Stimulatory effects of parathyroid hormone and 1,25-dihydroxyvitamin D_3 on insulin-like growth factor-binding protein-5 mRNA expression in osteoblastic UMR-106 cells: the difference between transient and continuous treatments

Masamichi Nasu, Toshitsugu Sugimoto*, Kazuo Chihara

Third Division, Department of Medicine, Kobe University School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe 650, Japan

Received 7 April 1997

Abstract The transient treatment with parathyroid hormone (PTH) for 12 h, followed by its removal for 36 h, stimulated insulin-like growth factor-binding protein (IGFBP)-5 mRNA expression more strongly than the continuous treatment for 48 h in osteoblastic UMR-106 cells. The transient but not continuous treatment with A23187 also stimulated it. In contrast, 1,25-dihydroxyvitamin D_3 stimulated it, irrespective of the treatment design. IGFBP-5 stimulated type-1 procollagen mRNA expression. The present study first indicated that the transient treatment with PTH more effectively stimulated IGFBP-5 mRNA expression than its continuous treatment partly via an increase in intracellular calcium and suggested that IGFBP-5 might be involved in the anabolic action of PTH in bone.

Key words: Parathyroid hormone; 1,25-Dihydroxyvitamin D_3; Insulin-like growth factor-I; Insulin-like growth factor-binding protein-5; Type-1 procollagen

1. Introduction

It is well known that parathyroid hormone (PTH) possesses a catabolic action in bone, but its anabolic action in bone has also been shown [1]. The previous studies [2,3] demonstrated that the intermittent administration of PTH to rats caused an increase in bone volume and stimulated bone formation.

Clinical study [4] also revealed that the intermittent administration of PTH increased the bone mineral density in osteoporotic patients. Moreover, a previous in vitro study [5] revealed that transient treatment with PTH increased the synthesis of type-1 collagen, whereas with continuous treatment with PTH it decreased. Thus, intermittent treatment with PTH seems to promote anabolic action in bone. However, its mechanism has not been clearly defined.

Insulin-like growth factor (IGF)-I is produced by osteoblasts and regulates bone formation in an autocrine and/or paracrine fashion [6,7]. Several IGF-binding proteins (IGFBP) are also produced by osteoblasts [8]. Among these IGFBPs, IGFBP-5 stimulates osteoblast proliferation directly or indirectly through IGF-I action [9,10]. Moreover, IGFBP-5 has a very strong affinity for hydroxyapatite [11], suggesting its important role in bone formation. Torring et al. [12] reported that PTH stimulated IGFBP-5 production in osteoblastic UMR-106 cells. It is, therefore, possible that the anabolic action of the intermittent treatment with PTH in vivo might be partly via its effect on IGFBP-5 production in osteoblasts. On the other hand, there has been evidence [13–15] that 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), another important calcium-regulating hormone, might stimulate bone formation partly via augmenting IGF-I activity.

The present study was, therefore, performed to compare the effect of transient or continuous treatment with PTH or $1,25\ (OH)_2D_3$ on IGFBP-5 mRNA expression in UMR-106 cells.

2. Materials and methods

2.1. Materials

UMR-106 cells were the generous gift from Dr. T.J. Martin (Melbourne, Australia). Human (h) PTH-(1–34) was purchased from Peptide Institute Inc. (Osaka, Japan). $1,25(OH)_2D_3$ was kindly provided by Chugai Pharma Co. Ltd. (Shizuoka, Japan). $N^6,O^{2\prime}$-dibutyryl adenosine 3′,5′-cyclic monophosphate (dbcAMP) and phorbol 12-myristate 13-acetate (PMA) were purchased from Sigma Chemical Co. (St. Louis, MO), A23187 from Hoechst Japan Co. (Tokyo, Japan), and recombinant human IGF-I and recombinant human IGFBP-5 from Cosmo Bio Co. (Tokyo, Japan). All other chemicals were of analytical grade.

2.2. Cell culture

UMR-106 cells were maintained in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal calf serum and penicillin G (100 IU/ml) in 5% CO_2–95% air atmosphere at 37°C, as previously described [16]. Cells were passed weekly, using 0.05% trypsin-0.02% EDTA solution. UMR-106 cells were cultured in 6-well plates. For the continuous treatment, these cells were incubated with each substance for 48 h. For the transient treatment, these cells were incubated with each substance for 12 h, the incubation medium was replaced by fresh medium without substance several times, and the cells were further incubated for 36 h.

2.3. Northern hybridization

The total RNA was extracted from cultured cells by means of the acid guanidinium thiocyanate-phenol-chloroform extraction method [17]. Twenty micrograms of total RNA was denatured, and run on a 1% agarose gel containing 2% formaldehyde, then transferred to a nitrocellulose membrane. This was hybridized to a ^{32}P-labeled DNA prove overnight at 42°C. The hybridization probe was either the 6.0 kb *Stul–Sac*II–*Hin*dIII fragment of the rat IGFBP-5 cDNA which was kindly provided by Dr. Shunichi Shimazaki (The Whitter Institute, La Jolla, CA), or the 2.8 kb *Eco*RI fragment of α1 gene of type-1 procollagen, a gift from Dr. Kimura (Osaka University, Japan). Also, a 1.2 kb β-actin cDNA was used as a reference. After hybridization, the filter was washed twice with 2× standard saline citrate (SSC) containing 0.5% sodium dodecyl sulfate (SDS), and subsequently twice with 0.1× SSC containing 0.5% SDS at 58°C for 1 h.

*Corresponding author. Fax: 78-371-6468

Abbreviations: PTH, parathyroid hormone; IGF-I, insulin-like growth factor-I; IGFBP-5, insulin-like growth factor-binding protein-5; $1,25(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; dbcAMP, $N^6,O^{2\prime}$-dibutyryl adenosine 3′,5′-cyclic monophosphate; PMA, phorbol 12-myristate 13-acetate

PII S0014-5793(97)00484-5

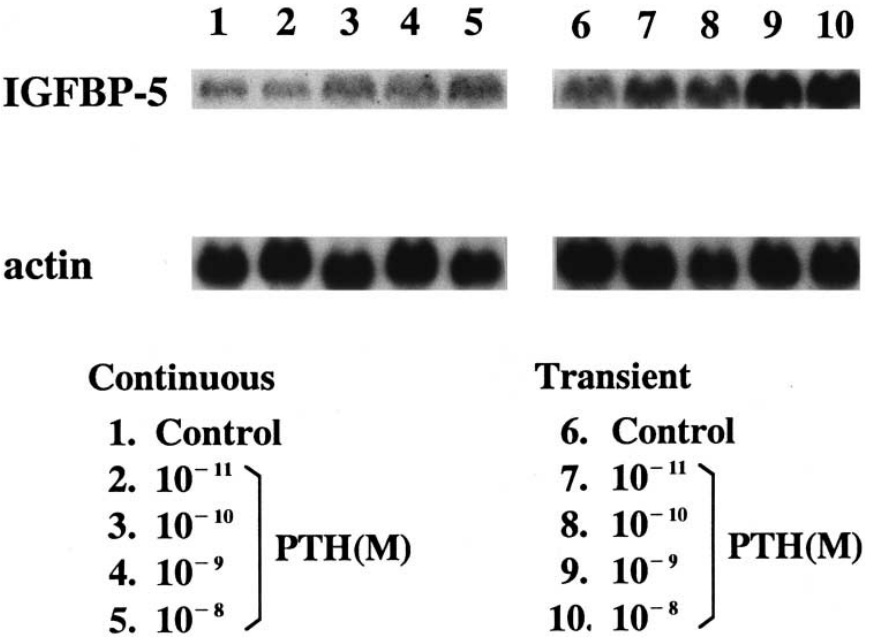

Fig. 1. Effects of continuous or transient treatment with PTH on IGFBP-5 mRNA expression in osteoblastic UMR-106 cells. Total RNA was isolated from UMR-106 cells after the continuous (for 48 h, left side) or transient (for 12 h, followed by its removal for 36 h, right side) treatment with indicated concentrations of hPTH-(1–34). The transferred RNA blots were hybridized first with ^{32}P-labeled rat IGFBP-5 cDNA and visualized by autoradiography. The probe was eluted and the blot was rehybridized with ^{32}P-labeled β-actin cDNA.

3. Results and discussion

First, we examined the effects of the transient or continuous treatment with 10^{-11} M to 10^{-8} M hPTH-(1–34) on IGFBP-5 mRNA expression in UMR-106 cells. As shown in Fig. 1, both transient and continuous treatments with hPTH-(1–34) dose-dependently stimulated the expression of IGFBP-5 mRNA in these cells, and the transient treatment with PTH was more potent to stimulate IGFBP-5 mRNA expression than the continuous one. The stimulation by PTH of IGFBP-5 mRNA expression was compatible with the previous evidence [12] that 24 h treatment with PTH increased IGFBP-

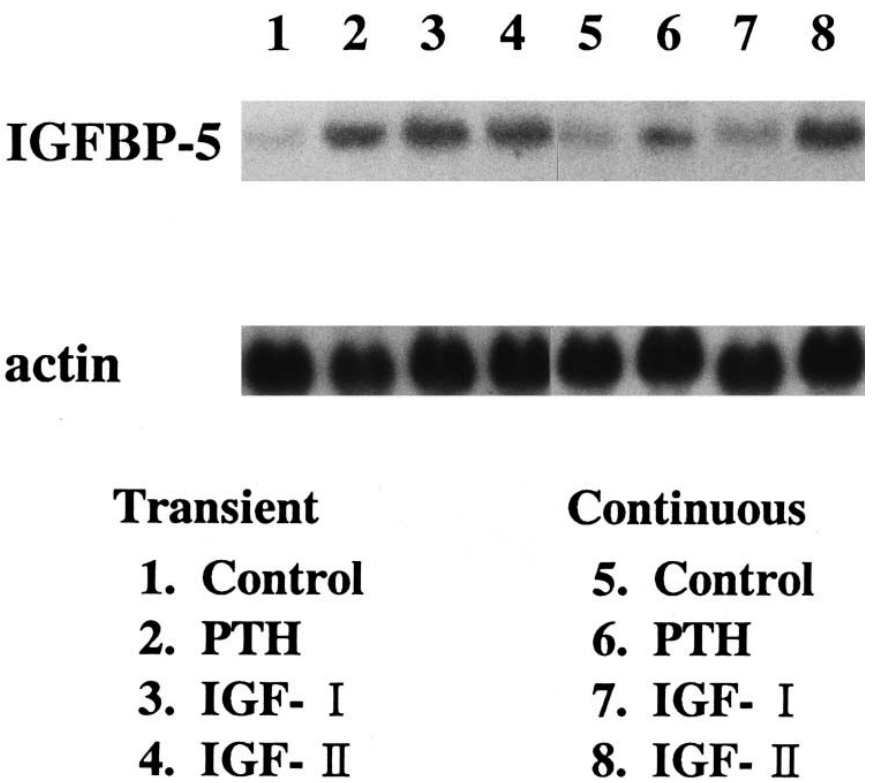

Fig. 2. Effects of transient or continuous treatment with IGF-I or IGF-II on IGFBP-5 mRNA expression in osteoblastic UMR-106 cells. The preparation and methods for RNA blotting were the same as Fig. 1. Before RNA extraction, UMR-106 cells were treated either transiently (for 12 h, followed by its removal for 36 h, left side) or continuously (for 48 h, right side) with 10^{-8} M hPTH-(1–34), 10 ng/ml IGF-I, or 10 ng/ml IGF-II.

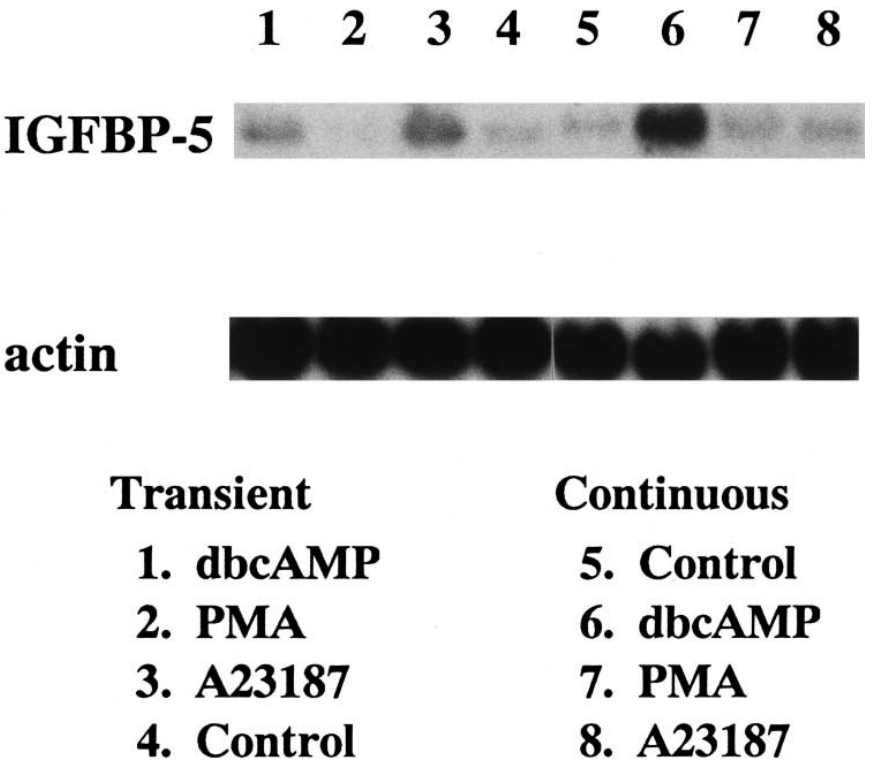

Fig. 3. Effects of transient or continuous treatment with second messenger analogues on IGFBP-5 mRNA expression in osteoblastic UMR-106 cells. The preparation and methods for RNA blotting were the same as Fig. 1. Before RNA extraction, UMR-106 cells were treated transiently (for 12 h, followed by its removal for 36 h, left side) or continuously (for 48 h, right side) with 10^{-4} M dbcAMP, 10^{-6} M PMA, or 10^{-6} M A23187.

5 production in UMR-106 cells, and Conover et al. [18] reported that the 6 h treatment with PTH stimulated IGFBP-5 mRNA expression more strongly than the 24 h treatment. However, the present study first demonstrated that transient treatment with PTH was more effective than continuous treatment to stimulate IGFBP-5 mRNA expression.

Linkhart et al. [19] reported that PTH stimulated IGF-I and IGF-II release from neonatal mouse calvaria. Moreover, PTH enhanced the transcript and polypeptide levels of IGF-I in osteoblast-enriched cultures from fetal rat bone [20]. Since IGF-I and IGF-II regulate the availability of IGFBP-5 in osteoblast-like cells [21], we also examined the effect of transient or continuous treatments with IGF-I and IGF-II on IGFBP-5 mRNA expression in UMR-106 cells. As shown in Fig. 2, the transient treatment with 10 ng/ml IGF-I stimulated the expression of IGFBP-5 mRNA in these cells, but the continuous treatment with it did not. In contrast, both transient and continuous treatments with 10 ng/ml IGF-II stimulated the expression of IGFBP-5 mRNA in these cells and the former was less effective than the latter. Since the effect of IGF-I was similar with that of PTH in the present study, IGF-I might be partly related to the effect of PTH by the transient treatment in UMR-106 cells.

There has been previous evidence that PTH possesses dual signal transduction systems, that is, cAMP-dependent protein kinase (PKA) and phospholipase C-coupled calcium/protein kinase C (Ca/PKC) [22]. Next, we examined the effect of the transient or continuous treatment with dbcAMP, a PKA ac-

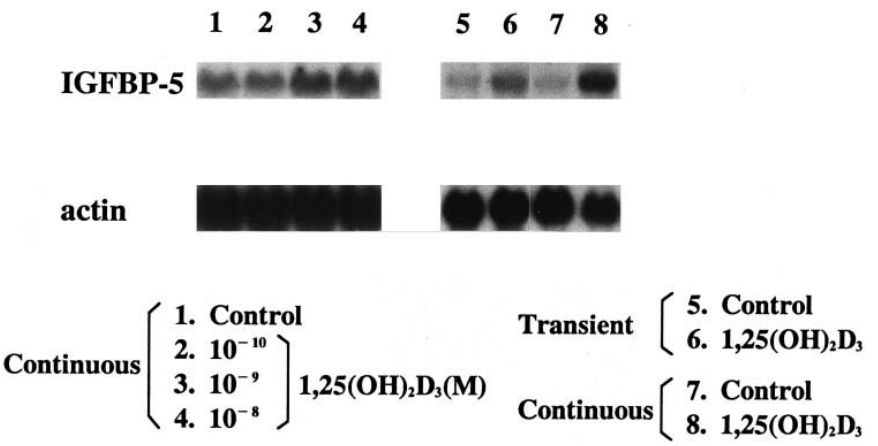

Fig. 4. Effects of transient or continuous treatment with $1,25(OH)_2D_3$ on IGFBP-5 mRNA expression in osteoblastic UMR-106 cells. The preparation and methods for RNA blotting were the same as Fig. 1. Before RNA extraction, UMR-106 cells were treated transiently (for 12 h, followed by its removal for 36 h) or continuously (for 48 h) with 10^{-8} M $1,25(OH)_2D_3$.

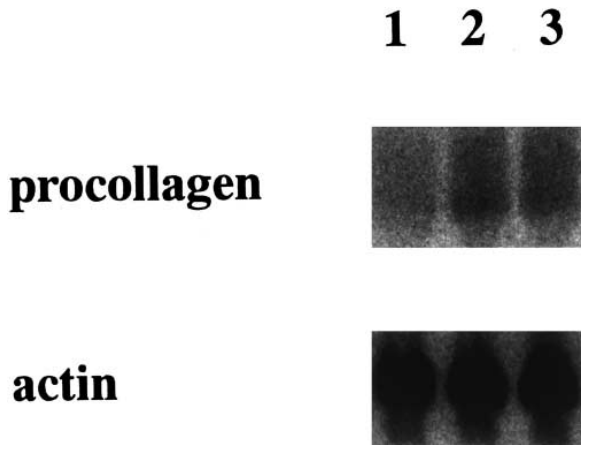

Fig. 5. Effects of IGFBP-5 and IGF-I on procollagen mRNA expression in osteoblastic UMR-106 cells. The preparation and methods for RNA blotting were the same as Fig. 1. Before RNA extraction, UMR-106 cells were treated with 230 ng/ml IGFBP-5, or 10 ng/ml IGF-I for 24 h. Lane 1, control; lane 2, IGFBP-5; lane 3, IGF-I.

tivator, PMA, a PKC activator, and A23187 which is thought to mimic myoinositol 1,4,5-trisphosphate by increasing intracellular calcium ($[Ca^{2+}]_i$). As shown in Fig. 3, the transient treatment with 10^{-6} M A23187 stimulated the expression of IGFBP-5 mRNA in these cells, but the continuous treatment did not affect it. In contrast, both transient and continuous treatments with 10^{-4} M dbcAMP stimulated the expression of IGFBP-5 mRNA in UMR-106 cells, and the former was less effective than the latter. Neither transient nor continuous treatment with 10^{-6} M PMA affected it. These findings suggest that an increase in $[Ca^{2+}]$i would be partly involved in the stimulatory effect of transient PTH treatment on IGFBP-5 mRNA expression in UMR-106 cells.

$1,25(OH)_2D_3$ is also an important calcium-regulating hormone and regulates IGFBPs secretion [14,23]. Schmid et al. [15] reported that the treatment with $1,25(OH)_2D_3$ for 24 h increased IGFBP-5 mRNA expression in cultured osteoblasts. We, therefore, examined the effect of the transient or continuous treatment with $1,25(OH)_2D_3$ on IGFBP-5 mRNA expression and compared its effects with those of PTH in UMR-106 cells. As shown in Fig. 4, $1,25(OH)_2D_3$ stimulated the expression of IGFBP-5 mRNA expression in a concentration-dependent manner and the maximum effective concentration was 10^{-8} M. Both transient and continuous treatments with 10^{-8} M $1,25(OH)_2D_3$ stimulated the expression of IGFBP-5 mRNA in these cells, but the transient treatment with $1,25(OH)_2D_3$ was less effective than the continuous one, which was different from the action of PTH.

The present study revealed that transient treatment with PTH caused a marked increase in mRNA expression of IGFBP-5, a stimulator IGF action, which was in concert with the stimulatory effect of intermittent PTH administration on bone mass in vivo [2–4]. Since there have also been several lines of evidence suggesting some role of IGFBP-5 in bone formation [9–11], the possibility was raised that IGFBP-5 might be partly involved in the anabolic action of PTH in bone. Type-1 collagen, the most abundant protein in bone matrix produced by osteoblasts, is an important marker of bone formation. We, therefore, examined whether IGFBP-5 would really increase the gene expression of type-1 procollagen in UMR-106 cells. As shown in Fig. 5, recombinant IGFBP-5 as well as IGF-I stimulated the expression of type-1 procollagen mRNA in these cells. Since there has been evidence that IGFBP-5 potentiates IGF-I action [10], it seems likely that IGFBP-5 stimulated the gene expression of type-1 procollagen through augmenting the action of endogenous IGF-I released from these cells. Alternatively, IGFBP-5 per se might stimulate it in a manner independent of IGF-I, as binding sites of IGFBP-5 have been reported to exist in osteoblasts [9]. The present findings might partly explain the mechanism of the anabolic action of the intermittent PTH administration on bone in vivo, although its precise mechanism remains to be clarified.

In conclusion, PTH as well as $1,25(OH)_2D_3$ stimulated the gene expression of IGFBP-5 in osteoblastic UMR-106 cells. The transient treatment with PTH, but not $1,25(OH)_2D_3$, more effectively stimulated this gene expression partly via an increase in $[Ca^{2+}]$i. IGFBP-5 might be partly involved in the anabolic action of PTH in bone.

Acknowledgements: This work was partly supported by SRF.

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