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Short carboxyl terminal parathyroid hormone peptides modulate human parathyroid hormone signaling in mouse osteoblasts

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Abstract

Background: Novel human parathyroid hormone (hPTH) peptides of unknown biological activity have recently been identified in the serum of subjects with normal renal function, chronic renal failure, and end-stage renal disease through the application of liquid chromatography-high resolution mass spectrometry.

Purpose of experiments: To determine the bioactivity of these peptides, we synthesized hPTH28–84, hPTH38–84, and hPTH45–84 peptides by solid phase peptide synthesis and tested their bioactivity in MC3T3-E1 mouse osteoblasts, either individually or together with the native hormone, hPTH1–84, by assessing the accumulation of 3′,5′-cyclic adenosine monophosphate (cAMP) and the induction of alkaline phosphatase activity.

Results: Increasing doses of hPTH1–84 (1–100 nM) increased the accumulation of cAMP and alkaline phosphatase activity in osteoblasts. hPTH28–84, hPTH38–84, and hPTH45–84 in concentrations of 1–100 nM were biologically inert. Surprisingly, 100 nM hPTH38–84 and hPTH45–84 increased the accumulation of cAMP in osteoblasts treated with increasing amounts of hPTH1–84. Human PTH28–84 had no effects on cAMP activity alone or in combination with hPTH1–84. Conversely, 100 nM hPTH38–84, hPTH45–84, and hPTH28–84 blocked the activation of alkaline phosphatase activity by hPTH1–84.

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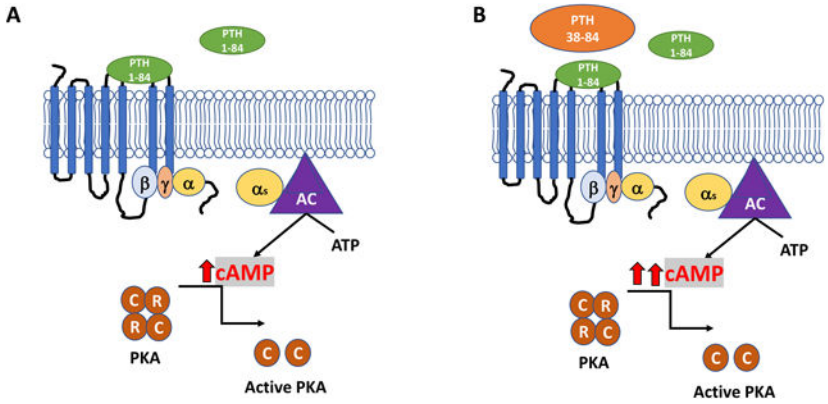
CONFLICT OF INTEREST STATEMENT

None of the authors has any conflicts of interest.

Conclusions: The data show that the short carboxyl-terminal hPTH peptides, hPTH38–84 and hPTH45–84, increase the amount of cellular cAMP generated in cultured osteoblasts in response to treatment with full-length hPTH1–84 when compared to full-length hPTH1–84 alone. Human PTH28–84 had no effect on cAMP activity alone or in combination with hPTH1–84. Human PTH28–84, hPTH38–84 and hPTH45–84 reduced the effects of hPTH1–84 in osteoblasts with respect to the induction of alkaline phosphatase activity compared to hPTH1–84 alone. Short carboxyl peptides of human PTH are biologically inert but when administered together with full-length hPTH1–84 modulate the bioactivity of hPTH1–84 in osteoblasts.

Graphical Abstract



Keywords

hPTH1-84; hPTH28-84; hPTH38-84; hPTH45-84; carboxyl-terminal hPTH peptides; cAMP; alkaline phosphatase; mouse osteoblasts

1. Introduction

We recently identified 8 novel human parathyroid hormone (hPTH) peptides (hPTH28–84, hPTH34–77, hPTH34–84, hPTH37–77, hPTH37–84, hPTH38–77, hPTH38–84, hPTH45–84) in the serum of subjects with normal renal function, chronic renal failure (CRF), and end-stage renal disease (ESRD) using liquid chromatography-high resolution mass spectrometry (LC-HRMS) [1]. We showed that concentrations of hPTH peptides increased dramatically when glomerular filtration rate decreased below 17–23 mL/min/1.73 m^2 [1]. Previous investigators have suggested the occurrence of some, but not all, of these peptides using less stringent analytical techniques such as matrix-assisted laser desorption mass spectrometry (MADI-TOF) and selected reaction monitoring-mass spectrometry following immunocapture and proteolysis of captured peptides [2,3]. Surprisingly, no information is available concerning the bioactivity of these hPTH peptides in cell culture or *in vivo*.

To characterize the bioactivity of these hPTH peptides, we synthesized 3 of the peptides (hPTH28–84, hPTH38–84, and hPTH45–84) by solid phase peptide synthesis [4,5] and tested their bioactivity in MC3T3-E1 osteoblasts alone or in combination with recombinant, full-length hPTH1–84. We examined the capacity of these hPTH peptides to alter the

accumulation of cAMP and their ability to alter alkaline phosphatase activity in mouse osteoblasts [6–8].

We demonstrate that carboxyl-terminal hPTH peptides (hPTH28–84, hPTH38–84, and hPTH45–84) are biologically inert in MC3T3-E1 mouse osteoblasts and are unable to increase cAMP production or alkaline phosphatase activity in the cells. We found that human PTH38–84 and hPTH45–84 potentiate the activity of full-length hPTH1–84 by increasing intra-cellular cAMP, whereas hPTH28–84 does not. cAMP generated in cells following treatment with hPTH1–84 is increased two-fold upon the pre-treatment of cells with hPTH38–84 and hPTH45–84. Alkaline phosphatase activity decreased upon pre-treatment of cells with hPTH28–84, hPTH38–84, and hPTH45–84 prior to the addition of recombinant, full length, hPTH1–84. The data demonstrate that short carboxyl terminal peptides of hPTH modulate the activity of full length hPTH1-84; and that increases in cAMP can be dissociated from changes in alkaline phosphatase activity in bone cells. The mechanism of action of these short carboxyl terminal hPTH peptides requires further investigation in cells and *in vivo*.

2. Materials and methods

2.1 Cell culture

MC3T3-E1 Subclone 4 cells were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA), and were maintained in α-MEM complete culture medium containing 10% fetal bovine serum (FBS), 100 μg/mL streptomycin, and 100 U/mL penicillin G sodium [9].

2.2 Reagents

Forskolin (>98% purity) and 3-isobutyl-methylxanthine (IBMX, >99% purity) were obtained from Sigma (St. Louis, MO, USA). Stock concentrations of forskolin and IBMX were prepared at concentrations of 1 mM and 45 mM in ethanol, respectively. hPTH1–84 was biosynthesized, purified (see below), and stored in low protein binding microcentrifuge tubes at −80 °C. hPTH28–84, hPTH38–84, and hPTH45–84 were prepared in the Mayo Clinic peptide core facility and were stored in 1% bovine serum albumin (BSA) in phosphate buffered saline (PBS) at −80 °C (stock concentration 1 μM).

2.3 Expression and purification of hPTH1–84

Human hPTH1–84 (SVSEIQLMHN LGKHLNSMER VEWLRKKLQD VHNFVALGAP LAPRDAGSQR PRKKEDNVLV ESHEKSLGEA DKADVNVLTK AKSQ) (GenBank NP_000306.1) was expressed in BL21 Star™ (DE3) *Escherichia coli* using a codon optimized DNA sequence with a N-terminal 6x-histidine tag and a Tobacco Etch Virus (TEV) protease site (ENLYFQ/S) 3’ in the pET-28a(+) plasmid vector (GenScript, Piscataway, NJ, USA) [10–13]. BL21 Star™ (DE3) *Escherichia coli* cells were transformed with the recombinant plasmid and plated on Luria broth (LB) agar plates containing 40 μg/mL kanamycin. Colonies from this plate were used to prepare bacterial starter cultures in three 2 L culture flasks, each containing 600 mL of 2x YT media. Bacterial starter culture flasks were grown at 37 °C for 24 hours with shaking at 240 rpm. Bacterial starter

cultures (50 mL) were used to inoculate 36 flasks, 2 L culture flasks each containing 600 mL of 2X YT media and 40 μg/mL kanamycin. The bacterial cultures were grown in a shaker incubator at 37 °C with 240 rpm shaking to an optical density of 0.8 at 600 nm. His-tagged hPTH1–84 expression was induced by the addition of 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) for 6 hours. Following protein induction, the bacterial cultures were centrifuged at 7000 × g, at 4 °C, for 15 minutes. Cells were resuspended in buffer A (50 mM Tris-base, 250 mM NaCl, 30 mM imidazole, 5 mM β-mercaptoethanol, 0.5 mM EDTA, 1 mM PMSF, pH 7.5), lysed with a BeadBeater containing 1 mm glass beads (BioSpec Products, Bartlesville, OK, USA) at 4 °C (20 cycles of 20 seconds on/2 minutes off with cooling using an ice jacket). The homogenate was centrifuged at 26000 g, 4 °C, for 30 minutes. The crude bacterial extract (supernatant) was incubated with 12 mL Ni Sepharose 6 Fast Flow beads (GE Healthcare Life Sciences; pre-equilibrated with wash buffer) at 4 °C, for 90 minutes. The resin beads were washed three times with buffer B (50 mM Tris-base, 250 mM NaCl, 30 mM imidazole, 5 mM β-mercaptoethanol, pH 7.5) and loaded onto a 2.5 × 10 cm chromatography column (DWK Life Sciences, Millville, NJ, USA) equilibrated with buffer B. The column of resin beads was initially washed with 100 mL of buffer B containing 5 mM ATP disodium trihydrate/5 mm $MgCl_2$ and then was extensively washed with 1000 mL of buffer B using a peristaltic pump at 4 °C. On-column cleavage of his-tagged hPTH1–84 was performed in a reaction buffer C (50 mM Tris-base, 200 mM NaCl, 20 mM imidazole, 1 mM DTT, 1 mM EDTA, pH 7.5). His-tagged TEV protease (Creative Enzymes, Shirley, NY, USA) reconstituted in buffer D (25 mM Tris-base, 200 mM NaCl, 10 mM imidazole, 1 mM DTT, 0.05 mM EDTA, 50% glycerol, pH 7.5) was added to the column, at a ratio of 1:1 (v/v), at 4 °C without shaking for 48 hours. The cleaved hPTH1–84 was collected in the flow through. The identity and purity of protein was confirmed by Coomassie stained sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE), peptide mapping using trypsin and AspN proteases, and mass spectrometry. The stock hPTH1–84 (1μM) was stored in low protein binding microcentrifuge tubes at −80 °C.

2.4 hPTH peptide synthesis

hPTH peptides were synthesized by Fmoc solid phase methods on preloaded glutamine Wang resin (Novabiochem) [4,5]. Each peptide chain was assembled from N^{α}-Fmoc protected amino acids on a Liberty Blue Microwave-Assisted Peptide Synthesizer (CEM Corp.) according to the manufacturer's coupling and deprotection protocols. After synthesis each peptide was cleaved from its resin support by acidolysis with a solution of trifluoroacetic acid containing 2.5 % water (v/v) and 2.5% triisopropylsilane (v/v) and 2.5% 3,6, dioxa-1,8-octanedithiol (v/v) for 30min at 42 °C. The crude peptide was then purified by preparative RP-HPLC using an aqueous acetonitrile gradient containing 1 % TFA (v/v) on a reverse-phase C_{18} column (Phenomenex Jupiter 15μ; 250 × 21.2 mm). The mass weight was verified by mass spectrometry on an Agilent InfinityLab LC/MSD instrument. Peptide homogeneity was confirmed by analytical RP-HPLC.

2.5 Quantitation of 3',5'-cyclic adenosine monophosphate (cAMP) in osteoblasts

MC3T3-E1 cells were cultured in 24-well plates at a seeding density of 3×10^4 cells per well and grown in a cell incubator with 5% CO_2 at 37 °C for 24 hours to 80–90%

confluence. Cells were treated with: 1) Culture medium; 2) 10 μM forskolin; 3) hPTH1–84 at concentrations of 1, 3, 10, 50, or 100 nM with or without 100 nM hPTH peptides (hPTH28–84, hPTH38–84, or hPTH45–84) or 4) hPTH28–84, hPTH38–84, or hPTH45–84 at concentrations of 1, 3, 10, 50, or 100 nM. All wells contained 1 mM IBMX to inhibit phosphodiesterase activity. Cells in hPTH1–84 plus hPTH peptide groups were pretreated with 500 μL of 100 nM hPTH peptides (hPTH28–84, hPTH38–84, or hPTH45–84) for 2 hours. Cells in other groups were pretreated with fresh culture media. Cells were then treated with either culture media, forskolin (10 μM), or hPTH1–84 (1, 3, 10, 50, 100 nM) with or without 100 nM hPTH peptide (hPTH28–84, hPTH38–84, or hPTH45–84) for 30 minutes. Following incubation, cell culture medium was removed from culture plate wells, and cells in each well were lysed with 500 μL of 1% Triton X-100 in 1 M HCl on the plate shaker at room temperature for 20 minutes. The cell lysates were transferred to 1.5 mL microcentrifuge tubes and centrifuged at 10000 × g for 5 minutes. The supernatants were collected for measurement of cAMP concentration using cAMP Complete ELISA Kit (Abcam, Cambridge, MA, USA) according to the manufacturer's protocol.

2.6 Quantitation of alkaline phosphatase activity in osteoblasts

MC3T3-E1 Subclone 4 cells were cultured in 24-well plates at a seeding density of 3×10^4 cells per well and grown in a cell incubator with 5% CO_2 at 37 °C for 48 hours in α-MEM complete culture medium containing 10% FBS, 100 μg/mL streptomycin, and 100 U/mL penicillin G sodium. After cells had reached 100% confluency, cell culture medium was replaced with differentiation medium (α-MEM with 10% FBS, 100 μg/mL ascorbic acid, 10 mM β-glycerophosphate, 1.5 mM K_3PO_4) every 48 hours. At seven days of differentiation, the medium was removed from culture plates and cells were treated with culture medium (negative control), forskolin (10 μM; positive control), or hPTH1–84 (1, 3, 10, 50, 100, 1000 nM) with or without 100 nM hPTH peptides (hPTH28–84, hPTH38–84, or hPTH45–84) for 24 hours. The medium was then removed from culture plates, and cells were rinsed twice with 1 mL of PBS per well and were frozen at −20 °C for 2 hours. For analysis, cells were chemically lysed with 500 μL of RIPA lysis buffer (50 mM Tris-base, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 1% sodium dodecylsulfate, pH 8.0) on the plate shaker at room temperature for 20 minutes. Cells were scraped from culture plates, transferred to 1.5 mL microcentrifuge tubes, and centrifuged at 10000 × g for 5 minutes. The supernatants were collected for measurement of alkaline phosphatase activity at 360/450 nm using QuantiFluo™ Alkaline Phosphatase Assay Kit (BioAssay Systems, Hayward, CA, USA) according to the manufacturer's protocol.

2.7 Statistical analysis

Results were expressed as the mean±SD. The significance of differences between two groups was assessed by the Student t-test in GraphPad Prism 9 software. P-values less than 0.05 were considered statistically significant.

3. Results

Despite overwhelming data concerning the presence of circulating carboxyl-terminal hPTH peptides in patients with normal and impaired renal function, no information has been

published concerning the bioactivity of these peptides [1–3]. Full-length hPTH1–84 is known to increase cAMP concentrations in responsive cells, including osteoblasts, by binding to the hPTH1 receptor, activating the G(s) α-subunit of G proteins and increasing adenylate cyclase and alkaline phosphatase activity [6–8,14–22]. We examined the bioactivity of hPTH28–84, hPTH38–84, and hPTH45–84 in MC3T3-E1 mouse osteoblasts by assessing the effect of peptide addition alone or together with full-length hPTH1–84 on intra-cellular cAMP and alkaline phosphatase. We found that the 3 hPTH peptides were biologically inert when administered individually to MC3T3-E1 osteoblasts -- no changes in cAMP concentration were observed following treatment of osteoblasts with the aforementioned hPTH peptides (Figure 1). As expected hPTH1–84 in concentrations of 1 to 100 nM showed robust increases in cAMP from 0 to 87.06±26.05 pmol/mL cAMP, respectively. The adenylyl cyclase stimulator, forskolin, increased intracellular cAMP generation.

To ascertain whether hPTH peptides altered the bioactivity of full-length hPTH1–84, we pre-treated MC3T3-E1 osteoblast cells with hPTH28–84, hPTH38–84, and hPTH45–84. To our surprise, we found that 100nM hPTH38–84 and hPTH45 −84 significantly increased the bioactivity of full-length hPTH1–84 ($p < 0.01$). cAMP concentrations in cells pre-treated with hPTH38–84 and hPTH45–84 before treatment with hPTH1–84, were almost twice as high as those obtained following treatment with hPTH1–84 alone. Pre-treatment of cells with hPTH28–84 had no significant effect on full-length hPTH1–84 cAMP response (Figure 2).

PTH1–84 increases alkaline phosphatase activity in cultured osteoblasts [6,8,23.]. We examined the effects of hPTH1–84 and carboxyl-terminal hPTH peptides on alkaline phosphatase activity in differentiated MC3T3-E1 cells. After 7 days of differentiation, cells were treated either with hPTH1–84 in increasing concentrations alone (1, 3, 10, 50, and 100 nM), with individual hPTH peptides (hPTH28–84, hPTH38–84, hPTH45–84), or a combination of hPTH1–84 and hPTH peptides. Increasing concentrations of hPTH1–84 enhanced alkaline phosphatase activity in MC3T3-E1 cells in a dose-dependent manner (Figure 3). Human PTH28–84, hPTH38–84, and hPTH45–84 did not change alkaline phosphatase activity when added individually to MC3T3-E1 cells (Figure 3). Pre-treatment of MC3T3-E1 cells with hPTH28–84, hPTH38–84 or hPTH45–84 blocked hPTH1–84 mediated increases in alkaline phosphatase activity following treatment with hPTH1–84 (Figure 4).

4. Discussion

We recently identified and quantified hPTH1–84 and hPTH peptides (hPTH28–84, hPTH34–77, hPTH34–84, hPTH37–77, hPTH37–84, hPTH38–77, hPTH38–84, hPTH45–84) using LC-HRMS in human subjects with varying renal function [1]. These peptides are generated by processing of full-length PTH1–84 in the liver and kidney. Human PTH is synthesized in parathyroid glands as 115-amino acid preproPTH molecule that is cleaved in the endoplasmic reticulum of the parathyroid cell to yield a 90-amino acid proPTH moiety. ProPTH is subsequently processed in the Golgi complex to yield bioactive full-length PTH1–84 [24] that is released into the circulation. Circulating PTH1–84 is cleared by

hepatic Kupffer cells which process PTH1–84 into amino-terminal and carboxyl-terminal PTH peptides [25,26]. The former peptides are degraded *in situ*, whereas carboxyl-terminal PTH peptides are released into the circulation to be cleared by the kidneys [27–29]. Carboxyl-terminal PTH peptides are also secreted by parathyroid glands [30]. Plasma hPTH peptide concentrations rise progressively in patients with CRF due to increased parathyroid function and impaired renal clearance [1,31,32]. Until recently, the systematic identification of highly purified circulating hPTH peptides derived from full-length hPTH1–84 had not been performed [1]. In a previous study, Zhang *et al.* identified 4 circulating hPTH peptides (hPTH34–84, hPTH37–84, hPTH38–84, hPTH45–84) in the plasma of patients with CRF and in normal subjects following parenteral hPTH1–84 administration [2]. In a subsequent study, Lopez *et al.* found similar hPTH peptides together with an additional five hPTH peptides (hPTH28–84, hPTH34–77, hPTH37–77, hPTH38–77, hPTH48–84) from the plasma of 12 healthy and 12 ESRD patients [3].

Interestingly, the biological activity of unequivocally identified, circulating hPTH peptides has not been previously reported. We used solid phase peptide synthesis to synthesize carboxyl-terminal hPTH peptides and bacterial protein expression methods to express full-length hPTH1–84. The peptides (hPTH28–84, hPTH38–84 and hPTH45–84) selected for synthesis encompassed the amino terminal and carboxyl terminal amino acids of the identified PTH peptide molecules.

In mouse osteoblasts, we demonstrated that hPTH28–84, hPTH38–84, and hPTH45–84 given individually did not alter cAMP concentrations nor alkaline phosphatase activity. However, hPTH38–84 and hPTH45–84, but not hPTH28–84, enhanced full-length hPTH1–84 mediated increases in cAMP generation in MC3T3-E1 cells when added concurrently with hPTH1–84. This finding suggests that short carboxyl-terminal PTH peptides modulate the effects of hPTH1–84 with respect to the generation of cAMP in osteoblasts. The mechanism(s) whereby hPTH38–84 and hPTH45–84 increase the bioactivity of hPTH1–84 is unknown. Reduction in hPTH1–84 degradation, and increases in cell-surface PTHR1 expression, Gsα expression, and adenylate cyclase activity are possibilities. It is unknown whether hPTH38–84 and hPTH45–84 bind specifically to PTHR1 receptors or have specific cell-surface receptors of their own. Indeed, previous publications have demonstrated possible specific receptors for carboxyl-terminal PTH peptides [33].

In contrast to the effects of hPTH peptides on intra-cellular cAMP concentrations, following pre-treatment of osteoblasts with hPTH28–84, hPTH38–84, and hPTH45–84, alkaline phosphatase activity induced by hPTH1–84 was blocked. These data show that the effects of full-length hPTH1–84 on alkaline phosphatase activity are blocked by short carboxyl terminal PTH peptides despite increases in cAMP concentrations in osteoblasts. The data also suggest that in CRF/ESRD where concentrations of carboxyl-terminal peptides of PTH and full-length hPTH1–84 are significantly increased, the cellular bioactivity of PTH1–84 on osteoblasts may be altered by short carboxyl terminal PTH peptides. The change in activity of PTH1–84 by short carboxyl terminal PTH peptides could contribute to the development of different types of bone disease seen in patients with CRF/ESRD. Thus, assessment of PTH bioactivity solely assessing serum intact PTH concentrations in CRF/ESRD may be misleading. Further investigations on the bioactivity of carboxyl-terminal

hPTH peptides *in vivo* and additional investigations regarding the mechanism of action of these peptides requires further investigation.

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References.

[1]. Kritmetapak K, Losbanos LA, Hines JM, O'Grady KL, Ulmer CZ, Vesper HW, Enders FT, Singh RJ, Kumar R, Chemical Characterization and Quantification of Circulating Intact PTH and PTH Fragments by High-Resolution Mass Spectrometry in Chronic Renal Failure, Clin Chem67 (2021) 843–853. 10.1093/clinchem/hvab013. [PubMed: 33693557]

[2]. Zhang CX, Weber BV, Thammavong J, Grover TA, Wells DS, Identification of carboxyl-terminal peptide fragments of parathyroid hormone in human plasma at low-picomolar levels by mass spectrometry, Anal Chem78 (2006) 1636–1643. 10.1021/ac051711o. [PubMed: 16503617]

[3]. Lopez MF, Rezai T, Sarracino DA, Prakash A, Krastins B, Athanas M, Singh RJ, Barnidge DR, Oran P, Borges C, Nelson RW, Selected reaction monitoring-mass spectrometric immunoassay responsive to parathyroid hormone and related variants, Clin Chem56 (2010) 281–290. 10.1373/clinchem.2009.137323. [PubMed: 20022981]

[4]. Fields GB, Introduction to peptide synthesis, Curr Protoc Protein Sci Chapter18 (2002) Unit 18 11. 10.1002/0471140864.ps1801s26.

[5]. Stawikowski M, Fields GB, Introduction to peptide synthesis, Curr Protoc Protein Sci Chapter18 (2012) Unit 18 11. 10.1002/0471140864.ps1801s69.

[6]. Smith DM, Johnston CC Jr., Cyclic 3', 5'-adenosine monophosphate levels in separated bone cells, Endocrinology96 (1975) 1261–1269. 10.1210/endo-96-5-1261. [PubMed: 164345]

[7]. Peck WA, Burks JK, Wilkins J, Rodan SB, Rodan GA, Evidence for preferential effects of parathyroid hormone, calcitonin and adenosine on bone and periosteum, Endocrinology100 (1977) 1357–1364. 10.1210/endo-100-5-1357. [PubMed: 191242]

[8]. Farley JR, Hall SL, Herring S, Tarbaux NM, Matsuyama T, Wergedal JE, Skeletal alkaline phosphatase specific activity is an index of the osteoblastic phenotype in subpopulations of the human osteosarcoma cell line SaOS-2, Metabolism40 (1991) 664–671. 10.1016/0026-0495(91)90081-7. [PubMed: 1651438]

[9]. Wang D, Christensen K, Chawla K, Xiao G, Krebsbach PH, Franceschi RT, Isolation and characterization of MC3T3-E1 preosteoblast subclones with distinct in vitro and in vivo differentiation/mineralization potential, J Bone Miner Res14 (1999) 893–903. 10.1359/jbmr.1999.14.6.893. [PubMed: 10352097]

[10]. Dubendorff JW, Studier FW, Controlling basal expression in an inducible T7 expression system by blocking the target T7 promoter with lac repressor, J Mol Biol219 (1991) 45–59. 10.1016/0022-2836(91)90856-2. [PubMed: 1902522]

[11]. Studier FW, Use of bacteriophage T7 lysozyme to improve an inducible T7 expression system, J Mol Biol219 (1991) 37–44. 10.1016/0022-2836(91)90855-z. [PubMed: 2023259]

[12]. Studier FW, Stable expression clones and auto-induction for protein production in E. coli, Methods Mol Biol1091 (2014) 17–32. 10.1007/978-1-62703-691-7_2. [PubMed: 24203322]

[13]. Sambrook J, Fritsch EF, Maniatis T, Molecular cloning: a laboratory manual, Cold spring harbor laboratory press1989.

[14]. Canterbury JM, Levey GS, Reiss E, Activation of renal cortical adenylate cyclase by circulating immunoreactive parathyroid hormone fragments, J Clin Invest52 (1973) 524–527. 10.1172/JCI107211. [PubMed: 4346008]

[15]. Chabardes D, Imbert M, Clique A, Montegut M, Morel F, PTH sensitive adenyl cyclase activity in different segments of the rabbit nephron, Pflugers Arch354 (1975) 229–239. 10.1007/BF00584646. [PubMed: 163468]

[16]. Forte LR, Nickols GA, Anast CS, Renal adenylate cyclase and the interrelationship between parathyroid hormone and vitamin D in the regulation of urinary phosphate and adenosine cyclic 3',5'-monophosphate excretion, J Clin Invest57 (1976) 559–568. 10.1172/JCI108311. [PubMed: 175088]

[17]. Kakuta S, Suda T, Sasaki S, Kimura N, Nagata N, Effects of parathyroid hormone on the accumulation of cyclic AMP in bone of vitamin D-deficient rats, Endocrinology97 (1975) 1288–1293. 10.1210/endo-97-5-1288. [PubMed: 171152]

[18]. Nielsen ST, Neuman WF, Effects of PTH and Ca2+ on renal adenyl cyclase, J Supramol Struct9 (1978) 391–398. 10.1002/jss.400090309. [PubMed: 748683]

[19]. Abou-Samra AB, Jueppner H, Westerberg D, Potts JT Jr., Segre GV, Parathyroid hormone causes translocation of protein kinase-C from cytosol to membranes in rat osteosarcoma cells, Endocrinology124 (1989) 1107–1113. 10.1210/endo-124-3-1107. [PubMed: 2537172]

[20]. Abou-Samra AB, Juppner H, Force T, Freeman MW, Kong XF, Schipani E, Urena P, Richards J, Bonventre JV, Potts JT Jr., et al., Expression cloning of a common receptor for parathyroid hormone and parathyroid hormone-related peptide from rat osteoblast-like cells: a single receptor stimulates intracellular accumulation of both cAMP and inositol trisphosphates and increases intracellular free calcium, Proc Natl Acad Sci U S A89 (1992) 2732–2736. 10.1073/pnas.89.7.2732. [PubMed: 1313566]

[21]. Abou-Samra AB, Zajac JD, Schiffer-Alberts D, Skurat R, Kearns A, Segre GV, Bringhurst FR, Cyclic adenosine 3',5'-monophosphate (cAMP)-dependent and cAMP-independent regulation of parathyroid hormone receptors on UMR 106–01 osteoblastic osteosarcoma cells, Endocrinology129 (1991) 2547–2554. 10.1210/endo-129-5-2547. [PubMed: 1718728]

[22]. Weinstein LS, Yu S, Warner DR, Liu J, Endocrine manifestations of stimulatory G protein alpha-subunit mutations and the role of genomic imprinting, Endocr Rev22 (2001) 675–705. 10.1210/edrv.22.5.0439. [PubMed: 11588148]

[23]. Yee JA, Stimulation of alkaline phosphatase activity in cultured neonatal mouse calvarial bone cells by parathyroid hormone, Calcif Tissue Int37 (1985) 530–538. 10.1007/BF02557837. [PubMed: 2415227]

[24]. Habener JF, Amherdt M, Ravazzola M, Orci L, Parathyroid hormone biosynthesis. Correlation of conversion of biosynthetic precursors with intracellular protein migration as determined by electron microscope autoradiography, J Cell Biol80 (1979) 715–731. 10.1083/jcb.80.3.715. [PubMed: 457765]

[25]. D'Amour P, Huet PM, Segre GV, Rosenblatt M, Characteristics of bovine parathyroid hormone extraction by dog liver in vivo, Am J Physiol241 (1981) E208–214. 10.1152/ajpendo.1981.241.3.E208. [PubMed: 7025656]

[26]. Rouleau MF, Warshawsky H, Goltzman D, Parathyroid hormone binding in vivo to renal, hepatic, and skeletal tissues of the rat using a radioautographic approach, Endocrinology118 (1986) 919–931. 10.1210/endo-118-3-919. [PubMed: 3948781]

[27]. Martin K, Hruska K, Greenwalt A, Klahr S, Slatopolsky E, Selective uptake of intact parathyroid hormone by the liver: differences between hepatic and renal uptake, J Clin Invest58 (1976) 781–788. 10.1172/JCI108529. [PubMed: 965485]

[28]. Bringhurst FR, Stern AM, Yotts M, Mizrahi N, Segre GV, Potts JT Jr., Peripheral metabolism of PTH: fate of biologically active amino terminus in vivo, Am J Physiol255 (1988) E886–893. 10.1152/ajpendo.1988.255.6.E886. [PubMed: 3202165]

[29]. Bringhurst FR, Stern AM, Yotts M, Mizrahi N, Segre GV, Potts JT Jr., Peripheral metabolism of [35S]parathyroid hormone in vivo: influence of alterations in calcium availability and parathyroid status, J Endocrinol122 (1989) 237–245. 10.1677/joe.0.1220237. [PubMed: 2769152]

[30]. D'Amour P, Rakel A, Brossard JH, Rousseau L, Albert C, Cantor T, Acute regulation of circulating parathyroid hormone (PTH) molecular forms by calcium: utility of PTH fragments/PTH(1–84) ratios derived from three generations of PTH assays, J Clin Endocrinol Metab91 (2006) 283–289. 10.1210/jc.2005-1628. [PubMed: 16219713]

[31]. Patel S, Barron JL, Mirzazedeh M, Gallagher H, Hyer S, Cantor T, Fraser WD, Changes in bone mineral parameters, vitamin D metabolites, and PTH measurements with varying chronic kidney disease stages, J Bone Miner Metab29 (2011) 71–79. 10.1007/s00774-010-0192-1. [PubMed: 20521154]

[32]. Kritmetapak K, Losbanos L, Berent TE, Ashrafzadeh-Kian SL, Algeciras-Schimnich A, Hines JM, Singh RJ, Kumar R, Hyperphosphatemia with elevated serum PTH and FGF23, reduced 1,25(OH)2D and normal FGF7 concentrations characterize patients with CKD, BMC Nephrol22 (2021) 114. 10.1186/s12882-021-02311-3. [PubMed: 33784965]

[33]. Nguyen-Yamamoto L, Rousseau L, Brossard JH, Lepage R, D'Amour P, Synthetic carboxyl-terminal fragments of parathyroid hormone (PTH) decrease ionized calcium concentration in rats by acting on a receptor different from the PTH/PTH-related peptide receptor, Endocrinology142 (2001) 1386–1392. 10.1210/endo.142.4.8093. [PubMed: 11250917]

Highlights:

- Human PTH38–84 and 45–84 enhance hPTH1–84-mediated osteoblast cAMP accumulation
- PTH28–84, 38–84 and 45–84 reduce hPTH1–84-mediated osteoblast alkaline phosphatase
- PTH28–84, 38–84, and 45–84 do not alter osteoblast cAMP or alkaline phosphatase
- Short carboxyl terminal PTH peptides might modulate PTH1–84 activity in osteoblasts

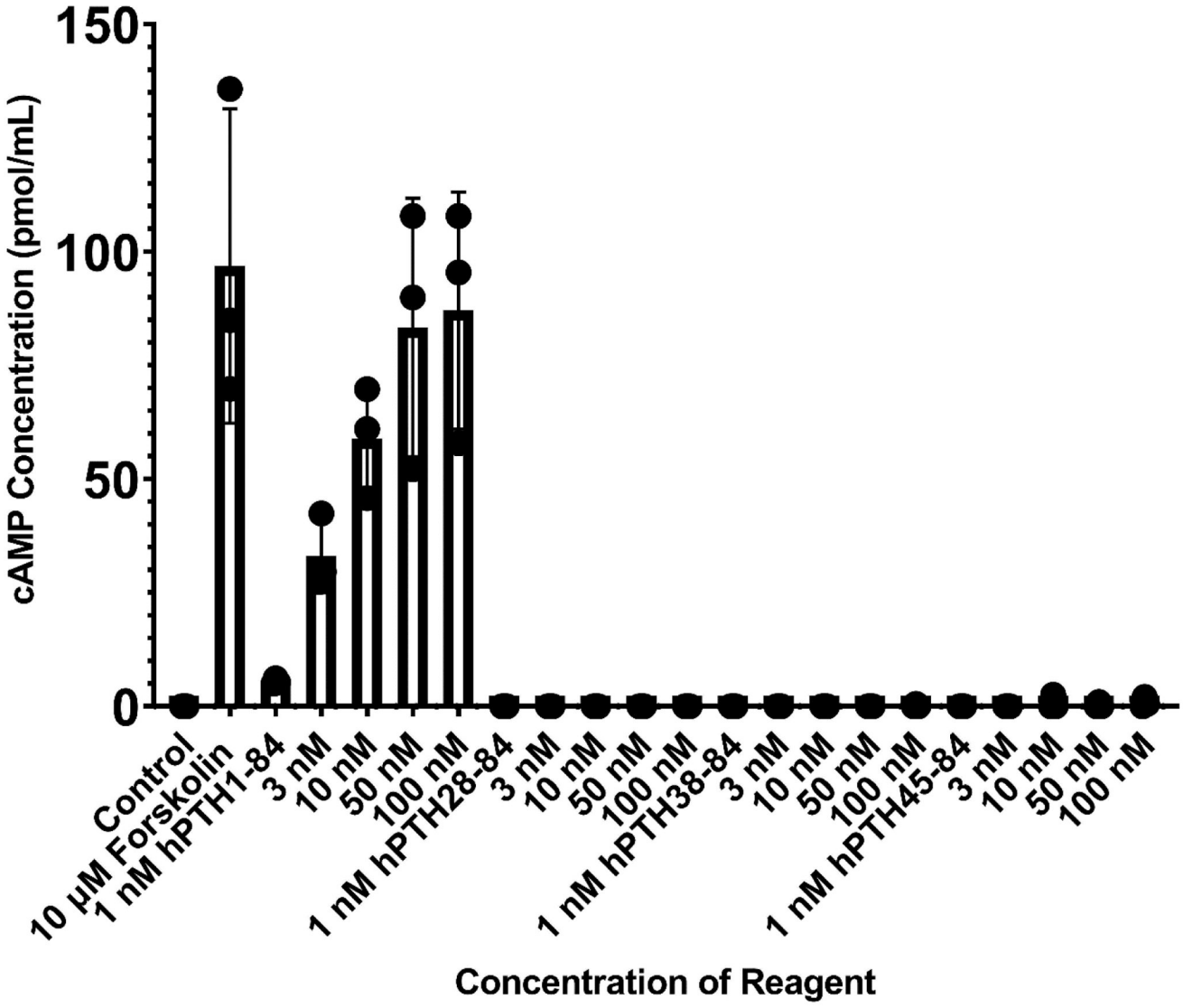

Figure 1.

Comparison of the effect of hPTH1–84 and hPTH peptides (hPTH28–84, hPTH38–84, hPTH45–84) on cAMP production in MC3T3-E1 cells. hPTH1–84 increased cAMP production in a dose-response manner. cAMP concentrations after treatment with 1, 3, 10, 50, and 100 nM hPTH1–84 were 5.50±0.63, 33.11±8.13, 58.86±12.13, 83.32±28.47, and 87.06±26.05 pmol/mL, respectively. The same concentration of hPTH peptides had no effect.

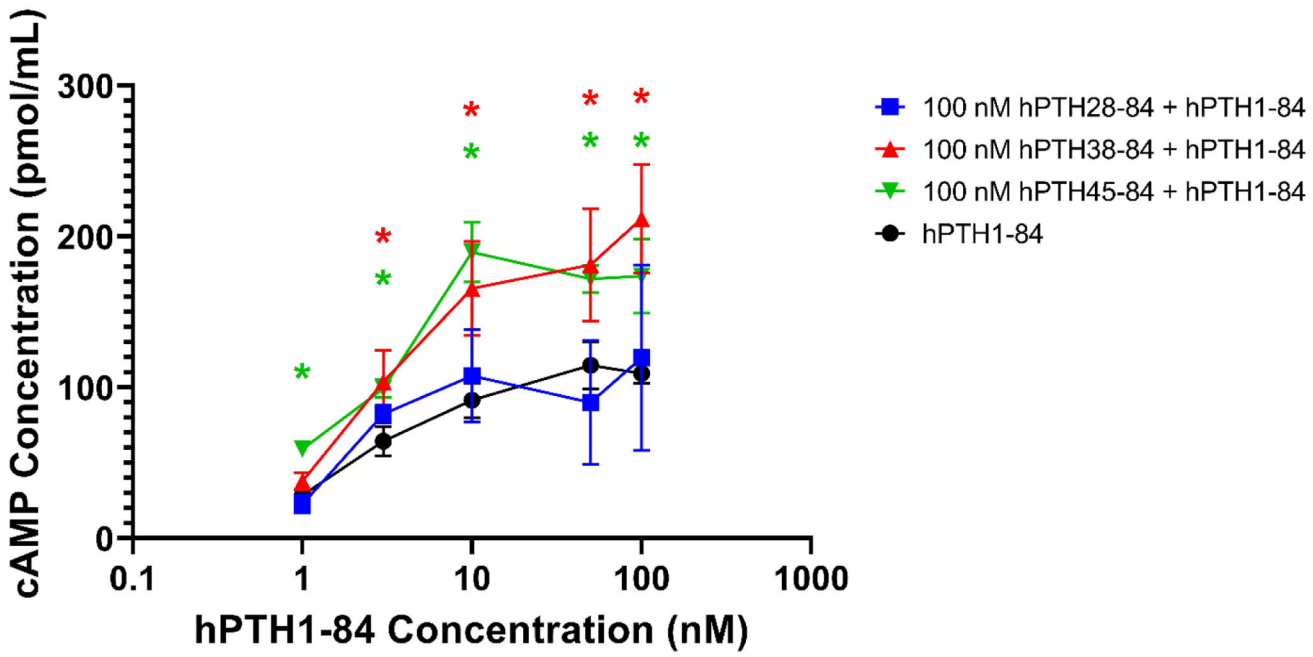

Figure 2.

Enhancement of cAMP production in MC3T3-E1 cells by hPTH1–84 at different concentrations (1, 3, 10, 50, 100 nM) plus either 100 nM hPTH38–84 or hPTH45–84. MC3T3-E1 cells were pretreated for 2 hours with either 100 nM hPTH28–84, hPTH38–84, or hPTH45–84, and then stimulated by hPTH1–84 at indicated concentrations together with 100 nM of individual hPTH peptides for 30 minutes. The control group (black line) was pretreated with fresh culture media and then stimulated by hPTH1–84 alone. Values are expressed as the mean±SD for triplicate determinations (*p <0.01 vs. hPTH1–84 alone).

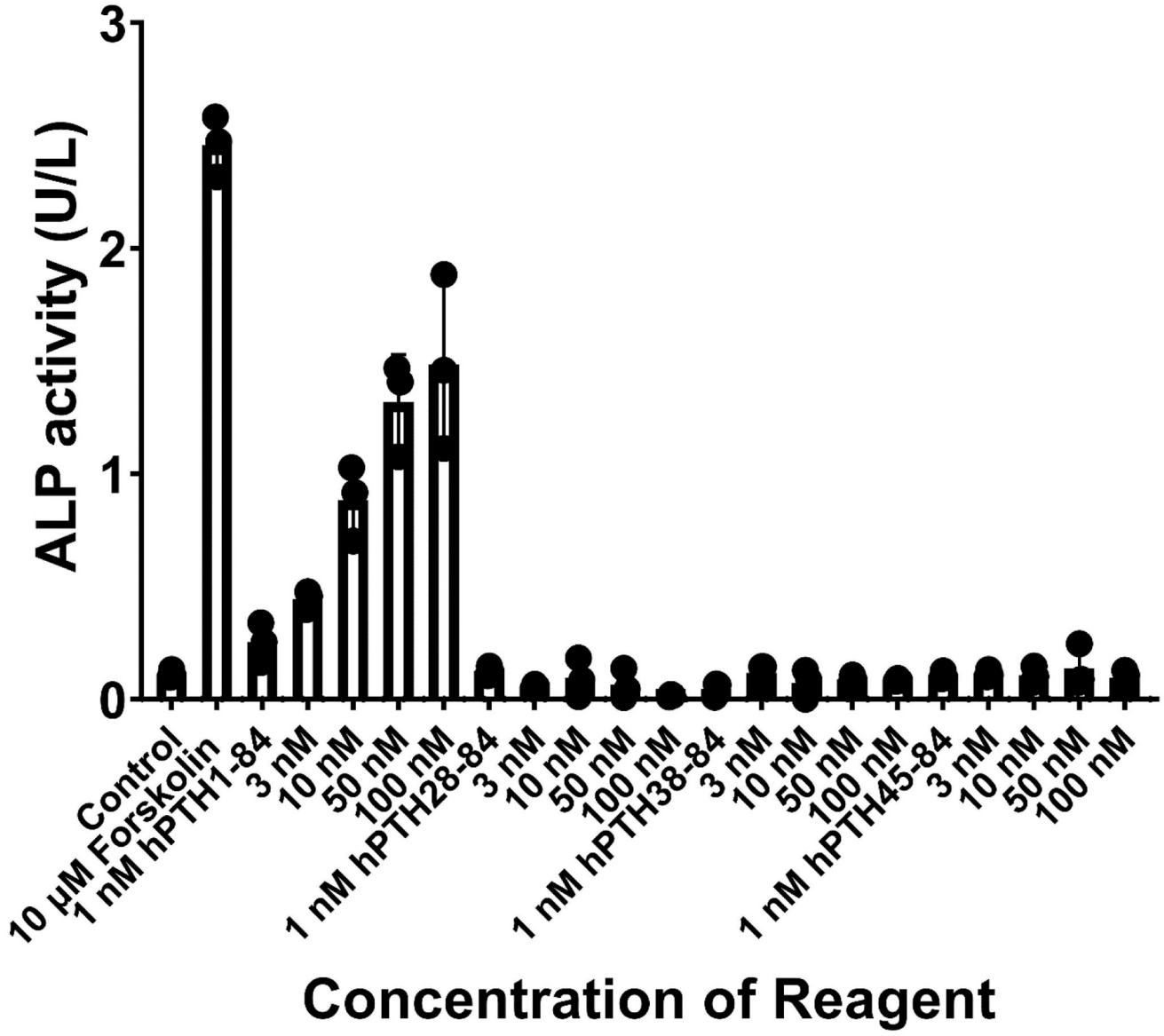

Figure 3.

Comparison of the effect of hPTH1–84 and hPTH peptides (hPTH28–84, hPTH38–84, hPTH45–84) on ALP activity in differentiated MC3T3-E1 cells. hPTH1–84 increased ALP activity in a dose-response manner. ALP activity after treatment for 24 hours using 1, 3, 10, 50, and 100 nM hPTH1–84 were 0.25±0.09, 0.44±0.04, 0.88±16, 1.32±0.21, and 1.48±0.39 U/L, respectively. The same concentration of hPTH peptides had no effect.

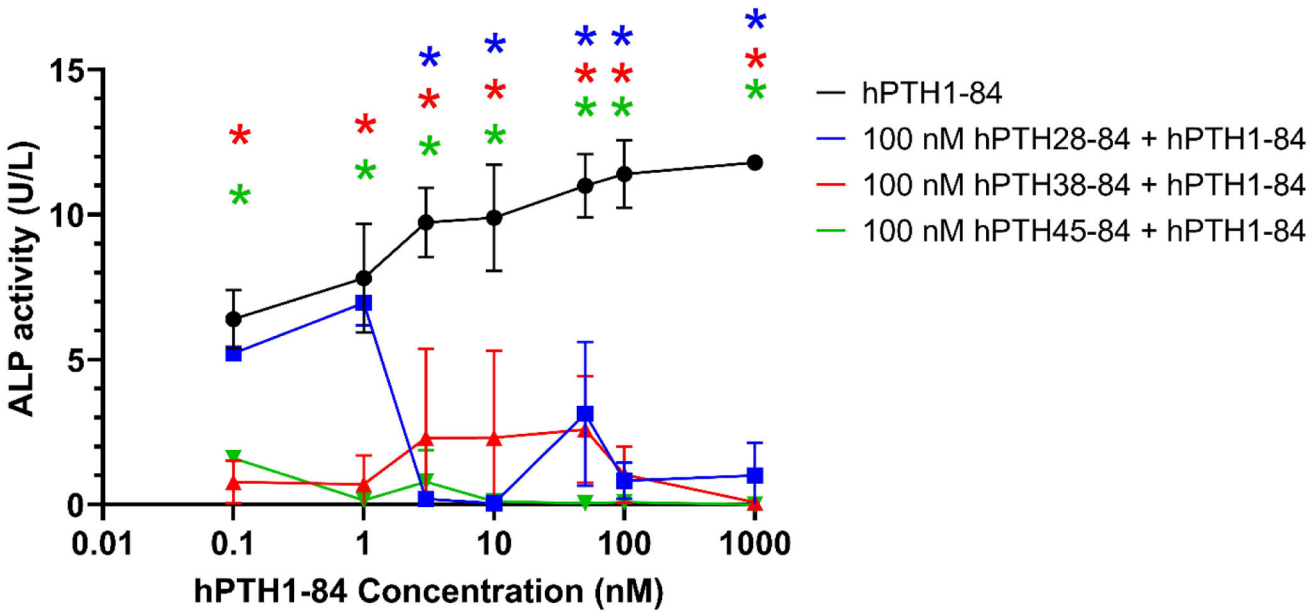

Figure 4.

Inhibition of ALP activity in differentiated MC3T3-E1 cells by 100 nM hPTH peptides (hPTH28–84, hPTH38–84, or hPTH45–84) together with hPTH1–84 at different concentrations (0.1, 1, 3, 10, 50, 100, 1000 nM). Differentiated MC3T3-E1 cells were treated for 24 hours using 100 nM of individual hPTH peptides together with hPTH1–84 at indicated concentrations. The control group (black line) was treated with hPTH1–84 alone. After 24 hours, ALP activity was assayed in cell lysates. hPTH1–84 alone increased ALP activity in a dose-response manner. However, ALP activity was significantly reduced in the presence of 100 nM of individual hPTH peptides. Values are expressed as the mean±SD for triplicate determinations (*p <0.01 vs. hPTH1–84 alone).