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Parathyroid Hormone Synergizes With Non-Cyclic AMP Pathways to Activate the Cyclic AMP Response Element

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ABSTRACT

Parathyroid hormone (PTH) activates multiple signaling pathways following binding to the PTH1 receptor in osteoblasts. Previous work revealed a discrepancy between cAMP stimulation and CRE reporter activation of truncated PTH peptides, suggesting that additional signaling pathways contribute to activation of the CRE. Using a CRE-Luciferase reporter containing multiple copies of the CRE stably transfected into the osteoblastic cell line Saos-2, we tested the ability of modulators of alternative pathways to activate the CRE or block the PTH-induced activation of the CRE. Activators of non-cyclic AMP pathways, that is, EGF (Akt, MAPK, JAK/STAT pathways); thapsigargin (intracellular calcium pathway); phorbol myristate acetate (protein kinase C, PKC pathway) induced minor increases in CRE-luciferase activity alone but induced dramatic synergistic effects in combination with PTH. The protein kinase A (PKA) inhibitor H-89 (10 μM) almost completely blocked PTH-induced activation of the CRE-reporter. Adenylate cyclase inhibitors SQ 22536 and DDA had profound and time-dependent biphasic effects on the CRE response. The MAPK inhibitor PD 98059 partially inhibited basal and PTH-induced CRE activity to the same degree, while the PKC inhibitor bisindolylmaleimide (BIS) had variable effects. The calmodulin kinase II inhibitor KN-93 had no significant effect on the response to PTH. We conclude that non-cAMP pathways (EGF pathway, calcium pathway, PKC pathway) converge on, and have synergistic effects on, the response of a CRE reporter to PTH. J. Cell. Biochem. 106: 887–895, 2009. © 2009 Wiley-Liss, Inc.

KEY WORDS: PARATHYROID HORMONE; OSTEOBLAST; CYCLIC AMP; CRE; MAPK; PKC

In osteoblasts, the bone anabolic drug parathyroid hormone (PTH) binds to the G-protein coupled PTH-1 receptor [Chorev, 2002] and initiates multiple intracellular signals, resulting in the rapid activation of a variety of protein kinases. In addition to the cyclic AMP-protein kinase A (cAMP-PKA) pathway, activated through Gαs, PTH can also, via Gαq, stimulate the phospholipase C (PLC) pathway, which bifurcates on the one hand into an IP3-mediated intracellular calcium signal, and on the other hand into the diacylglycerol signal that in turn activates protein kinase C (PKC). PTH can also, via Gα12/Gα13, activate phospholipase D [Singh et al., 2005], resulting in protein kinase C alpha (PKCα) translocation to the membrane [Radeff et al., 2004], and PTH has also been reported to induce the phosphorylation of c-src [Izbicka et al., 1994]. The effects of PTH on activation of the mitogen-activated protein kinase (MAPK) pathway are cell-type specific and apparently more complex. PTH has been reported to either activate [Verheijen and Defize, 1997; Gentili et al., 2001, 2002; Swarthout et al., 2001; Fujita et al., 2002; Ahmed et al., 2003; Bacic et al., 2003; Syme et al., 2005], or inhibit [Verheijen and Defize, 1995; Fujita et al., 2002; Homme et al., 2004] MAPK, the effect being variously attributed to cyclic AMP [Verheijen and Defize, 1997; Gentili et al., 2001; Fujita et al., 2002], Gαq signaling [Syme et al., 2005], PKC [Miao et al., 2001; Swarthout et al., 2001], PI3K [Gentili et al., 2002], MKP-1[Homme et al., 2004] PTHR1 internalization [Syme et al., 2005], and PTH-dependent transactivation of the EGFR [Ahmed et al., 2003; Syme et al., 2005].

In vivo studies in rodents using PTH fragments with selective signaling properties suggest that cyclic AMP is the major mediator of the skeletal effects of PTH in vivo, and may be essential for the anabolic effect of the hormone [Ahmed et al., 2003]. Not only do truncated fragments of PTH that have a limited ability to stimulate cAMP have a similarly limited ability to induce bone formation [Hilliker et al., 1996; Mohan et al., 2000], but drugs that can stimulate cAMP without activating a membrane receptor, such as the phosphodiesterase inhibitor rolipram, can mimic the effects of parathyroid hormone in vivo, albeit less efficaciously [Kinoshita

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et al., 2000]. Many of the changes in gene expression that are induced by PTH appear to be either dependent upon PKA activation, or inducible with cAMP agonists [Mauro et al., 1996; Swarthout et al., 2002; Kawane et al., 2003; Pirih et al., 2003; Ozkurt et al., 2004] reviewed in Murrills [2006], emphasizing the important role cAMP has in PTH signaling. Additionally, genetic studies in mice also indicate that the cyclic AMP pathway has profound effects on osteogenesis, as evidenced by a high bone mass phenotype in mice expressing constitutively active Gαs in osteoblasts [Hsiao et al., 2008].

By contrast, PTH fragments that activate only the PKC pathway, and have little or no ability to activate cAMP (or intracellular calcium) such as PTH(28–48), can induce only minor bone anabolic activity, inasmuch as they can increase DNA incorporation and cortical width in immature mice and rats [Somjen et al., 1991; Rihani-Bisharat et al., 1998; Kim et al., 2002], but cannot increase bone mineral density or mechanical strength in ovariectomized (OVX) rats with established osteopenia [Murrills et al., 2004]. These studies suggest that PKC activation may play a role in the proliferative response to PTH [Swarthout et al., 2002], but that this response is insufficient of itself for inducing trabecular bone formation. There is evidence from in vitro and in vivo studies that PKC plays a role the catabolic actions of PTH. In vitro, inhibition of PKC diminished PTH-induced bone resorption and induction of bone resorptive cytokines IL-6, TNFα, and IL-1β [Bos et al., 1992; Nagy et al., 2001]. In vivo, PTH(1–31) (Ostabolin), a C-terminally truncated PKA selective analog of PTH which has reduced activation of PKC, induces reduced hypercalcemia compared to PTH, and also reduced serum markers of bone resorption [Mohan et al., 2000]. Studies of gene expression have identified a number of genes whose regulation by PTH can be altered by protein kinase C inhibitors, but these are few compared with those genes whose regulation is influenced by the cAMP pathway (reviewed in Murrills, 2006). The role of the PKC pathway in the proliferative and catabolic response to PTH has recently been probed using transgenic mice expressing constitutively active Gαq in osteoblasts. These animals have a low bone mass phenotype due to impaired osteoblast differentiation, with no apparent effect on osteoblast proliferation or bone resorption [Ogata et al., 2007] suggesting that Gαq and Gαs have opposing roles in osteoblast differentiation, and that Gαq does not play a role in bone resorption, although this does not rule out a potential role in bone resorption for other PKC-activating pathways such those downstream of Gα12/Gα13.

Downstream of receptor and protein kinase activation, a variety of transcription factors are phosphorylated following PTH exposure, particularly the cyclic AMP response element binding-protein (CREB), for which phosphorylation at Ser133 is required for CREB-mediated transcription [Shaywitz and Greenberg, 1999]. Interestingly, CREB can be phosphorylated on Ser133 not only by PKA, but also by PKC, MAPK, and Ca^{2+}/calmodulin kinase [Shaywitz and Greenberg, 1999]. However, it is not known how such non-cyclic AMP phosphorylation of CREB would affect the transcriptional response to PTH, or whether PTH itself can use non-cyclic AMP pathways to promote transcription of genes with cyclic AMP response elements.

Given the evidence that non-cyclic AMP pathways can result in CREB phosphorylation, we investigated, using a CRE-luciferase reporter assay, whether non-cAMP pathways, activated either by PTH or other agonists, influence the CRE transcriptional response to PTH. Our results show that the PKC, calcium and EGF-induced signaling pathways synergistically activate the CRE in combination with PTH. In addition, non-cAMP pathways activated by PTH itself may contribute the CRE response to PTH.

MATERIALS AND METHODS

PTH AND SIGNALING INHIBITORS

We purchased hPTH(1–34) from Bachem (Torrance, CA); H-89, PD98059, forskolin, phorbol myristate acetate (PMA), 4-α-phorbol 12, 13 didecanoate, rolipram, isobutylmethylxanthine (IBMX), thapsigargin, EGF and A23187 from Sigma (St. Louis, MO); SQ 22536 and KN-93 from Calbiochem (San Diego, CA); and 2′ 5′-dideoxyadenosine (DDA) from Biomol (Plymouth Meeting, PA).

CRE-LUCIFERASE ASSAY

We assayed CRE-reporter activity in Saos-2 human osteosarcoma cells that had been stably transfected with 15 copies of the CRE (TGACGTCA) linked to a luciferase reporter, which we call Saos-H3 cells. We have previously confirmed that Saos-H3 cells respond to PTH and other cAMP agonists with an increase in luciferase activity [Murrills et al., 2004]. We grew and maintained the cells in McCoy's 5A modified medium containing L-glutamine (Invitrogen), 10% heat-inactivated fetal calf serum (FCS), 1% (v/v) penicillin–streptomycin and 2 mM Glutamax-1. For storage, we harvested cells at ~80% confluence using trypsin (0.05%)-EDTA (0.53 mM) (Gibco) before centrifugation and re-suspension in freezing medium (95% FBS + 5% DMSO), and storage in a −150 freezer. For assay, we thawed an aliquot of cells, diluted it into phenol red free DMEM and then plated at 10,000 cells per well into the wells of a 96-well white plate. We typically allowed the cells to adhere for 4 h before adding PTH or other cAMP agonists. In some experiments, we extended this to overnight, which resulted in a lower basal level of CRE-reporter activity, and hence a larger PTH response. We added signaling inhibitors 1 h before adding PTH. The phosphodiesterase inhibitor IBMX was added to each well to a final concentration of 10 μM to enhance the CRE-luciferase signal. After incubating the cells with agonists and inhibitors for a further 24 h, we detected luciferase activity using a LucScreen kit (Tropix, Bedford, MA) and measured relative luminescent units (RLUs) using a TopCount luminescence counter (Packard). We performed each treatment using triplicate wells along with appropriate vehicle controls.

GRAPHING AND STATISTICS

We analyzed data, with or without log transformation as appropriate, using ANOVA [SAS Excel or JMP (SAS Institute, Corey, NC)]. We determined significant differences using LSD, Dunnett's test or Tukey-Kramer HSD, as appropriate. We graphed data (means ± SEM of three replicates) using Sigmaplot (Systat Software, San Jose CA).

RESULTS

SYNERGY OF PTH WITH EGF AND STIMULATORS OF THE CALCIUM/PKC PATHWAYS

Epidermal growth factor. EGF, which does not stimulate cAMP, but activates multiple pathways including the MAPK, JAK/STAT, and Akt pathways via its membrane receptor, had little effect on the CRE when used alone (Fig. 1). In combination with PTH, however, EGF induced a synergistic and dose-dependent stimulation of CRE-Luciferase activity, approximately 60–70% greater than that induced by PTH alone (Fig. 1).

Phorbol ester. Phorbol myristate acetate (PMA), an activator of PKC that cannot stimulate cAMP, induced a modest increase in CRE-luciferase activity when used alone, which was occasionally significant. However, when PMA was incubated together with PTH, together they induced a robust and synergistic increase in CRE-luciferase activity (Fig. 2a). Similar synergistic increases were seen when PMA was co-incubated with the cAMP agonists forskolin or rolipram, (Fig. 2b). The PKC-inactive analog of PMA, 4-α-phorbol 12, 13 didecanoate, was inactive alone and in combination with PTH (data not shown). Non-cAMP-activating fragments of PTH, that is, PTH(3–34) and PTH(7–34), that have been reported to activate PKC, were however inactive in the CRE-luciferase assay when used alone and showed antagonistic, not synergistic, properties when incubated with PTH(1–34) (data not shown), presumably because of their known ability to compete with PTH(1–34) at the PTH1 receptor [Murrills et al., 2004].

Thapsigargin. Thapsigargin, a known stimulator of capacitative calcium entry in osteoblasts [Labelle et al., 2007] had modest effects on CRE activity when used alone (Fig. 1d). However, in combination with PTH or the cAMP agonist forskolin, thapsigargin induced a strong, dose-dependent, synergistic response (Fig. 3).

EFFECTS OF INTRACELLULAR SIGNALING PATHWAY INHIBITORS ON THE PTH RESPONSE

PTH is known to be capable of stimulating MAPK and Ca/PKC pathways, in addition to the cAMP pathway. Therefore, we examined the potential role of these additional pathways in the response of the CRE to PTH, to determine whether or not activation of these pathways played an enhancing role in the CRE's response to PTH itself. The results were complex, exhibiting both dose- and time-dependent biphasic effects.

Cyclic AMP pathway inhibitors. H-89, the well-known PKA inhibitor, produced an almost complete inhibition of the PTH response at doses of 10 μM and above (Fig. 4a), confirming the important role of PKA in PTH's activation of the CRE.

The adenylate cyclase (AC) inhibitors SQ22536 (100 μM to 1 mM) and DDA (500 μM) surprisingly induced both inhibitory and stimulatory effects on the PTH response, in an apparently time-dependent manner. Significant stimulatory effects of AC inhibitors were noted in 8/10 overnight cultures, while significant inhibitory effects were noted in 3 of 4 shorter cultures (2.5–4.5 h). This appeared to be due to the time-course of the PTH response, which showed an initial, rapid period of CRE-luciferase induction followed by a leveling off or reduction in luciferase activity. Inhibition of each phase by AC inhibitors would result in an initial reduction, followed by an apparent stimulation of CRE activity (Fig. 4b).

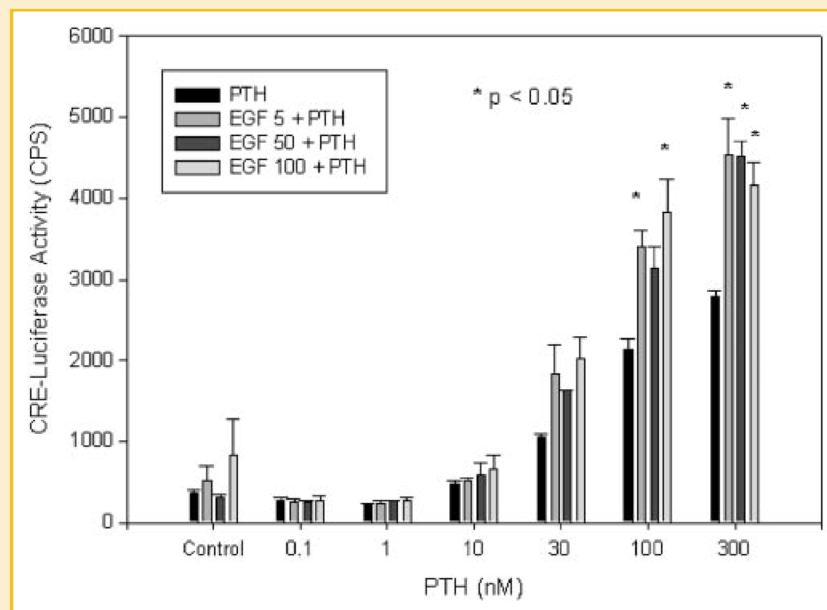

Fig. 1. Effects of EGF on PTH-induced CRE-luciferase activity. EGF (5–100 ng/ml) had little effect alone but synergized with PTH. (*) EGF-treatments significantly different from PTH alone. PTH alone was significantly active versus control at doses of 100–300 nM in this experiment.

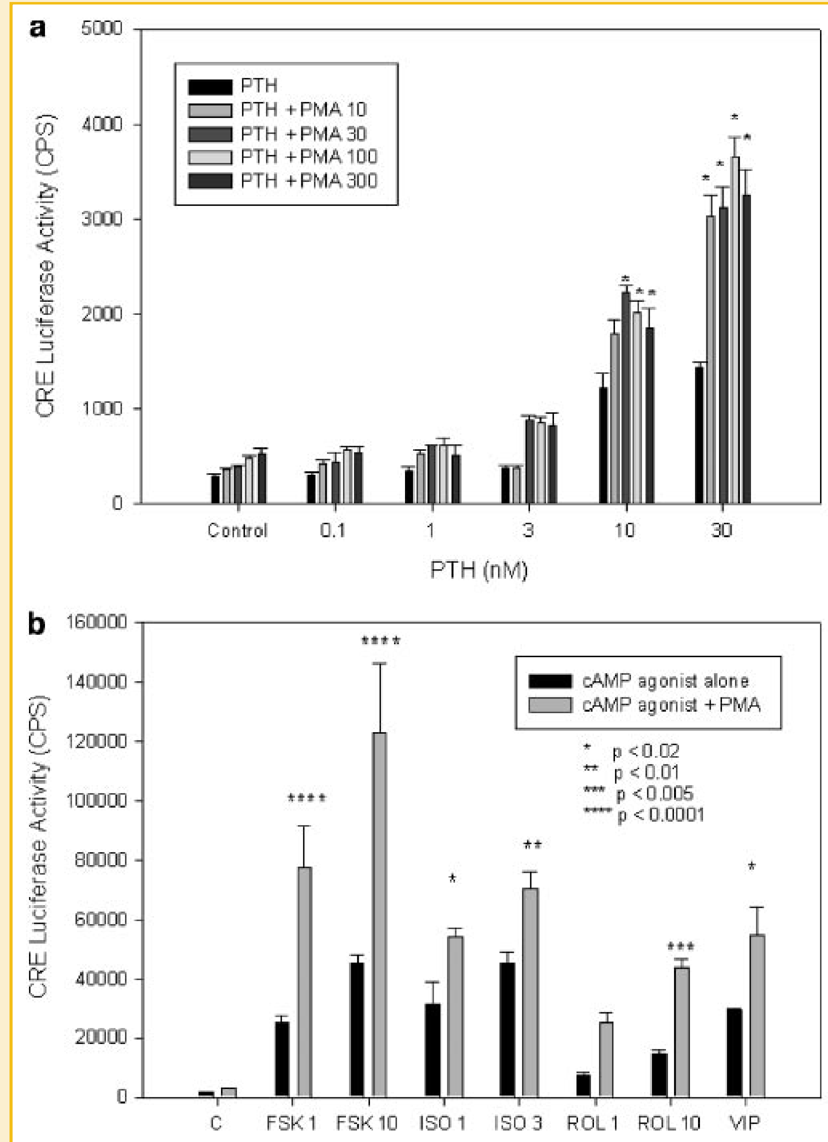

Fig. 2. a: Synergy of PMA with PTH on CRE-luciferase activity. Cells were incubated 2.5 h in the presence of PTH and PMA (10–300 nM) $^{*}P < 0.05$ significantly different from corresponding PTH value. PTH itself was significantly different from control at doses of 10 nM and 30 nM. b: Synergy of PMA (100 nM) with cAMP agonists forskolin (1 and 10 μM), isoproterenol (1 and 3 μM), rolipram (1 and 10 μM) and vasoactive intestinal peptide (1 nM). Asterisks indicate significant differences from agonist alone.

MEK inhibitor. The MEK inhibitor, PD98059, inhibited both basal and PTH-induced CRE-Luciferase activity at doses of 10–100 μM (Fig. 5). We did not observe any time-dependence for the inhibition by PD98059, inhibition being observed in both short and overnight cultures.

Protein kinase C inhibitor. The protein kinase C inhibitor, bisindolylmaleimide (Bis), had variable effects on PTH-induced CRE activity. Although significant inhibition occurred in two of five experiments (data not shown), the average inhibition of 5 μM Bis on 10 nM PTH was only 20.2%.

Calmodulin kinase inhibitor. The calmodulin kinase inhibitor KN-93 had no significant effects on PTH-induced CRE-luciferase activity (data not shown).

DISCUSSION

Our results show that activators of non-cAMP pathways (namely the PKC, calcium and EGF-stimulated pathways) synergize with PTH in inducing CRE-mediated transcription, despite having little or no effect on the CRE when used alone. This observation has implications not only for the modulation of the PTH response by endocrine or local factors, but also for the role of the calcium, PKC and MAPK signaling in the transduction of the PTH response itself. Recent research on intracellular signaling pathways has revealed that what were previously viewed as separate, compartmentalized pathways, in fact are closely tied to one another through cross-talk or convergence onto common elements [Franceschi et al., 2003].

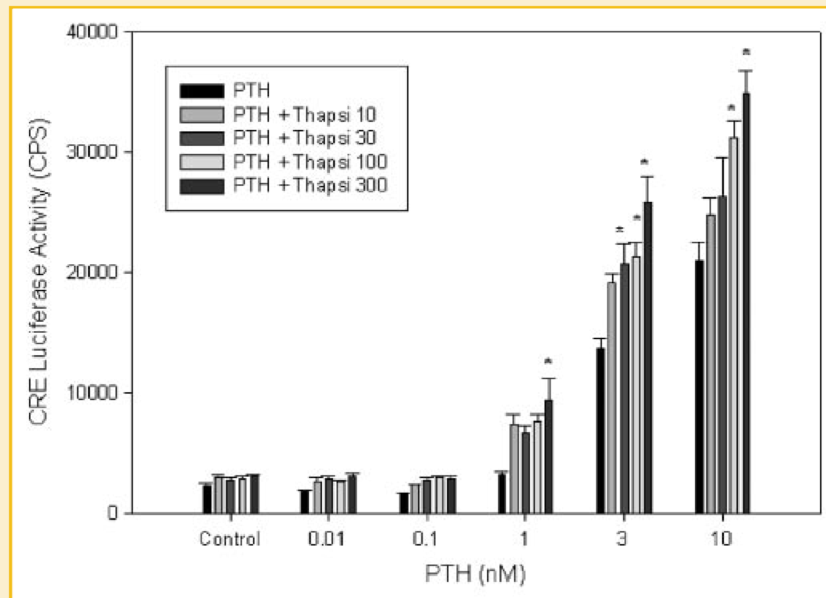

Fig. 3. Synergy between thapsigargin (10–300 nM) and PTH (0.01–10 nM). *Significantly different from PTH alone, $P < 0.05$.

Synergy between PMA and PKC pathways has been noted previously in the transcriptional response to osteoblast genes regulated by PTH, albeit in few studies. A synergistic effect on osteocalcin promoter activity and protein levels was noted when PTH-treated cells were also treated with phorbol ester [Boguslawski et al., 2000], and synergy between PMA and forskolin was noted in regulating osteocalcin mRNA and alkaline phosphatase activity in neonatal rat calvarial osteoblasts [Ishizuya et al., 1997].

Current understanding of the biology of CREB, the intermediary between activated PKA and the CRE, is consistent with our observations of synergy. Whereas cAMP signals that result in phosphorylation of CREB at Ser133 are sufficient to trigger CRE-dependent transcription, mitogen and stress signals resulting from growth factor or phorbol ester stimulation, which induce phosphorylation of CREB at the same site (Ser 133) and to a similar degree, alone have very little effect on CRE-dependent transcription [Brindle et al., 1995]. The mechanism for such signal discrimination appears to be related to the cAMP-specific dephosphorylation and nuclear migration of latent cytoplasmic co-activators called transducers of regulated CREB (TORCs), particularly TORC2, which associate with CBP and increase the expression of cAMP-responsive genes [Ravnskjaer et al., 2007]. Similarly, our experience with TPA, EGF, and thapsigargin, when administered alone, revealed little consistent effect on CRE-mediated transcription, despite the well known ability of these pathways to phosphorylate CREB. In our assay, this scenario would predict that in the presence of a cAMP agonist to recruit TORC2 to the nucleus, a synergistic effect on CRE-mediated transcription might result provided that phosphorylated CREB was rate-limiting. Indeed, we did observe that non-cyclic AMP signaling pathways, that are known to phosphorylate CREB, had mostly minimal effects on CRE-mediated transcription by themselves but had a synergistic effect on CRE-mediated transcription in the presence of PTH or other cAMP agonists.

Our studies addressed the question as to whether or not PTH uses non-cAMP signaling pathways in its activation of CRE-mediated transcription. Despite evidence that PTH can activate the PKC, calcium and MAPK pathways [Reid et al., 1987; Abou-Samra et al., 1989; Ljunggren et al., 1992; Whitfield et al., 2001; Swarthout et al., 2002; Murrills et al., 2004; Yang et al., 2006] there is no consensus on the roles and relative importance of the non-cAMP pathways. The cAMP pathway appears to be the primary pathway: there are numerous reports of PTH-induced gene expression being inhibited by the PKA inhibitor H-89 but fewer reporting an effect of the PKC or MAPK pathway inhibitors, while several report no effect of PKC inhibitors (see Murrills, 2006, for review). In fact, cAMP signaling appears to be required for all of the genes examined to date [Murrills, 2006]. Several genes have been reported to be solely dependent upon cAMP signaling, with no apparent contribution from the PKC pathway, for example BAALC [Robinson et al., 2006], connexin 43 [Schiller et al., 1997], IL-18 [Raggatt et al., 2008], and TGFβ-2 [Wu and Kumar, 2000]. However, several genes have also been shown to be at least partially dependent upon PKC signaling, for example β-catenin [Tobimatsu et al., 2006], IGFBP-5 [Erclik and Mitchell, 2002], Smad3 [Sowa et al., 2003], and TGFβ-1 [Wu and Kumar, 2000]. For several commonly investigated genes, such as alkaline phosphatase, IL-6, osteocalcin and Runx2, the data is mixed, some reports favoring no role for PKC while others detect an influence of PKC signaling. Together, these data suggest a supporting role for PKC signaling for at least some PTH-regulated genes, the extent of which may depend upon cell context. While it is not appropriate to extrapolate from an effect on a CRE to an effect on a gene, which may have other response elements in its promoter, the available

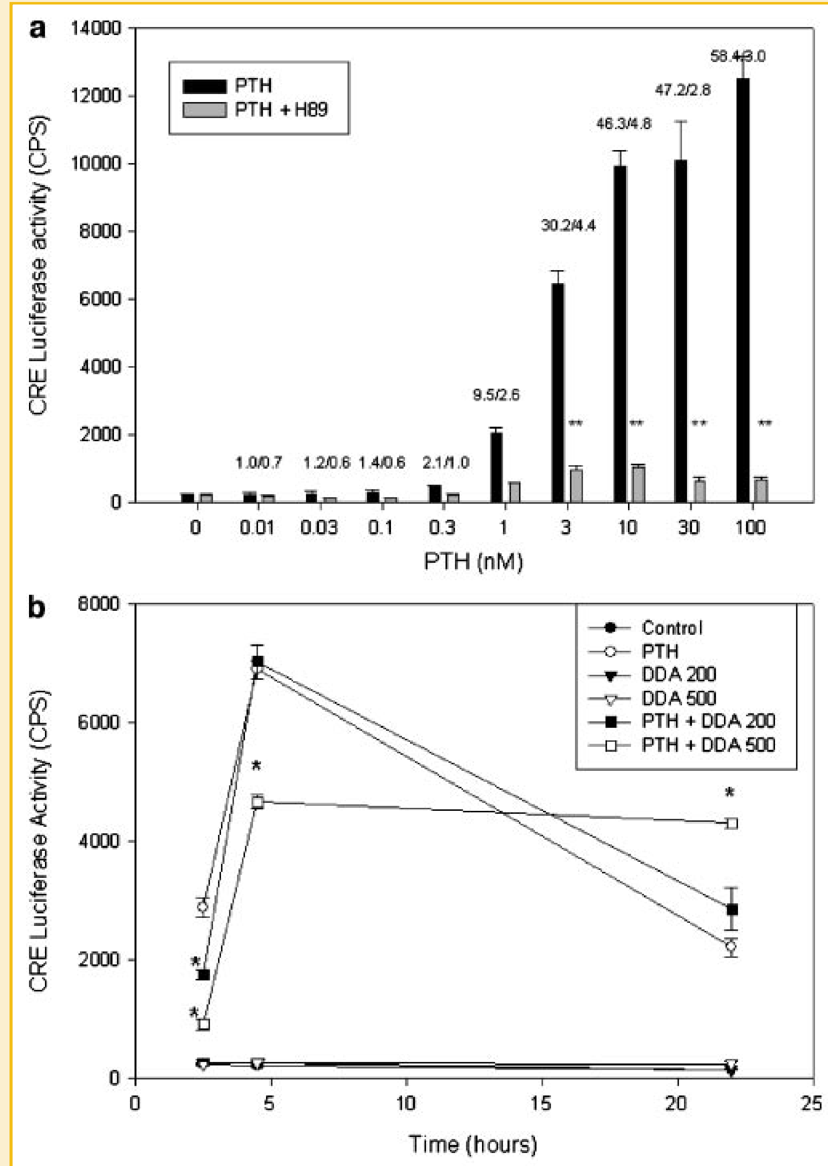

Fig. 4. a: Effect of the PKA inhibitor H-89, 10 μM, on the PTH response of Saos-H3 cells. Values above bars indicate fold increase over control for PTH alone/PTH + H89. b: Time-course of the effect of the adenylate cyclase inhibitor DDA (200 and 500 μM) on the response to 30 nM PTH. *Significantly different from PTH alone $P < 0.001$.

literature on the role of PKA and PKC signaling pathways in PTH-induced gene regulation appears to be consistent with the effects of PTH and PKC agonists on CRE-mediated transcription described in the present study.

In keeping with the published gene expression data, we found a robust inhibition of PTH-induced CRE activity using the PKA inhibitor H-89. The effect of inhibiting adenylyl cyclase using SQ22536 or DDA was profound but biphasic, which presumably reflects a major role for cAMP in both activating and down-regulating phases of the CRE response to PTH. Results using the MAPK inhibitor PD98059 suggest an important, if partial, role for the MAPK pathway in the PTH response but interpretation of these studies was complicated by an inhibitory effect of PD98059 on basal CRE-luciferase activity. Our studies using bisindolylmaleimide as a selective inhibitor of PKC suggested a possible partial role for PTH-induced activation of the PKC pathway in the CRE transcriptional response, but the effect of bisindolylmaleimide was variable. This could conceivably reflect variable coupling of the PTH receptor to PKC-activating G-proteins such as Gαq and Gα12/Gα13 in this system. Despite thapsigargin's profound synergy with PTH, we were unable to demonstrate any effect of the calmodulin kinase (CaMKII) inhibitor on the PTH response. It should be noted that it is advisable to exercise caution when interpreting the results of experiments using currently available "selective" signaling inhibitors, particularly kinase inhibitors, as most kinase inhibitors will also target other kinases [Davies et al., 2000; Brehmer et al., 2004] and often

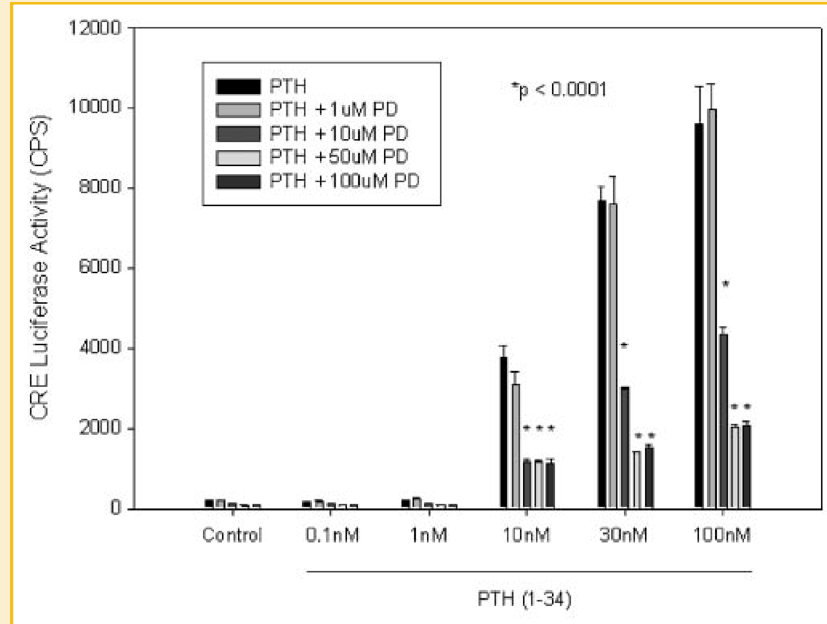

Fig. 5. PD98059 (10–100 μM) inhibited PTH-induced CRE-Luciferase activity. PD98059 (10–100 μM) also inhibited basal CRE-luciferase activity, this effect being statistically significant only after appropriate log transformation of the data.

other proteins, albeit at higher doses. Furthermore, it is difficult to be certain, from biochemical enzyme IC50 studies, what extracellular concentration of an inhibitor will be required to effectively inhibit its intracellular target and whether or not the selectivity observed in a biochemical assay will be maintained in a cellular environment in which concentrations of substrate and enzyme may be very different. For example, bisindolylmaleimide, originally described as a PKC-selective staurosporine derivative [Toullec et al., 1991], has been shown through further enzyme assays [Davies et al., 2000] and also proteomics studies [Brehmer et al., 2004], to bind to and inhibit other kinases such as GSK3-β, Rsk2, and others.

In summary, we have observed that multiple signaling pathways converge synergistically on the cyclic AMP response element and that this may play an important role in determining the magnitude of the transcriptional response to PTH. This observation is likely to be of importance in elucidating the roles of non-cyclic AMP pathways in the response to PTH itself in the cell, and also in the various responses to PTH that can be observed in different bone envelopes or in different patients treated with PTH.

REFERENCES

Abou-Samra AB, Jueppner H, Westerberg D, Potts JT, Jr., Segre GV. 1989. Parathyroid hormone causes translocation of protein kinase-C from cytosol to membranes in rat osteosarcoma cells. Endocrinology 124:1107–1113.

Ahmed I, Gesty-Palmer D, Drezner MK, Luttrell LM. 2003. Transactivation of the epidermal growth factor receptor mediates parathyroid hormone and prostaglandin F2 alpha-stimulated mitogen-activated protein kinase activation in cultured transgenic murine osteoblasts. Mol Endocrinol 17:1607–1621.

Bacic D, Schulz N, Biber J, Kaissling B, Murer H, Wagner CA. 2003. Involvement of the MAPK-kinase pathway in the PTH-mediated regulation of the proximal tubule type IIa Na+/Pi cotransporter in mouse kidney. Pflugers Arch 446:52–60.

Boguslawski G, Hale LV, Yu XP, Miles RR, Onyia JE, Santerre RF, Chandrasekhar S. 2000. Activation of osteocalcin transcription involves interaction of protein kinase A- and protein kinase C-dependent pathways. J Biol Chem 275:999–1006.

Bos MP, Most W, van Leeuwen JP, Herrmann-Erlee MP. 1992. Role of protein kinase C (PKC) in bone resorption: Effect of the specific PKC inhibitor 1-alkyl-2-methylglycerol. Biochem Biophys Res Commun 184:1317–1323.

Brehmer D, Godl K, Zech B, Wissing J, Daub H. 2004. Proteome-wide identification of cellular targets affected by bisindolylmaleimide-type protein kinase C inhibitors. Mol Cell Proteomics 3:490–500.

Brindle P, Nakajima T, Montminy M. 1995. Multiple protein kinase A-regulated events are required for transcriptional induction by cAMP. Proc Natl Acad Sci USA 92:10521–10525.

Chorev M. 2002. Parathyroid hormone 1 receptor: Insights into structure and function. Receptor Channel 8:219–242.

Davies SP, Reddy H, Caivano M, Cohen P. 2000. Specificity and mechanism of action of some commonly used protein kinase inhibitors. Biochem J 351:95–105.

Erclik MS, Mitchell J. 2002. The role of protein kinase C-delta in PTH stimulation of IGF-binding protein-5 mRNA in UMR-106-0 cells. Am J Physiol Endocrinol Metab 282:E534–E541.

Franceschi RT, Xiao G, Jiang D, Gopalakrishnan R, Yang S, Reith E. 2003. Multiple signaling pathways converge on the Cbfa1/Runx2 transcription factor to regulate osteoblast differentiation. Connect Tissue Res 44 (Suppl 1): 109–116.

Fujita T, Meguro T, Fukuyama R, Nakamuta H, Koida M. 2002. New signaling pathway for parathyroid hormone and cyclic AMP action on extracellular-regulated kinase and cell proliferation in bone cells. Checkpoint of modulation by cyclic AMP. J Biol Chem 277:22191–22200.

Gentili C, Morelli S, Boland R, de Boland AR. 2001. Parathyroid hormone activation of map kinase in rat duodenal cells is mediated by 3′,5′-cyclic AMP and Ca(2+). Biochim Biophys Acta 1540:201–212.

Gentili C, Morelli S, Russo De Boland A. 2002. Involvement of PI3-kinase and its association with c-Src in PTH-stimulated rat enterocytes. J Cell Biochem 86:773–783.

Hilliker S, Wergedal JE, Gruber HE, Bettica P, Baylink DJ. 1996. Truncation of the amino terminus of PTH alters its anabolic activity on bone in vivo. Bone 19:469–477.

Homme M, Schmitt CP, Mehls O, Schaefer F. 2004. Mechanisms of mitogen-activated protein kinase inhibition by parathyroid hormone in osteoblast-like cells. J Am Soc Nephrol 15:2844–2850.

Hsiao EC, Boudignon BM, Chang WC, Bencsik M, Peng J, Nguyen TD, Manalac C, Halloran BP, Conklin BR, Nissenson RA. 2008. Osteoblast expression of an engineered Gs-coupled receptor dramatically increases bone mass. Proc Natl Acad Sci USA 105:1209–1214.

Ishizuya T, Yokose S, Hori M, Noda T, Suda T, Yoshiki S, Yamaguchi A. 1997. Parathyroid hormone exerts disparate effects on osteoblast differentiation depending on exposure time in rat osteoblastic cells. J Clin Invest 99:2961–2970.

Izbicka E, Niewolna M, Yoneda T, Lowe C, Boyce B, Mundy G. 1994. pp60 c-src expression and activity in MG-63 osteoblastic cells modulated by PTH but not required for PTH-mediated adenylate cyclase response. J Bone Miner Res 9:127–132.

Kawane T, Mimura J, Yanagawa T, Fujii-Kuriyama Y, Horiuchi N. 2003. Parathyroid hormone (PTH) down-regulates PTH/PTH-related protein receptor gene expression in UMR-106 osteoblast-like cells via a 3′,5′-cyclic adenosine monophosphate-dependent, protein kinase A-independent pathway. J Endocrinol 178:247–256.

Kim TY, Vargas V, Mayer H, Somjen D, Kaye AM. 2002. Selective anabolic effects of muteins of mid-region PTH fragments on skeletal tissues of prepubertal rats. Bone 30:78–84.

Kinoshita T, Kobayashi S, Ebara S, Yoshimura Y, Horiuchi H, Tsutsumimoto T, Wakabayashi S, Takaoka K. 2000. Phosphodiesterase inhibitors, pentoxifylline and rolipram, increase bone mass mainly by promoting bone formation in normal mice. Bone 27:811–817.

Labelle D, Jumarie C, Moreau R. 2007. Capacitative calcium entry and proliferation of human osteoblast-like MG-63 cells. Cell Prolif 40:866–884.

Ljunggren O, Johansson H, Lerner UH, Lindh E, Ljunghall S. 1992. Effects of parathyroid hormone on cyclic AMP-formation and cytoplasmic free Ca2+ in the osteosarcoma cell line UMR 106-01. Biosci Rep 12:207–214.

Mauro LJ, Olmsted EA, Davis AR, Dixon JE. 1996. Parathyroid hormone regulates the expression of the receptor protein tyrosine phosphatase, OST-PTP, in rat osteoblast-like cells. Endocrinology 137:925–933.

Miao D, Tong XK, Chan GK, Panda D, McPherson PS, Goltzman D. 2001. Parathyroid hormone-related peptide stimulates osteogenic cell proliferation through protein kinase C activation of the Ras/mitogen-activated protein kinase signaling pathway. J Biol Chem 276:32204–32213.

Mohan S, Kutilek S, Zhang C, Shen HG, Kodama Y, Srivastava AK, Wergedal JE, Beamer WG, Baylink DJ. 2000. Comparison of bone formation responses to parathyroid hormone(1–34), (1–31), and (2–34) in mice. Bone 27:471–478.

Murrills RJ. 2006. Parathyroid hormone and bone cells. Clin Rev Bone Miner Metab 4:233–258.

Murrills RJ, Matteo JJ, Samuel RL, Andrews JL, Bhat BM, Coleburn VE, Kharode YP, Bex FJ. 2004. In vitro and in vivo activities of C-terminally truncated PTH peptides reveal a disconnect between cAMP signaling and functional activity. Bone 35:1263–1272.

Nagy Z, Radeff J, Stern PH. 2001. Stimulation of interleukin-6 promoter by parathyroid hormone, tumor necrosis factor alpha, and interleukin-1beta in UMR-106 osteoblastic cells is inhibited by protein kinase C antagonists. J Bone Miner Res 16:1220–1227.

Ogata N, Kawaguchi H, Chung UI, Roth SI, Segre GV. 2007. Continuous activation of G alpha q in osteoblasts results in osteopenia through impaired osteoblast differentiation. J Biol Chem 282:35757–35764.

Ozkurt IC, Pirih FQ, Tetradis S. 2004. Parathyroid hormone induces E4bp4 messenger ribonucleic acid expression primarily through cyclic adenosine 3′,5′-monophosphate signaling in osteoblasts. Endocrinology 145:3696–3703.

Pirih FQ, Nervina JM, Pham L, Aghaloo T, Tetradis S. 2003. Parathyroid hormone induces the nuclear orphan receptor NOR-1 in osteoblasts. Biochem Biophys Res Commun 306:144–150.

Radeff JM, Singh AT, Stern PH. 2004. Role of protein kinase A, phospholipase C and phospholipase D in parathyroid hormone receptor regulation of protein kinase Calpha and interleukin-6 in UMR-106 osteoblastic cells. Cell Signal 16:105–114.

Raggatt LJ, Qin L, Tamasi J, Jefcoat SC, Jr., Shimizu E, Selvamurugan N, Liew FY, Bevelock L, Feyen JH, Partridge NC. 2008. Interleukin-18 is regulated by parathyroid hormone and is required for its bone anabolic actions. J Biol Chem 283:6790–6798.

Ravnskjaer K, Kester H, Liu Y, Zhang X, Lee D, Yates JR III, Montminy M III. 2007. Cooperative interactions between CBP and TORC2 confer selectivity to CREB target gene expression. EMBO J 26:2880–2889.

Reid IR, Civitelli R, Halstead LR, Avioli LV, Hruska KA. 1987. Parathyroid hormone acutely elevates intracellular calcium in osteoblastlike cells. Am J Physiol 253:E45–E51.

Rihani-Bisharat S, Maor G, Lewinson D. 1998. In vivo anabolic effects of parathyroid hormone (PTH) 28-48 and N-terminal fragments of PTH and PTH-related protein on neonatal mouse bones. Endocrinology 139:974–981.

Robinson JA, Susulic V, Liu YB, Taylor C, Hardenburg J, Gironda V, Zhao W, Kharode Y, McLarney S, Bai Y, Malone DP, Murrills R, Bex F. 2006. Identification of a PTH regulated gene selectively induced in vivo during PTH-mediated bone formation. J Cell Biochem 98:1203–1220.

Schiller PC, Roos BA, Howard GA. 1997. Parathyroid hormone up-regulation of connexin 43 gene expression in osteoblasts depends on cell phenotype. J Bone Miner Res 12:2005–2013.

Shaywitz AJ, Greenberg ME. 1999. CREB: A stimulus-induced transcription factor activated by a diverse array of extracellular signals. Annu Rev Biochem 68:821–861.

Singh AT, Gilchrist A, Voyno-Yasenetskaya T, Radeff-Huang JM, Stern PH. 2005. G{alpha}12/G{alpha}13 subunits of heterotrimeric G proteins mediate parathyroid hormone activation of phospholipase D in UMR-106 osteoblastic cells. Endocrinology 146:2171–2175.

Somjen D, Schluter KD, Wingender E, Mayer H, Kaye AM. 1991. Stimulation of cell proliferation in skeletal tissues of the rat by defined parathyroid hormone fragments. Biochem J 277(Pt 3): 863–868.

Sowa H, Kaji H, Iu MF, Tsukamoto T, Sugimoto T, Chihara K. 2003. Parathyroid hormone-Smad3 axis exerts anti-apoptotic action and augments anabolic action of transforming growth factor beta in osteoblasts. J Biol Chem 278:52240–52252.

Swarthout JT, Doggett TA, Lemker JL, Partridge NC. 2001. Stimulation of extracellular signal-regulated kinases and proliferation in rat osteoblastic cells by parathyroid hormone is protein kinase C-dependent. J Biol Chem 276:7586–7592.

Swarthout JT, D'Alonzo RC, Selvamurugan N, Partridge NC. 2002. Parathyroid hormone-dependent signaling pathways regulating genes in bone cells. Gene 282:1–17.

Syme CA, Friedman PA, Bisello A. 2005. Parathyroid hormone receptor trafficking contributes to the activation of extracellular signal-regulated kinases but is not required for regulation of cAMP signaling. J Biol Chem 280:11281–11288.

Tobimatsu T, Kaji H, Sowa H, Naito J, Canaff L, Hendy GN, Sugimoto T, Chihara K. 2006. Parathyroid hormone increases beta-catenin levels through Smad3 in mouse osteoblastic cells. Endocrinology 147:2583–2590.

Toullec D, Pianetti P, Coste H, Bellevergue P, Grand-Perret T, Ajakane M, Baudet V, Boissin P, Boursier E, Loriolle F, Duhamel L, Charon D,

Kirilovsky J. 1991. The bisindolylmaleimide GF 109203X is a potent and selective inhibitor of protein kinase C. J Biol Chem 266:15771–15781.

Verheijen MH, Defize LH. 1995. Parathyroid hormone inhibits mitogen-activated protein kinase activation in osteosarcoma cells via a protein kinase A-dependent pathway. Endocrinology 136:3331–3337.

Verheijen MH, Defize LH. 1997. Parathyroid hormone activates mitogen-activated protein kinase via a cAMP-mediated pathway independent of Ras. J Biol Chem 272:3423–3429.

Whitfield JF, Isaacs RJ, Chakravarthy B, Maclean S, Morley P, Willick G, Divieti P, Bringhurst FR. 2001. 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. J Bone Miner Res 16:441–447.

Wu Y, Kumar R. 2000. Parathyroid hormone regulates transforming growth factor beta1 and beta2 synthesis in osteoblasts via divergent signaling pathways. J Bone Miner Res 15:879–884.

Yang D, Guo J, Divieti P, Bringhurst FR. 2006. Parathyroid hormone activates PKC-delta and regulates osteoblastic differentiation via a PLC-independent pathway. Bone 38:485–496.