Parathyroid hormone enhances bone morphogenetic protein activity by increasing the intracellular 3', 5'-cyclic adenosine monophosphate accumulation in osteoblastic MC3T3-E1 cells

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Introduction

Daily injections of parathyroid hormone (PTH) amino-terminal peptide 1-34, or the full length protein PTH (1-84), increase bone mass and reduce the fracture risk in postmenopausal women, elderly men, and women with glucocorticoid-induced osteoporosis.

Previously we have reported that elevation of intracellular cAMP by phosphodiesterase inhibitors (pentoxifylline and rolipram), permeable analogue of cAMP (dibutyl cAMP) and activator of adenylate cyclase (prostaglandin E2, EP4 agonist (ONO-4819)) enhanced BMP action and caused an increase in ALP activity of osteoblastic cells and BMP-induced bone mass in experimental animals. Then we hypothesized that PTH exert anabolic effects on bone through enhancement of BMP signaling, by PTH-induced cAMP production. To substantiate this hypothesis, we here investigated the interaction between BMP and PTH signaling in mouse osteoblastic cell line, MC3T3-E1-cells.

Materials and methods

- Cell culture: Mouse osteoblastic cell line MC3T3-E1 was obtained from the Riken Cell Bank. Cells were cultured in α-minimal essential medium containing 10% FBS.
- Experimental protocol for BMP and/or PTH treatment: Upon achieving confluence, they were divided into six groups to the mode of treatment to rhBMP-2 and hPTH (1-34): control, rhBMP-2 treatment, hPTH (1-34) treatment, rhBMP-2 + hPTH (1-34) treatment, and rhBMP-2 treatment. The Up-regulated expression of Smad6 mRNA by the treatment with hPTH (1-34) to enhance BMP signaling by PTH, which was further up-regulated by cyclic treatments with PTH, whereas single treatment of PTH had no effect on the BMP induced transcriptional activity in the Id1 promoter assay.

In MC3T3-E1 cells transfected with the luciferase gene linked to non-functional Id1 promoter with a mutated BMP responsive element, no significant change in luciferase expression was noted following treatments with rhBMP-2, PTH, or both.

Enhancement of rhBMP-2 induced mRNA expression of ALP, Runx2

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Intelligence

Enhancement of intracellular cAMP signaling by PTH... significant differences between treatment groups were analyzed using Fisher’s PLSD test. Values of P<0.05 were considered statistically significant.

Results

Elevation of intracellular cAMP levels by hPTH (1-34) and other chemicals

Low dose (50ng/ml) rhBMP-2 significantly induced ALP activity in MC3T3-E1 cells. Although single high (10^-6 M) or cyclic low dose (10^-9 M) of PTH did not alter the ALP activity, BMP-2-induced ALP expression was enhanced after cyclic PTH stimulation. In order to investigate intracellular signaling pathway involved in the mechanism to enhance BMP signaling by PTH, the effect of PKA and PDE inhibitors were examined. PKA inhibitor (H89) abolished the PTH action almost completely. Meanwhile PDE inhibitor (IBMX) enhanced the PTH action.

Discussion

In summary, this study suggests that the intracellular cAMP level was increased significantly during PTH cyclic treatment and about 2h after PTH cyclic treatment.

Id1 promoter driven luciferase expression by rhBMP-2 and/or hPTH (1-34)

The relative luciferase expression assay in MC3T3-E1 cells transfected with promoter, of Id1, an early response gene to BMPs, showed elevation of transcriptional activity of luciferase in response to rhBMP-2 treatment, which was further up-regulated by cyclic treatments with PTH, whereas single treatment of PTH had no effect on the BMP induced transcriptional activity in the Id1 gene promoter assay. In MC3T3-E1 cells transfected with the luciferase gene linked to non-functional Id1 promoter with a mutated BMP responsive element, no significant change in luciferase expression was noted following treatments with rhBMP-2, PTH, or both.

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by the treatment with hPTH (1-34). Suppression of rhBMP-2 induced Smad6 expression by the treatment with hPTH (1-34)

From the real time RT-PCR analysis, the expression of ALP mRNA was increased in a time-dependent manner. In day 3, the expression of ALP mRNA was increased by rhBMP-2 treatment, although PTH single treatment slightly stimulated expression of ALP mRNA induced by BMP-2. Meanwhile, cyclic PTH treatments dramatically enhanced the expression of ALP mRNA induced by BMP-2. BMP-2-induced Runx2 mRNA expression peaked in Day1, which was also enhanced by the cyclic PTH treatment. Smad6 mRNA expression was increased by rhBMP-2 treatment. The Up-regulated expression of Smad6 mRNA peaked in 12 h and lasted 2 days. Single treatment of PTH slightly suppressed the expression of Smad6 mRNA. The mRNA expression was further suppressed by the cyclic PTH treatments.

ALP induction by rhBMP-2 and/or hPTH (1-34)

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