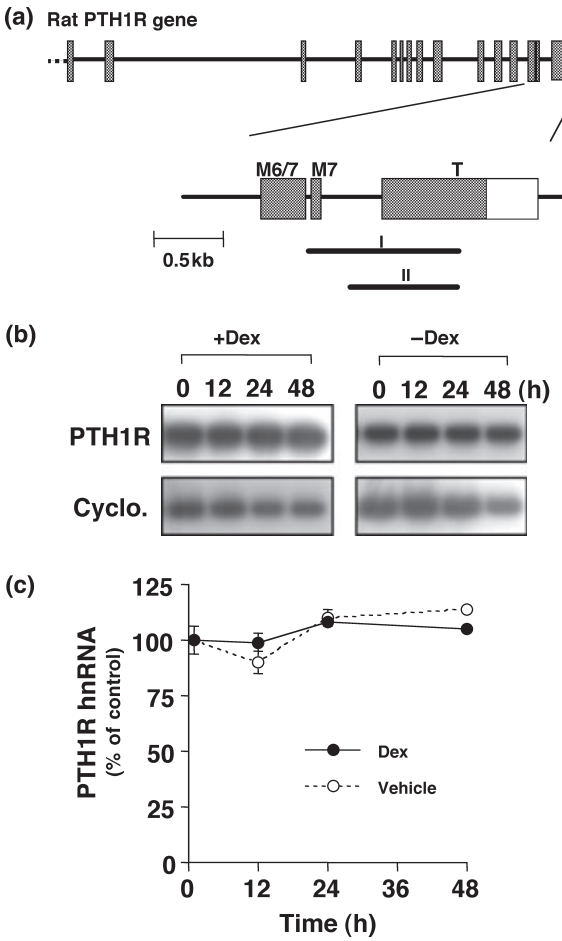

Figure 4 Time course of PTH1R hnRNA concentrations by dexamethasone (Dex) in UMR-106 cells. Serum-depleted cells were treated with 10^{-6} M Dex (●) or vehicle (○) for the time periods indicated. Total RNA from the cells was reverse-transcribed and amplified by PCR. (a) Schematic representation of the structure of the rat PTH1R gene. The gene between exons M6/7 and T was expanded; and these exons are shown in boxes. An open reading frame is indicated by boxes with reticulation, while the 3′-untranslated region is shown as an open box. The nucleotide sequence of region I was determined and region II was amplified by PCR for determination of PTH1R hnRNA concentrations. (b) Total RNA was subjected to RT-PCR, and products were analyzed by Southern blotting. (c) Quantitative determination of PTH1R hnRNA. Results were normalized to those for cyclophilin expression, and are given as mean ± s.e.m. of triplicate determinations

immunosuppression to prevent organ transplant rejection. However, side effects of potent glucocorticoids are dramatic. Sustained high concentrations of glucocorticoids obtained by treatment with Dex are known to inhibit skeletal growth and bone remodeling. Bone loss (glucocorticoid-induced osteoporosis) resulting in incapacitating fractures can negate the benefits of steroid therapy (Reid, 1997; Epstein *et al*, 2003). As mechanisms underlying glucocorticoid-induced osteoporosis are poorly understood, we examined regulation of PTH1R expression by Dex in osteoblast-like UMR-106 cells.

The expression of PTH1R is typically downregulated by a relatively short exposure to several hormones and growth factors. Expression is controlled not only by homologous downregulation seen in treatment with PTH or PTHrP, but also in treatment with unrelated agonists such as insulin-like growth factor (IGF)-I (Kawane and Horiuchi, 1999), prostaglandin E_1 (Civitelli *et al*, 1992), calcitonin (Guo *et al*, 1997) and $1,25(OH)_2D_3$ (Gonzalez and Martin, 1996), which act at different receptors. We have previously shown that homologous desensitization to PTH in UMR-106 cells results from reduced expression of the PTH1R gene (Kawane *et al*, 2001). Inhibition of PTH1R expression already was maximal in UMR-106 cells at 2 h after initiation of treatment with rat PTH(1-34), and was maintained for up to 24 h. Furthermore, cycloheximide, a protein synthesis inhibitor, did not affect the suppression of receptor mRNA. Homologous downregulation therefore was rapid and did not require *de novo* protein synthesis. Our previous report indicated that PTH-induced downregulation of receptor mRNA was mediated by a mechanism dependent on cAMP produced by AC linked with PTH1R (Kawane *et al*, 2003). In contrast, heterologous desensitization to PTH in UMR-106 cells, such as that induced by IGF-I, resulted from reduced expression of the PTH1R gene. Maximal inhibition of PTH1R mRNA expression by IGF-I, observed 6 h after treatment in UMR-106 cells, required *de novo* protein synthesis. IGF-I suppressed PTH1R gene transcription via signal transduction of the ERK1/2MAP kinase pathway (Kawane *et al*, 2005). Mechanisms of downregulation of PTH1R gene by PTH thus differ from those induced by IGF-I.

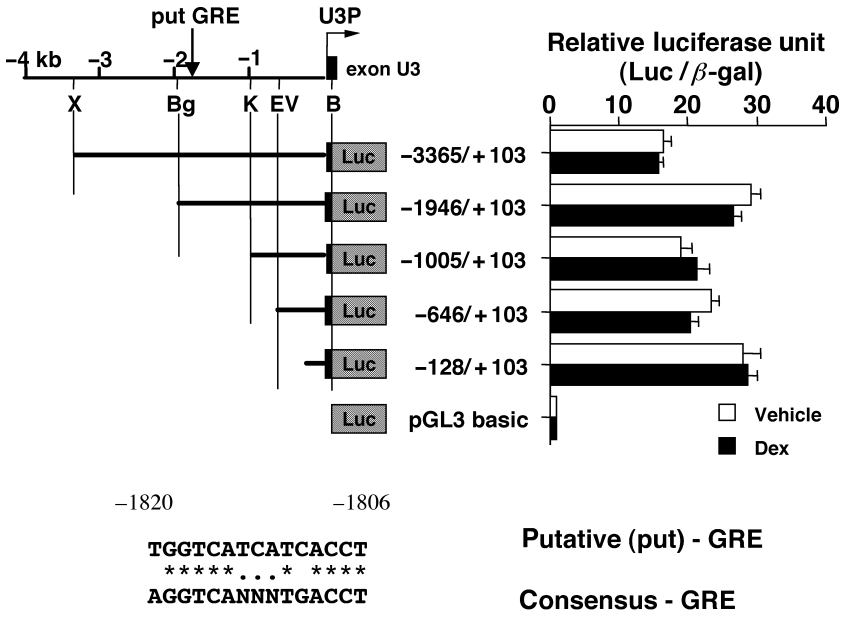

Figure 5 Effect of dexamethasone (Dex) on transcriptional activity of the PTH1R gene in UMR-106 cells. Left: Schematic representation of deletion mutants of the 5′-flanking region nucleotides of rat PTH1R gene (from −3365 bp to +103 bp). Numbers in the name of each deletion mutant indicate 5′ and 3′ ends. Restriction enzyme sites are indicated as: X, *Xho*I; Bg, *Bgl*II; K, *Kpn*I; EV, *Eco*RV; and B, *Bam*HI. Right: Luciferase activity of the 5′-flanking region of the gene transfected into UMR-106 cells without or with 10^{-6} M Dex. Luciferase activity was measured and normalized for transcription efficiency using a cotransfected β-gal reporter. Relative luciferase activity is shown as units relative to promoter-less plasmid pGL-3 basic vector. Sequences of putative (put) and consensus GRE also are shown (bottom). Data are given as mean ± s.e.m. of triplicate determinations

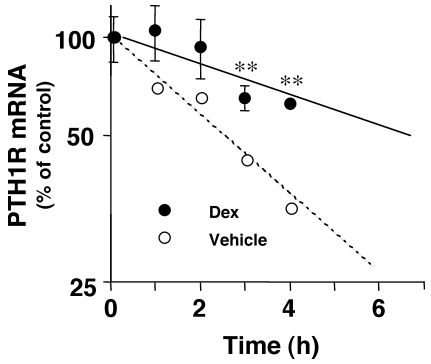

Figure 6 Stability changes in PTH1R mRNA induced by dexamethasone (Dex) in UMR-106 cells. Serum-depleted cells were exposed to 10^{-6} M Dex (●) or vehicle (○) for 24 h before the addition of 75 μM DRB, a transcriptional inhibitor. At the indicated time point after the addition of DRB, total RNA was subjected to Northern blot analysis. PTH1R mRNA normalized to cyclophilin mRNA was quantified by densitometry. Data are expressed as mean ± s.e.m. of triplicate determinations. $^{**}P < 0.01$ compared with vehicle-treated control at each time point

In contrast to the general pattern of downregulation, several studies in our laboratory and others (Rodan *et al*, 1984; Yamamoto *et al*, 1988) showed that treatment of osteoblast-like cells such as UMR-106 and ROS17/2.8 with glucocorticoids resulted in time- and dose-dependent increases in PTH1R mRNA expression and PTH-stimulated cAMP accumulation. The present study investigated mechanisms of PTH1R mRNA accumulation by Dex in osteoblast-like UMR-106 cells.

Much information concerning regulation of functional PTH receptor expression in osteoblastic cells has been obtained by ligand-receptor binding studies and by determination of PTH-induced accumulation of cAMP (Rodan *et al*, 1984; Yamamoto *et al*, 1988; Kawane and Horiuchi, 1999). We demonstrated that upregulation of functional PTH receptor expression assessed by AC activity was observed 24–72 h after treatment with Dex in UMR-106 cells. Northern blot analysis confirmed that Dex dose-dependently augmented PTH1R mRNA accumulation. We next sought to establish whether Dex directly enhanced PTH1R gene transcription or stabilized receptor mRNA. As concentrations of hnRNA correlate well with transcription rates (Buttice and Kurkinen, 1993), we monitored the expression of PTH1R hnRNA in UMR-106 cells over 48 h in the presence and absence of Dex. The expression of PTH1R hnRNA did not change with Dex treatment. In addition, we cloned the promoter region of the rat PTH1R gene and transiently transfected chimeric deletion constructs containing the 5′-flanking region (3365 bp), and the luciferase gene into UMR-106 cells. Arguing again against a transcription increase, Dex did not alter luciferase activity of various deletion constructs in transfected cells. We found a putative GRE of the rat PTH1R gene in the present study, but Dex did not affect luciferase activity in constructs containing this putative GRE in the promoter. Rather, Dex enhanced PTH1R stability in transcriptionally arrested UMR-106 cells by strikingly prolonging the half-life of the mRNA. The data presented here demonstrate that upregulation of PTH1R mRNA expression in osteoblast-like UMR-106 cells involved posttranscriptional regulation, increasing stability of the mRNA. Accumulation of PTH1R mRNA and elevated activity of PTH-stimulated AC by Dex required a long interval compared with appearance of homologous and heterologous downregulation by PTH and IGF-I (Kawane *et al*, 2003).

In humans and rats, transcripts encoding the PTH1R gene are derived from at least three promoters located at a region including four promoter-specific untranslated exons (U1, U2, U3, and U4) (Minagawa *et al*, 2000; Kawane *et al*, 2003): the upstream promoter (U1P) is a tissue-specific promoter active in the kidney and the ovary (Joun *et al*, 1997); the middle promoter (U3P) is

ubiquitous and functions in a variety of tissues including bone (Amizuka *et al*, 1999); and a downstream promoter (U4P), adjacent to exon S, is known to be active in osteoblast-like cells in humans and rats. Activity of the upstream promoter (U1P) is very weak or absent in bone (Joun *et al*, 1997). We recently showed that PTH and IGF-I clearly repressed activities of U3P and U4P in UMR-106 cells (Kawane *et al*, 2003). In the present study we chose U3P to examine how Dex raises PTH1R mRNA concentrations in UMR-106 cells. The results showed that the major promoter of the rat PTH1R gene in osteoblasts, U3P, was not affected by treatment of Dex, which, therefore, did not exert transcriptional control of the gene.

Intracellular abundance of an mRNA is determined by rates of both synthesis and degradation. Especially, in cases of downregulation, we have demonstrated mechanisms that control PTH1R gene transcription. However, posttranscriptional mechanisms also are important. AU-rich elements (AREs) which include AUUUA sequences, polyA tracts, and UUAUUUAAU sequences occur in the 3′-untranslated region of several mRNAs including cyclooxygenase-2 mRNA, and represent important determinants of mRNA turnover (Shaw and Kamen, 1986) and targets of RNA-binding proteins such as AUF1 (Burd and Dreyfuss, 1994). AREs within the 3′-untranslated region of cyclooxygenese-2 mRNA were found to affect mRNA stability and Dex-regulated degradation of mRNA in the presence of an anti-cancer drug (Subbaramaiah *et al*, 2003). A recent study reported posttranscriptional enhancement of tissue factor mRNA stability by Dex in monocytes (Reddy *et al*, 2004), most likely by increasing stability of a transcript consisting of the final 300 nucleotides of the mRNA, a region containing several AREs. Rat PTH1R mRNA has a relatively short 3′-untranslated region that contains a few AU-rich sequences, although strictly defined AREs are not present in the region. At present, the mechanism by which Dex stabilizes PTH1R mRNA has not been defined. Dex may regulate binding of RNA-binding proteins such as HuR (Subbaramaiah *et al*, 2003) and AUF1 (Burd and Dreyfuss, 1994) to these AU-rich sequences in the 3′-untranslated region. In such a manner, Dex may control synthesis of these proteins in osteoblasts.

Glucocorticoid-induced osteoporosis is a common variety of secondary osteoporosis that exhibits two phases contributing to bone loss. The first phase is characterized by increased bone resorption, while the second phase shows decreased bone formation (probably the predominant effect). Evidence has accumulated that osteoblasts bear the brunt of the insult from glucocorticoid excess. Glucocorticoids have been shown to inhibit expression of a variety of genes critical for osteoblast function such as those encoding type I collagen, transforming growth factor-β, IGF-I, and RANKL, that promote osteoclast formation (Patschan *et al*, 2001). Glucocorticoids induce not only low-turnover bone loss but also decreased lifespan of osteoblasts by mechanisms that accelerate apoptosis; such findings may predominate in the second phase of the disease. In contrast, the pathophysiology of excessive remodeling in the initial stage of glucocorticoid-induced osteoporosis is not fully understood. This bone loss can be explained by a decrease in sex steroids and by indirect effects upon PTH secretion. Glucocorticoid-induced osteoporosis can lead to secondary hyperparathyroidism, but the contribution of secondary hyperparathyroidism to steroid-associated osteoporosis is controversial (Rubin and Bilezikian, 2002).

Another way to explain increased bone resorption in the first stage would be increased sensitivity to circulating PTH. Our current findings support the concept as glucocorticoids such as Dex enhanced the sensitivity to PTH exposure by means of increased expression of PTH receptor in osteoblasts. High expression of the functional PTH receptor induced by Dex in osteoblasts therefore appears to be related to stimulation of bone resorption by PTH, which can be a cause of glucocorticoid-induced osteoporosis.

The relationship between glucocorticoid exposure and aggravation of periodontitis is well documented in rats, where experimental periodontitis was induced by tying a silk ligature around the neck of molar teeth. Administration of corticosterone increased the development of periodontitis, while adrenoectomy reduced disease severity (Breivik *et al*, 2000a). Chronic administration of the glucocorticoid antagonist RU 486 reduced damage in ligature-induced and naturally occurring periodontitis (Breivik *et al*, 2000b). Most of the loss of attachment in periodontitis is thought to result from the immune response to dental plaque microorganisms. A compensatory elevation in infiltration by phagocytic polymorphonuclear leukocytes may occur in gingival tissue and alveolar bone during glucocorticoid-induced immune suppression in periodontitis, where the disease may be aggravated by accelerated bone-resorptive activity of osteoblasts resulting from increased PTH sensitivity involving elevated expression of functional PTH receptors. At least partially, then, Dex may accelerate progression of periodontal disease through increased expression of PTH1R mRNA in osteoblasts.

In conclusion, Dex greatly enhances PTH1R mRNA abundance and PTH-stimulated AC activity in osteoblast-like UMR-106 cells. Upregulation of PTH1R mRNA expression is caused by involvement of posttranscriptional mechanisms such as increased stability of the mRNA, as opposed to transcriptional activation of the gene. Glucocorticoid-induced osteoporosis thus results at least, in part, from excessive responsiveness to PTH that stimulates bone resorption.

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