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Stimulation of Protein Kinase C Activity in Cells Expressing Human Parathyroid Hormone Receptors by C- and N-Terminally Truncated Fragments of Parathyroid Hormone 1-34

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ABSTRACT

The parathyroid hormone (PTH) fragment PTH(1-34) stimulates adenylyl cyclase, phospholipase C (PLC), and protein kinase C's (PKCs) in cells that express human, opossum, or rodent type 1 PTH/PTH-related protein (PTHrP) receptors (PTHR1s). Certain carboxyl (C)-terminally truncated fragments of PTH(1-34), such as human PTH(1-31) [hPTH-(1-31)NH_2], stimulate adenylyl cyclase but not PKCs in rat osteoblasts or PLC and PKCs in mouse kidney cells. The hPTH(1-31)NH_2 peptide does fully stimulate PLC in HKRK B7 porcine renal epithelial cells that express 950,000 transfected hPTHR1s per cell. Amino (N)-terminally truncated fragments, such as bovine PTH(3-34) [bPTH(3-34)], hPTH(3-34)NH_2, and hPTH(13-34), stimulate PKCs in Chinese hamster ovary (CHO) cells expressing transfected rat receptors, opossum kidney cells, and rat osteoblasts, but an intact N terminus is needed to stimulate PLC via human PTHR1s in HKRK B7 cells. We now report that the N-terminally truncated analogs bPTH(3-34)NH_2 and hPTH(13-34)OH do activate PKC via human PTHR1s in HKRK B7 cells, although less effectively than hPTH(1-34)NH_2 and hPTH(1-31)NH_2. Moreover, in a homologous human cell system (normal foreskin fibroblasts), these N-terminally truncated fragments stimulate PKC activity as strongly as hPTH(1-34)NH_2 and hPTH(1-31)NH_2. Thus, it appears that unlike their opossum and rodent equivalents, hPTHR1s can stimulate both PLC and PKCs when activated by C-terminally truncated fragments of PTH(1-34). Furthermore, hPTHR1s, like the PTHR1s in rat osteoblasts, opossum kidney cells, and rat PTHR1-transfected CHO cells also can stimulate PKC activity by a mechanism that is independent of PLC. The efficiency with which the N-terminally truncated PTH peptides stimulate PKC activity depends on the cellular context in which the PTHR1s are expressed. (J Bone Miner Res 2001;16:441–447)

Key words: parathyroid hormone, receptors, adenylyl cyclase, protein kinase C

INTRODUCTION

INTERMITTENT SUBCUTANEOUS injections of parathyroid hormone (PTH) or of N-terminal fragments such as human PTH(1-34) [hPTH-(1–34)NH_2] or hPTH(1-31)NH_2 stimulate bone growth in rodents, monkeys, and osteoporotic humans, but fragments that lack an intact N terminus do not stimulate bone growth, at least in rats.[1] Because differences in PTH receptor signal transduction induced by these various peptides are presumed to account for these obser-

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vations, it is important to find out what signals are generated when these fragments activate human receptors.

Jouishomme et al.[2,3] found that hPTH fragments needed amino acids 29–32 to stimulate membrane-associated protein kinase-C (PKC) activity in ROS 17/2 rat osteosarcoma cells. Thus, although N-terminally truncated fragments could not stimulate adenylyl cyclase, they could stimulate PKC activity provided residues 29–32 were present. In contrast, the C-terminally truncated hPTH(1-31)NH_2 peptide fully stimulated adenylyl cyclase but not PKC activity. Increasing the bioactivity of this peptide (as indicated by a 6-fold lower concentration for half-maximally stimulating adenylyl cyclase, the EC_{50}) by replacing Lys^{27} with Leu and linking Glu^{22} to Lys^{26} to produce the [Leu^{27}]cyclo(Glu^{22}-Lys^{26})hPTH(1-31)NH_2 lactam still did not enable it to stimulate PKC activity in ROS 17/2 cells.[4,5]

By contrast, certain N-terminally modified or truncated PTH fragments that are not osteogenic (at least in rats) such as 1-desamino-hPTH(1-34), bovine PTH(3-34) [bPTH(3-34)], hPTH(28-34), hPTH(13-34), hPTH(28-48), and hPTH(3-84) had little or no ability to stimulate adenylyl cyclase; yet all of them, especially the more potent hPTH(13-34), strongly stimulated PKC activity in ROS 17/2 cells.[1–3] In rat osteosarcoma cells, PTH(3-34) and PTH(7-34) cause a rapid cytosolic Ca^{2+} surge although they do so less effectively than rat PTH(1-34).[6] PTH(3-34) can stimulate phospholipase C (PLC; as indicated by increased inositol-1,4,5-tris phosphate [IP_3]) as much as PTH(1-34), and both PTH(3-34) and PTH(7-34) can stimulate PKC activity in UMR 106–01 rat osteosarcoma cells.[7] PTH(3-34), PTH(28-42), and PTH(28-48) also strongly stimulate PKC activity but not adenylyl cyclase activity, in Chinese hamster ovary (CHO) AP-1 cells expressing transfected rat type 1 PTH/PTH-related protein (PTHrP) receptors (PTHR1s).[8]

These signaling patterns are not limited to rat osteoblasts or hamster cells with transfected rat PTHR1s. hPTH(1-31)NH_2 stimulates adenylyl cyclase fully, but cannot activate PLC in mouse kidney cells although neither bPTH(3-34) nor hPTH(7-34) can stimulate adenylyl cyclase or PKC activity in these cells.[9] hPTH(28-48) stimulates the expression of insulin-like growth factor I (IGF-I) and cartilage growth in neonatal mice.[10] hPTH(3-34), PTH(28–42), PTH(28-48), and PTH(28-34) can stimulate PKC activity as effectively as PTH(1-34)[11,12] whereas hPTH(1-30) can stimulate adenylyl cyclase but not PKC activity in OK opossum kidney cells.[11–13]

It is clear from these observations that PTHR1s on mouse and opossum kidney cells and those on neoplastic rat osteoblasts respond similarly to C-terminally truncated PTH fragments. Moreover, the receptors on mouse bone and cartilage cells, opossum kidney cells, rat osteoblasts, and CHO cells expressing rat receptors, though not the receptors on mouse kidney cells, also respond similarly to N-terminally truncated PTH fragments. The question remains as to what signals hPTHR1s use to stimulate bone formation in PTH-treated osteoporotic humans.

One approach to answering this question has been to use porcine LLC-PK1 kidney cells, which have no endogenous PTHR1s, to compare the signaling properties of exogenous stably transfected hPTHR1 and rat PTHR1 receptors.[14,15] When LLC-PK1 cells express heterologous rat receptors, hPTH(1-31)NH_2 and hPTH(1-34)NH_2 equally stimulate adenylyl cyclase.[14] However, in contrast to the inability of hPTH(1-31)NH_2 to stimulate PKC activity in ROS 17/2 rat cells, hPTH(1-31)NH_2 can stimulate PLC (IP_3 production) via rat PTHR1s in the porcine cells, although only 25–30% as effectively as hPTH(1-34)NH_2.[14] In LLC-PK1 cells expressing human PTHR1s the two fragments are equipotent stimulators of adenylyl cyclase and IP_3 production.[15] Importantly, PTH also needs an unmodified N terminus to stimulate IP_3 production in PTHR1-expressing porcine cells.[16] Thus, removing the α-amino group from Ser^1 of hPTH(1-34)NH_2 or removing the N-terminal Ser^1 eliminates PLC stimulation without affecting binding to PTHR1 or adenylyl cyclase activation.[15,16] Thus, N-terminally truncated PTH fragments do not stimulate IP_3 production in LLC-PK1 cells expressing large numbers of hPTHR1s or, surprisingly, rat PTHR1s.[15]

Because PLC activation is a common mechanism for activating PKC, and because both PLC and PKC can be activated by recombinant PTHR1s, these disparities in PLC versus PKC activation by N- and C-truncated PTH fragments seem surprising. They could arise from intrinsic differences between hPTHR1s and opossum and rat PTHR1s, from differences in cellular PTHR1 expression or effector coupling or in the case of N-truncated fragments from PLC-independent PKC-activating mechanisms. To address these questions, we have measured the activation of membrane PKC activity by hPTH fragments in LLC-PK1 cells expressing hPTHR1s, for which adenylyl cyclase and PLC responses are known in detail and in a homologous human foreskin fibroblast system (fibroblasts have PTH/PTHrP receptors[17–22]). We will show that PLC-stimulating[14–16] C-terminally truncated fragments such as hPTH(1-31)NH_2 and hPTH(1–28)NH_2 stimulate PKC activity as efficiently as hPTH(1-34)NH_2 in LLC-PK1 cells, whereas N-terminally truncated fragments that do not detectably stimulate IP_3 production in LLC-PK1 cells[14–16] stimulate PKC activity in human fibroblasts.

MATERIALS AND METHODS

PTH fragments

hPTH(1-34)NH_2, hPTH(1-31)NH_2, and hPTH(1–28)NH_2 were synthesized in the Institute for Biological Science using the Fmoc protocol and a continuous-flow peptide synthesizer (model 9050; PerSeptive Biosystems, Framingham, MA, USA) as described previously.[23] Bovine bPTH(3-34)NH_2 and hPTH(13-34)OH were purchased from Bachem (Torrance, CA, USA). [Tyr^{34}]hPTH(3-34)NH_2 was synthesized by the Peptide and Oligonucleotide Core Laboratory of the Massachusetts General Hospital's Endocrine Unit.

Cell culture: HKRK B7 cells

The derivation of HKRK B7 cells, stably expressing about 950,000 transfected PTHR1s/cell, from LLC-PK1 porcine renal epithelial cells, has been described previous-

ly.[14,15] The cells were grown in an antibiotic-free complete medium consisting of 93% (vol/vol) high-glucose (4.5 g/liter) Dulbecco's modified Eagle's medium (DMEM; Sigma Chemical Co., St. Louis, MO, USA) and 7% (vol/vol) heat-inactivated fetal bovine serum.[15] The cultures were incubated at 37°C in 5% CO_2 and 95% air.

For each experiment, 0.5 or 0.6×10^6 cells in 6 ml of medium were plated in 60-mm plastic dishes and the cultures were allowed to grow 3 days or 4 days until they were nearly confluent. Two hours before adding a PTH fragment, the complete medium was discarded, the cultures were washed twice with phosphate-buffered saline (PBS), the complete medium was replaced with 3 ml of serum-free DMEM, and cultures were returned to 37°C in the 5% CO_2/air atmosphere. The cells were exposed to a PTH fragment for 3 minutes (the time of peak activity) at 37°C. The cultures were then washed with ice-cold PBS. The PBS was discarded and the culture dishes put on ice and 1.2 ml of lysis buffer[2] was added. Two minutes later, the cells were scraped with a rubber policeman. The resulting suspensions were put into a 15-ml centrifuge tube and vortexed for 2 minutes. The lysates were centrifuged at 600*g* for 10 minutes at 4°C and the postnuclear supernatant fractions were pelleted by centrifugation at 100,000*g* for 10 minutes at 4°C. The PKC activity associated with these membranes was measured without prior extraction, reconstitution, or artificial activation according to Chakravarthy et al.[24,25] The level of PKC activity in equal amounts of membrane protein was indicated by the extent of phosphorylation by the pelleted membranes of the PKC-specific peptide Ac-FKKSFKL (acetyl-Phe-Lys-Lys-Ser-Phe-Lys-Leu-NH_2), which corresponds to residues 160–166 of the membrane-associated myristoylated alanine-rich C-kinase substrate (MARCKS) protein, the standard marker of PKCs activation in intact cells.[22] The extent of phosphorylation of this peptide substrate was determined as described by Chakravarthy et al.[24,25] and Whitfield et al.[26] and expressed as counts per minute of ^{32}P radioactivity per microgram of Ac-FKKSFKL substrate. As expected, the phosphorylation of the Ac-FKKSFKL substrate by the membranes from control and PTH-treated cells was prevented by including in the assay mixture the RFARKGALRQKVNHEVKN peptide corresponding to the autoinhibitory 19–36 pseudosubstrate domain of PKCs at concentrations that are known not to affect other protein kinases in intact cell membranes such as those used in this study. Cultures used as positive controls were exposed to the standard PKC activator 12–0-tetradecanolyphorbol-13-acetate (TPA), at a concentration of 1 μM.

This assay measures activity of membrane-associated PKCs in situ and not a shift of activated PKCs from the cytosol to membranes. Substantial fractions of cellular PKCs may reside on membranes in an inactive but stimulatable state, and the extent of translocation of enzyme activity from cytosol to membranes may not be an accurate or sufficiently sensitive measure of PKC stimulation.[25] Therefore, we did not measure the cytosolic PKCs in the present experiments because the actual peak membrane–associated PKC activities in the PTH- or TPA-stimulated cells were the sums of the activities of the PKCs that were already in the membranes at the time of exposure to PTH or TPA and the PKCs that had subsequently moved from the cytosol to the membrane.

The peaks, troughs, and relative increases of PKC activity in the treated cultures varied from experiment to experiment depending on unavoidable fluctuations in the states of the cells.

Cell culture: human dermal fibroblasts

Human dermal fibroblasts from newborns' foreskins were provided by Dr. Eric DesRosiers of Apotex Research, Inc. (Weston, Ontario, Canada) as frozen second-passage cells. They were incubated at 37°C in a complete medium consisting of 90% low-glucose DMEM and 10% heat-inactivated fetal bovine serum in an atmosphere of 5% CO_2. For an experiment, cells from confluent cultures in T25 flasks were plated in 60-mm dishes. The cultures were then left until they were completely confluent before starting an experiment.

The assay for membrane-associated PKC activity in the human fibroblasts was basically the same as that for the activity in HKRK B7 cells. However, the fibroblasts were much more resistant to the lysis buffer and needed two, 5-s sonications to be lysed in the buffer. We used 4 μg instead of 10 μg of protein per assay and the time of peak PKC activity was 10 minutes instead of the 3 minutes in HKRK B7 cells.

The adenylyl cyclase activity in human fibroblasts was expressed as the rate of formation of [^{3}H]cyclic adenosine monophosphate (cAMP) from the cellular adenosine triphosphate (ATP) pool that had been labeled with [^{3}H]adenine before the cells were exposed to a PTH fragment.[2] The cells were incubated for 10 minutes after adding a peptide. The reaction was stopped with 10% trichloroacetic acid, and the [^{3}H]cAMP was then separated and measured.[2]

Statistical analysis

All data were expressed as means ± SEM. Comparisons were made by one-way analysis of variance (ANOVA). When significant effects were found, Scheffe's or Fisher's projected least significant difference (PLSD) tests were used for multiple comparisons and $p < 0.05$ was considered to be significant.

RESULTS

HKRK B7 cells

We first determined the optimal time at which to measure membrane-associated PKC activity after exposing HKRK B7 cells to TPA or PTH fragments. The stimulation of PKC activity by the positive control agent, 1 μM TPA, was 1.7-fold by 5 minutes, after which it increased again to 2.7-fold higher than controls at 7 minutes (Fig. 1). The PKC activity peaked around 3 minutes after adding 50 nM hPTH(1–28)NH_2 or hPTH(1-34)NH_2, and it returned to the control level by 5 minutes. Therefore, measurements of the

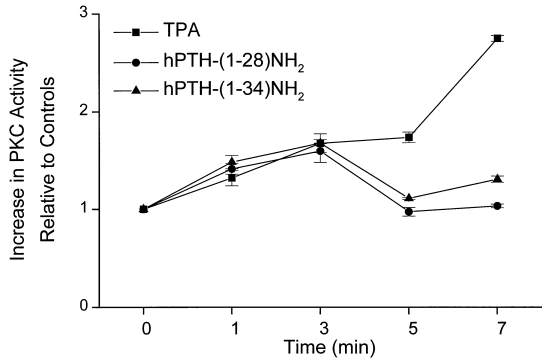

FIG. 1. Changes in PKC activity in HKRK B7 cells with time after addition of 1 μM TPA, 50 nM hPTH(1-34)NH_2, or 50 nM hPTH(1–28)NH_2. The points are the combined means ± SEMs of the results from three separate experiments.

effects of various PTH fragments on PKC activity were carried out 3 minutes after their addition to the cultures, a time when IP_3 production also peaks in PTH-treated HKRK B7 cells.[16]

The response to increasing concentrations of hPTH(1–28)NH_2 tended to be biphasic, as in ROS 17/2 rat cells.[2] The activity reached peak values at 0.25 nM (1.78 ± 0.21-fold higher than controls) and again at 25 nM (1.79 ± 0.26-fold higher than controls; Fig. 2). The response to hPTH(1-31)NH_2 was also biphasic. The activity peaked at 0.25 nM at 1.54 ± 0.10 times the control value, dropped between 0.25 nM and 5 nM, and then rose again to 1.83 ± 0.24 times the control activity at 500 nM (Fig. 2). There was a similar biphasic response after exposure to various concentrations of hPTH(1-34)NH_2 (Fig. 2). The three peptides equally stimulated PKC activity when tested in the same experiment at a concentration of 500 nM (data not shown). Smaller peptides hPTH(1–27)NH_2 and hPTH(1–26)NH_2 did not increase PKC activity at any concentration between 0.1 and 500 nM (data not shown).

Bovine PTH(3-34)NH_2, which stimulates PKC activity in ROS 17/2 and other cells,[2,8] significantly ($p < 0.05$) increased PKC activity in HKRK B7 cells 1.29 ± 0.02-fold at 50 nM (Fig. 3). Similar results (not shown) were observed with [Tyr^{34}]hPTH(3-34)NH_2, a peptide that does not activate PLC in HKRK B7 cells.[15,16] hPTH(13-34)OH, which was the most potent stimulator of PKC activity in ROS 17/2 cells,[2] increased PKCs activity 1.16 ± 0.03-fold at 25 nM (Fig. 3).

Human dermal fibroblasts

hPTH(1-34)NH_2 significantly ($p < 0.05$) increased PKC activity 2.69 ± 0.36-fold at 0.5 nM in human foreskin fibroblasts (Fig. 4); however, the response was not biphasic like it was in HKRK B7 cells. As in the HKRK B7 cells, there was a similar response to hPTH(1-31)NH_2 (Fig. 4). In contrast to the PKC activity in HKRK B7 cells, PKC activity in human fibroblasts was stimulated as strongly by hPTH(3-34)NH_2 as by hPTH(1-34)NH_2 or hPTH(1-31)NH_2 (Fig. 5). In fact, the strongest stimulator of dermal fibroblast

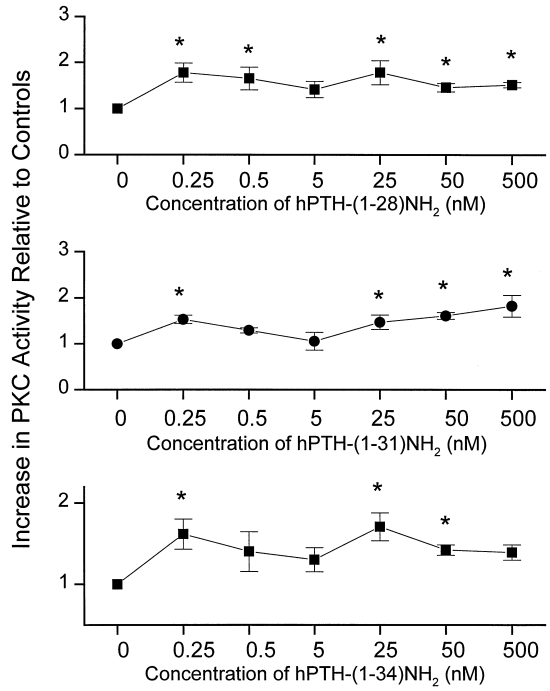

FIG. 2. Stimulation of PKC activity in HKRK B7 cells by hPTH(1-34)NH_2 and two of its C-terminally truncated fragments hPTH(1-31)NH_2 and hPTH(1–28)NH_2. Each point is the combined mean ± SEM of measurements of PKC activity from three separate experiments. The actual membrane-associated PKC activities in the untreated control cultures were 2322 ± 266 cpm, 1961 ± 79 cpm, and 2552 ± 138 cpm of ^{32}P radioactivity per microgram of FKKSFKL peptide substrate, respectively. The (*) superscript indicates that the value is significantly different ($p < 0.05$) from control.

PKC activity was hPTH(13-34)OH (Fig. 5). For example, the PKC activity in cultures exposed to 10 nM hPTH(13-34)OH was 4.83 ± 0.16-fold greater than in control cultures.

We considered the possibility that the enhanced PKC responses to the N-terminally truncated PTH fragments could have been triggered by a PTH receptor different from PTHR1 such as the type 2 PTH receptor (the receptor for the distantly related neuropeptide tuberoinfundibular protein [TIP] 39), which is not activated by PTHrP, or maybe the PTH receptor in keratinocytes and lymphocytes, which stimulates PKC activity but cannot stimulate adenylyl cyclase.[26,27] However, both possibilities were ruled out by the nearly equal abilities of hPTH(1-34)NH_2 and hPTHrP(1-34)NH_2 to stimulate adenylyl cyclase in these cells (Fig. 6).

DISCUSSION

The ability of N-terminally truncated PTH fragments such as bPTH(3-34)NH_2 and hPTH(13-34)OH to stimulate PKC activity, even if only weakly, in HKRK B7 porcine cells with their high density of hPTHR1s contrasts with the inability of such N-terminally truncated hPTH fragments to

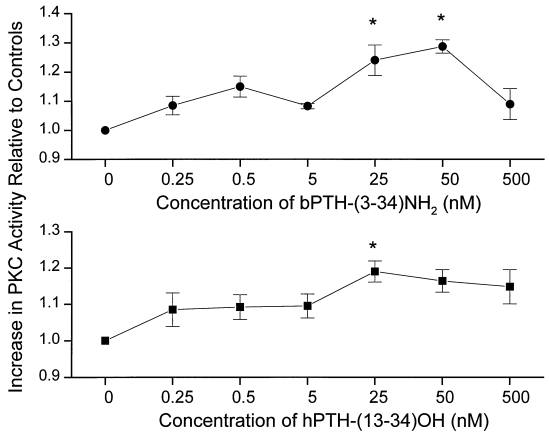

FIG. 3. Stimulation of PKC in HKRK B7 cells by the N-terminally truncated fragments bPTH(3-34)NH_2 and hPTH(13-34)OH. The points are the combined means ± SEMs of the results of three or four separate experiments. The actual PKC activities in the untreated control cultures were 1957 ± 156 cpm and 2577 ± 53 cpm of ^{32}P radioactivity per microgram of FKKSFKL peptide substrate, respectively. The (*) superscript indicates that the value is significantly different ($p < 0.05$) from control.

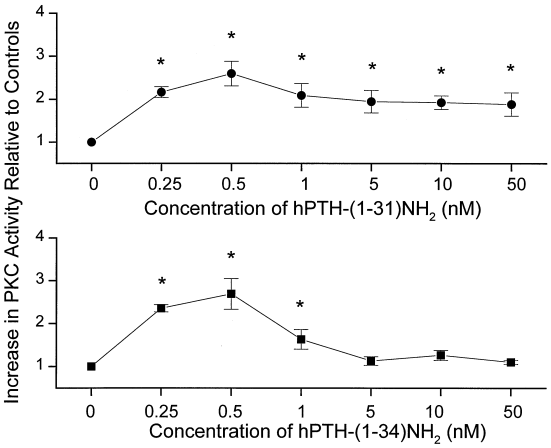

FIG. 4. Ability of hPTH(1-31)NH_2 and hPTH(1-34)NH_2 to stimulate PKC activity in normal early passage human dermal fibroblasts. The curves are combined means ± SEMs from three and five separate experiments, respectively. The actual PKC activities in the untreated control cultures were 1852 ± 99 cpm and 4104 ± 111 cpm of ^{32}P radioactivity per microgram of FKKSFKL peptide substrate, respectively. The (*) superscript indicates that the value is significantly different ($p < 0.05$) from control.

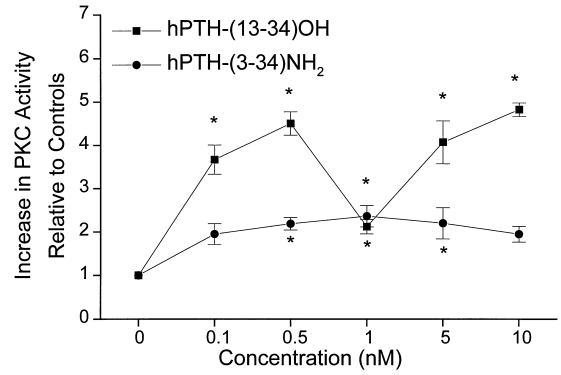

FIG. 5. Ability of [Tyr^{34}]hPTH(3-34)NH_2 and hPTH(13-34)OH to stimulate PKC activity in human dermal fibroblasts. The results are the combined means ± SEMs from three separate experiments. The actual PKC activities in the untreated control cultures were 3661 ± 303 cpm and 2232 ± 72 cpm of ^{32}P radioactivity per microgram of FKKSFKL peptide substrate, respectively. The (*) superscript indicates that the value is significantly different ($p < 0.05$) from control.

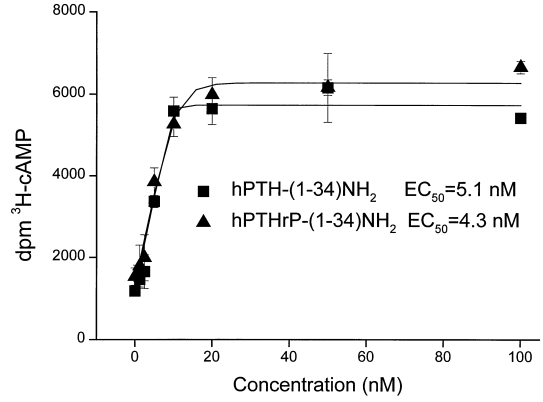

FIG. 6. Ability of hPTH(1-34)NH_2 and hPTHrP(1-34)NH_2 to stimulate adenylyl cyclase in normal human dermal fibroblasts. Points are the combined means ± SEMs from four separate experiments.

stimulate IP_3 production in these cells.[15–16] The ability of hPTH(3-34)NH_2 to activate the PTHR1 receptors also is indicated by the ability of the fragment to stimulate mitogen-activated protein (MAP) kinase activity and moderately stimulate the proliferation of HKRK B7 cells.[28] The failure of the N-terminally truncated fragments to stimulate IP_3 production but at the same time stimulate PKC and MAP kinase activities and the proliferation of these cells points to the existence of a PLC-independent mechanism of PKC activation by N-terminally truncated PTH fragments via the PTHR1. The existence of such a mechanism also is suggested by the fact that despite the stimulation of PKC activity by both N- and C-terminally truncated fragments in human fibroblasts, we have been unable to detect either a rise in IP_3 or the Ca^{2+} surge that should have been triggered if there had been an increase in IP_3 production (data not shown). The absence of a detectable rise in IP_3 in these fibroblasts could have been caused by them having a low PTHR1 density, which is known to reduce the efficiency of PLC activation.[14] Indeed, when competitive binding assays were carried out as in Whitfield et al.[29] we found that PTH binding, hence the receptor density, was too low for an accurate Scatchard analysis (data not shown). The efficiency of such an alternative mechanism appears to depend on the type of cell expressing the PTHR1 because the same N-terminally truncated PTH fragments stimulated PKC activity at least as strongly as the N-terminally intact hPTH(1-34)NH_2 in the dermal fibroblasts.

As noted previously, the ability of a PTH peptide to stimulate PLC or PKC is a function of receptor density,[14] the receptor species, and the availability within the target cell of the components needed to couple the receptor to this mechanism. The dependence of PTHR1's signaling properties on the nature of the target cell is shown by the ability of an N-terminally truncated PTH fragment to stimulate PKC strongly (relative to hPTH(1-34)NH_2) in rat osteoblasts with rat receptors,[2,7] CHO hamster cells expressing rat receptors,[8] and dermal fibroblasts expressing normal levels of endogenous human receptors, but only weakly in LLC-PK1 porcine cells expressing large numbers of human receptors. An example of the influence of the target cell and receptor origin is the fact that the C-terminally truncated hPTH(1-31)NH_2 activates PKC as strongly in HKRK B7 cells and human dermal fibroblasts as hPTH(1-34)NH_2, but it fails to activate PKCs in ROS 17/2 rat osteoblastic cells, murine proximal tubular cells, or probably human fetal osteoblasts.[3,9,30] In this respect, the hPTHR1s in the HKRK B7 cells and dermal fibroblasts behave like the unconventional PTH receptors on rat spleen lymphocytes.[5]

These various observations suggest that there may be at least two mechanisms by which an activated hPTHR1 can stimulate PKC. One of these, like the stimulation of adenylyl cyclase, requires an interaction of the receptor with a PTH fragment's unmodified N terminus[16] and may proceed via activation of PLC when conditions favor this response. It seems that cells of an established line of human fetal osteoblasts lack this mechanism because they cannot stimulate PKC-dependent processes when exposed to hPTH(1-31)NH_2.[30] The other mechanism can be activated by a portion of the PTH ligand that does not include the N terminus and can proceed without measurable PLC activation. The PLC-independent mechanism might involve the stimulation of phospholipase A_2 and/or phospholipase D.[9,31–33] However, Bringhurst and Singh[34] have reported that hPTH(3-34)NH_2 does not stimulate PKC activity via phospholipase (PLD) in LLC-PK1 cells and we have not been able to show a stimulation of PLD in the human fibroblasts (data not shown). LLC-PK1 cells are relatively deficient in what human and rat PTH/PTHrP receptors need to stimulate the second mechanism when they are activated by N-terminally truncated PTH fragments whereas CHO cells, human fibroblasts, opossum kidney tubule cells, and rat osteoblasts can provide the necessary environment to enable PTHR1s to activate fully PKC on interaction with N-terminally truncated PTHs.

An important practical question arising from the present experiments is what signals would be given by hPTH(1-31)NH_2 to induce osteoblasts to start making bone in an osteoporotic patient? (From the clinical standpoint whether or not N-terminally truncated PTH fragments can stimulate PKCs is unimportant—they cannot stimulate adenylyl cyclase, which [at least in rats] is a sine qua non for inducing new bone formation.[35,36]) According to both the present results and those of Takasu and Bringhurst[14] the effects of hPTH(1-31)NH_2 and hPTH(1-34)NH_2 in humans should be the same, but they are not. According to Fraher et al.[37] hPTH(1-31)NH_2 subcutaneously infused into human volunteers stimulates adenylyl cyclase as effectively as hPTH(1-34), as indicated by an equally increased urinary cAMP concentration. However, the truncated peptide is far less effective than hPTH(1-34) in stimulating bone resorption, as indicated by its failure to increase either the plasma calcium level or the urinary cross-linked N-terminal collagen telopeptides. The same result has been observed in mice: hPTH(1-31)NH_2 stimulates osteoblast activity as effectively as hPTH(1-34)NH_2, but it is only half as effective a stimulator of osteoclast activity as hPTH(1-34)NH_2.[38] The results of experiments on cells of an established line of human fetal osteoblasts suggest that these cells respond to C-terminally truncated PTHs like ROS osteoblasts rather than the primary human fibroblasts. In these human osteoblasts, hPTH(1-31) can stimulate the cAMP/PKA-dependent expression of transforming growth factor β2 (TGF-β2) but not the PKC-dependent expression of TGF-β1 whereas hPTH(1-34) can stimulate the expression of both TGF-βs.[30] Clearly, the next step is to find out what signals are in fact given to normal primary or very early passage human osteoblasts by osteogenic fragments such as hPTH(1-31)NH_2 and how these signals are related to the processes of bone nodule formation and osteoclast induction by osteoblastic cells.

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