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PARATHYROID HORMONE AND 1,25-DIHYDROXYVITAMIN D SYNERGISTICALLY INDUCE THE 1,25-DIHYDROXYVITAMIN D-24-HYDROXYLASE IN RAT UMR-106 OSTEOBLAST-LIKE CELLS

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We have studied the effect of parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D$) on the expression of the 1,25-dihydroxyvitamin D-24-hydroxylase (24-hydroxylase) in rat UMR-106 osteoblastic cells. $1,25(OH)_2D$ (10^{-7}M) alone produced a gradual increase in mRNA levels of the 24-hydroxylase cytochrome P450 component (P450cc24) during 12 hours of incubation. Rat PTH 1-34 (250 nM) in the presence of $1,25(OH)_2D$ further increased P450cc24 mRNA levels 7-10 fold after 3 hours. PTH alone had no significant effect. The action of PTH was mimicked by forskolin but not by phorbol ester. The 24-hydroxylase enzymatic activity in UMR cells was increased over 5-fold by PTH and $1,25(OH)_2D$ together compared to $1,25(OH)_2D$ alone after 6 hours. This synergistic regulation of the 24-hydroxylase in bone cells is in contrast to the regulation in the kidney, where PTH and $1,25(OH)_2D$ have opposite effects on 24-hydroxylase expression.

Parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D$), the hormonal form of vitamin D, are the major hormonal regulators of vitamin D metabolism in intestine, bone, and kidney. An important enzyme in vitamin D metabolism is the $1,25(OH)_2D$-24-hydroxylase (24-hydroxylase), which inactivates $1,25(OH)_2D$ in its target tissues (1). In renal and intestinal cells, $1,25(OH)_2D$ stimulates 24-hydroxylase activity (2,3) and markedly increases mRNA levels of the cytochrome P450 component of the 24-hydroxylase (P450cc24)

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Abbreviations: 24-hydroxylase, 1,25-dihydroxyvitamin D-24-hydroxylase; P450cc24, 24-hydroxylase cytochrome P450; TPA, phorbol 12-myristate 13-acetate; PKA, protein kinase A; PKC, protein kinase C.

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(4,5). On the other hand, PTH has the opposite effect of $1,25(OH)_2D$ in the kidney. PTH has been shown to decrease the production of $24,25(OH)_2D$ in both renal slices (6) and in primary renal cell cultures (7). PTH also inhibits the action of $1,25(OH)_2D$ in the kidney but not in the intestine with regard to P450cc24 mRNA expression (8).

Compared to renal and intestinal cells, less is known about the regulation of the 24-hydroxylase in bone cells. Therefore, we have studied the expression of P450cc24 mRNA and 24-hydroxylase activity in UMR-106 cells. These osteoblast-like cells are derived from the rat and contain functional PTH and $1,25(OH)_2D$ receptors. We found that $1,25(OH)_2D$ and PTH synergistically increased both 24-hydroxylase mRNA levels and 24-hydroxylase activity in UMR cells. This is in marked contrast to the opposing actions of $1,25(OH)_2D$ and PTH in the kidney.

METHODS

The UMR-106 cell line was obtained from the American Type Culture Collection. This cell line is a cloned derivative of a transplantable rat osteogenic sarcoma (9). Cells were cultured in T25 flasks with Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. Cells reached confluency in 3-5 days and were used within 30 passages of receipt.

To study the effects of PTH and $1,25(OH)_2D$, confluent cells were deprived of serum for 24 hours. PTH was rat PTH (1-34) (Bachem, Torrance, CA), and $1,25(OH)_2D$ was a kind gift of Dr. Milan Uskokovic (Hofmann-LaRoche, Nutley, NJ). At the end of the experiment, cells were washed and stored frozen until isolation of RNA using RNAzol (Tel-Test, Inc., Friendswood, TX).

The P450cc24 mRNA levels were measured by dot blot using a cDNA probe for rat P450cc24 as previously described (4,5). The full length clone for rat P450cc24 (p108, 3.2 Kb) was kindly supplied by Drs. Y. Ohyama and K. Okuda (Hiroshima University School of Dentistry, Hiroshima, Japan) (10). Radiolabeled probe was prepared by random priming using a DECAprime labeling kit, and hybridization to the filter was performed at 42 degrees C overnight. Filters were then washed extensively and exposed to x-ray film. The dots on the x-ray film were quantitated by densitometry, using a linear region of the dilution curve. Dot blots were routinely stripped and re-hybridized with a probe for beta-actin (Oncor, Inc., Gaithersburg, MD). This checked for uniformity of sample loading and the specificity of the $1,25(OH)_2D$ response. Based on actin rehybridization, sample loading was quite uniform. Therefore, data were routinely normalized to the amount of total RNA applied, which was determined spectrophotometrically.

24-Hydroxylase activity was measured using radiolabeled 25(OH)D as a substrate, as described for Caco-2 cells (3). Briefly, cells were cultured to confluency in T75 flasks, washed twice with serum-free medium, and then pre-incubated for one hour in serum-free medium. Cells were then incubated for one hour with 50 nM radiolabeled $25(OH)D_3$ (25-hydroxy(26(27)-methyl-^{3}H)cholecalciferol, Amersham Corp., Arlington Heights, IL). The tritiated vitamin D products were identified by HPLC, using a Zorbax-Sil column (0.5 x 25 cm) and a solvent system of hexane:methylene chloride:methanol (77:17.25:5.75). The only radiolabeled metabolite consistently detected under these conditions comigrated with $24,25(OH)_2D_3$. This metabolite was at least 90% $24,25(OH)_2D$ as determined by rechromatography using other solvent systems (11) and was reduced more than 80% by periodate treatment. Thus, the $24,25(OH)_2D$ product was routinely taken to be the radioactivity comigrating with $24,25(OH)_2D_3$.

Data are reported as the mean $\pm$ SE of the indicated number of flasks. Two-tailed Student's t-test was used to determine statistical significance, and $P < 0.05$ was considered significant.

RESULTS

The effects of $1,25(OH)_2D$ and PTH on P450cc24 mRNA levels were examined as a function of time (Fig. 1). Flasks of cells were treated with $1,25(OH)_2D$ or $1,25(OH)_2D$ plus PTH for up to 12 hours, and P450cc24 mRNA levels were determined by dot blot (Fig. 1-left). P450cc24 mRNA levels were almost undetectable in untreated cells (0 hour). Treatment with $1,25(OH)_2D$ resulted in a linear increase in P450cc24 mRNA levels with time (Fig. 1-right). Treatment with PTH in the presence of $1,25(OH)_2D$ resulted in a marked increase in P450cc24 mRNA levels compared to $1,25(OH)_2D$ alone. The action of PTH was rapid with a significant effect at 1 hour and a maximal level at 3 hours. At 3 hours, P450cc24 mRNA levels were over 20-fold higher in cells treated with PTH in the presence of $1,25(OH)_2D$ compared to cells treated with $1,25(OH)_2D$ alone. After 12 hours, P450cc24 mRNA levels were still much higher in the PTH-treated cells compared with those treated with $1,25(OH)_2D$ alone. Since the effect of $1,25(OH)_2D$ alone was maximal at 12 hours (data not shown), this demonstrated that PTH increased the maximal response to $1,25(OH)_2D$ as well as the rapidity of the response. Finally, these effects of $1,25(OH)_2D$ and PTH were specific in that the hormones had no effect on beta-actin mRNA levels in the same studies (data not shown).

To determine if PTH increased P450cc24 mRNA levels in a dose-dependent manner, the effect of PTH concentration was studied. Cells were incubated for 3 hours with varying concentrations of PTH in the presence or absence of $1,25(OH)_2D$ (Fig. 2). In the presence of $1,25(OH)_2D$, PTH increased P450cc24 mRNA levels in a sigmoidal fashion with a maximal effect seen at 250 nM. PTH was effective at concentrations as low as 2.5 nM with an EC_{50} of about 10 nM. In the absence of $1,25(OH)_2D$, PTH alone had no significant effect, indicating the synergistic nature of the interaction of PTH with $1,25(OH)_2D$.

In osteoblasts, PTH may activate both the cAMP-dependent protein kinase A (PKA) and protein kinase C (PKC) pathways (12). Therefore, activators of these pathways were tested

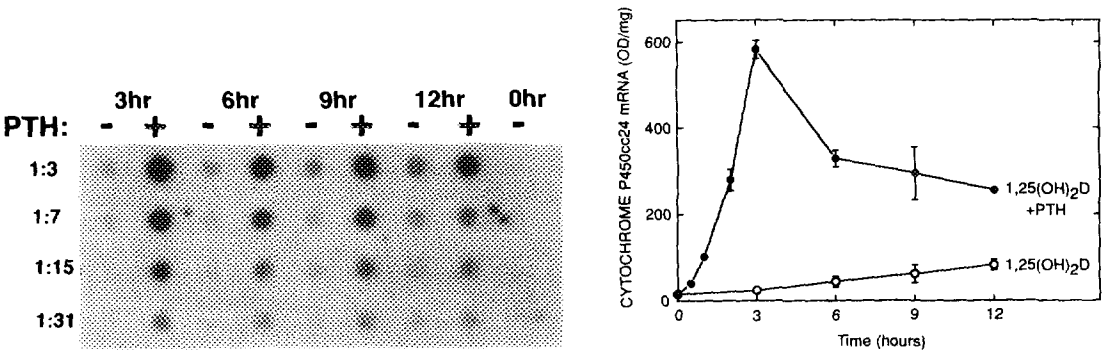

FIGURE 1. Effect of $1,25(OH)_2D$ and PTH on Cytochrome P450cc24 mRNA Levels. Cells were treated with $1,25(OH)_2D$ (10^{-7}M) (-) or PTH (250 nM) plus $1,25(OH)_2D$ (+) for the indicated time. Total RNA was dotted serially, probed for P450cc24 mRNA, autoradiographed, and quantitated by densitometry. Autoradiograph of dot blot (left) is of the pooled flasks from each time point. Data points on graph (right) are the mean $\pm$ SE of 3-6 individual flasks.

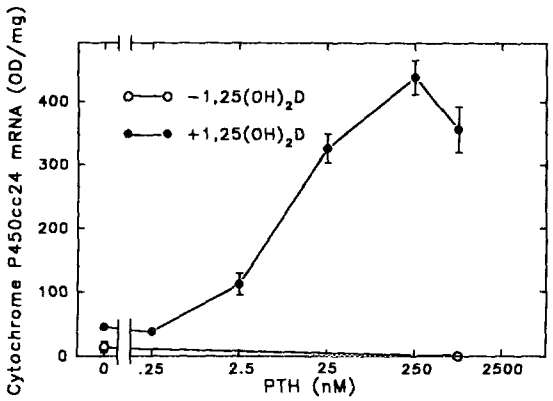

FIGURE 2. Effect of PTH Dose on Cytochrome P450cc24 mRNA Levels. Cells were treated with the indicated concentrations of PTH in the presence or absence of $1,25(OH)_2D$ (10^{-7}M) for 3 hours. P450cc24 mRNA levels were quantitated by dot blot and are the mean ± SE of 3 flasks.

for their capacity to increase P450cc24 mRNA levels (Table 1). Forskolin, an activator of PKA, increased mRNA to the same level seen with PTH. Like PTH, forskolin had no effect in the absence of $1,25(OH)_2D$. The phorbol ester TPA, an activator of PKC, had no effect on P450cc24 mRNA levels in either the presence or absence of $1,25(OH)_2D$ in this cell line. This is in contrast to the action of TPA in renal (4) and intestinal cells (5), where it markedly potentiates the effect of $1,25(OH)_2D$.

Finally, the effect of $1,25(OH)_2D$ and PTH on the 24-hydroxylase activity in UMR cells was determined (Fig. 3). $1,25(OH)_2D$ alone produced a progressive increase in enzyme activity after a 3 hour delay. PTH together with $1,25(OH)_2D$ markedly increased 24-hydroxylase activity at all time points compared with $1,25(OH)_2D$ alone. The effect of PTH was rapid with the activity at 3 hours being 5-fold higher than that with $1,25(OH)_2D$ alone. This effect of PTH was still observed after 12 hours of treatment.

TABLE 1

EFFECT OF PTH, FORSKOLIN, AND PHORBOL ESTER ON CYTOCHROME P450cc24 mRNA LEVELS

AGONIST	CYTOCHROME P450cc24 mRNA LEVELS (OD/mg)	
	- $1,25(OH)_2D$	+ $1,25(OH)_2D$
CONTROL	n.d	31 ± 8
PTH	n.d	247 ± 31
FORSKOLIN	n.d	212 ± 36
TPA	n.d	16 ± 9

Table entries are the mean ± SE of 3-6 flasks. Concentrations were $1,25(OH)_2D$=100 nM, PTH=25 nM, forskolin=100 nM, and TPA=200nM. Cells were incubated for 3 hours. n.d.=not detectable.

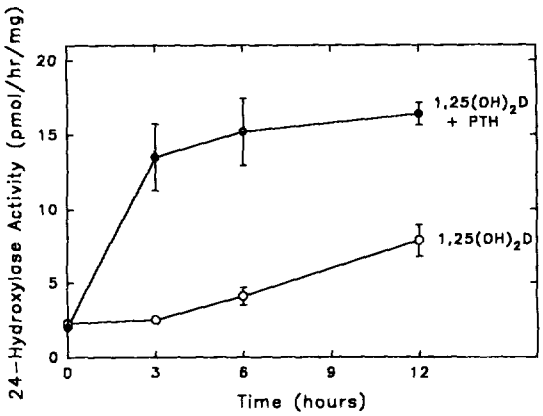

FIGURE 3. Effect of 1,25(OH)$_2$D and PTH on 24-Hydroxylase Activity. Cells were treated with 1,25(OH)$_2$D (10^{-7}M) in the presence or absence of 25 nM PTH for the indicated period of time. 24-Hydroxylase activity was measured by incubating with 50 nM of radiolabeled 25(OH)D for 1 hour and separating out the radiolabeled products by HPLC. Data points are the mean $\pm$ SE of 3 flasks.

DISCUSSION

These studies demonstrate that 1,25(OH)$_2$D and PTH synergistically increase P450cc24 mRNA levels in this osteoblast-like cell line. PTH increases both the rapidity and the maximal effect of the 1,25(OH)$_2$D action. The increase in P450cc24 mRNA levels (Fig. 1) is paralleled by increases in 24-hydroxylase enzymatic activity (Fig. 3), suggesting that this regulation by 1,25(OH)$_2$D and PTH may have physiological importance. The synergistic action in UMR cells is in marked contrast to the action of PTH in the kidney. In the kidney, PTH decreases renal 24,25(OH)$_2$D production (13), and it blocks the capacity of 1,25(OH)$_2$D to increase renal 24-hydroxylase activity and P450cc24 mRNA levels (8). There is also evidence from a recent in vivo study suggesting that the 24-hydroxylase is regulated differently in kidney and bone. Feeding rats a low calcium diet, which increases both serum 1,25(OH)$_2$D and PTH, increases the 24-hydroxylase activity in bone but decreases the activity in the kidney (14).

The physiological role of the 24-hydroxylase in bone is still under investigation. It may be that the bone cell 24-hydroxylase functions to degrade 1,25(OH)$_2$D in this target cell as it does in other target cells. If this is the case, it would be appropriate for 1,25(OH)$_2$D and PTH to upregulate the 24-hydroxylase together. This is because increased serum PTH levels, in general, lead to increased serum 1,25(OH)$_2$D levels in mammals. On the other hand, the function of the 24-hydroxylase in bone may be to produce 24,25(OH)$_2$D from circulating 25(OH)D. It has been suggested that 24,25(OH)$_2$D has a special role in bone metabolism (15).

Interestingly, it has recently been reported that PTH, either alone or in combination with 1,25(OH)$_2$D, has no effect on P450cc24 mRNA levels in the rat osteoblastic cell lines C-26 and C-11 (14). In this study, mRNA levels were measured 24 hours after hormone treatment. However, in our studies, the action of PTH in UMR cells peaked at 3 hours (Fig. 1) and was

not detectable after 24 hours (data not shown). Thus, if PTH had an early effect in the C-26 and C-11 cell lines, it would not be detected at 24 hours. On the other hand, the effect of PTH may simply vary with the osteoblastic cell line, although PTH markedly stimulates cAMP production in the C-11 cells (14).

The mechanism by which PTH potentiates the action of $1,25(OH)_2D$ is still unclear. Since the action of PTH is mimicked by forskolin, these studies suggest that PTH may be acting directly or indirectly through its cAMP-dependent pathway. One possibility is that PTH acts via cAMP to increase transcription of the P450cc24 gene. It has been suggested that the promoter region of this gene has two cAMP-response sites (16), but it has not yet been demonstrated that they are true response elements. Alternatively, PTH may work indirectly to enhance $1,25(OH)_2D$ action. PTH has been reported to increase expression of the vitamin D receptor in UMR-106 cells (17). This cell line should be a useful model in which to study the dual regulation of vitamin D metabolism by PTH and $1,25(OH)_2D$.

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