Effect of parathyroid hormone on phospholipid metabolism in osteoblast-like rat osteogenic sarcoma cells

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Previous results have shown that 1,25-dihydroxycholecalciferol [$1,25(\text{OH})_2\text{D}_3$] enhances the synthesis of phosphatidylserine (PS) and suppresses the synthesis of phosphatidylethanolamine (PE) in osteoblast-like rat osteogenic sarcoma UMR 106 cells [Matsumoto, Kawanobe, Morita & Ogata (1985) *J. Biol. Chem.* **260**, 13704–13709]. In the present study, the effect of parathyroid hormone (PTH) on phospholipid metabolism is examined by using these cells. Treatment of UMR 106 cells with human PTH-(1–34)-peptide suppresses the synthesis of phosphatidylethanolamine in a dose- and time-dependent manner without affecting the synthesis of PS. The maximal effect on PE synthesis is obtained with 2.4 nM-human PTH-(1–34)-peptide when the cells are treated for 48 h or longer. In addition, when human PTH-(1–34)-peptide is added together with the maximal dose of $1,25(\text{OH})_2\text{D}_3$, there is a further decline in PE synthesis, whereas the stimulation of PS synthesis by $1,25(\text{OH})_2\text{D}_3$ is not altered. Because methylation of PE is suggested to affect hormone receptor–adenylate cyclase coupling, the observed change in PE metabolism by PTH and $1,25(\text{OH})_2\text{D}_3$ may be, at least in part, involved in the development of desensitization phenomenon to PTH in these cells.

INTRODUCTION

PTH has been shown to stimulate formation (Tam *et al.*, 1982) and resorption (Raisz, 1976) of bone, and the net consequences of prolonged PTH treatment *in vivo* is an increase in bone turnover (Malluche *et al.*, 1982; Podbesek *et al.*, 1983). However, specific binding of radiolabelled PTH can be demonstrated on osteoblast membranes, but not on osteoclast membranes (Silve *et al.*, 1982). Thus Rodan & Martin (1981) put forth the hypothesis that osteoblasts are the main target cells for bone-resorbing hormones and that osteoblasts control osteoclastic bone resorption. Studies using osteoblast-like cells have revealed that PTH has diverse effects on these cells, such as enhancement of cyclic AMP production (Peck & Klahr, 1979) and cyclic AMP-dependent protein kinase activity (Livesey *et al.*, 1982), induction of a change in cell shape (Miller *et al.*, 1976), stimulation of plasminogen activator (Hamilton *et al.*, 1985), inhibition of collagen synthesis (Rodan & Rodan, 1983), suppression of alkaline phosphatase activity (Majeska & Rodan, 1982) and inhibition of cell proliferation (Partridge *et al.*, 1985). However, the mechanism by which PTH controls formation and resorption of bone through osteoblasts remains unclear.

By using a clonal rat osteogenic sarcoma cell line of osteoblastic phenotype UMR 106 (Livesey *et al.*, 1982; Partridge *et al.*, 1983, 1985; Hamilton *et al.*, 1985), we have demonstrated that $1,25(\text{OH})_2\text{D}_3$ stimulates the synthesis of PS and suppresses the synthesis of PE (Matsumoto *et al.*, 1985a). Because PS is shown to play an important role on bone mineralization process through formation of Ca^{2+} –PS–phosphate complexes (Wuthier, 1982), these findings suggested that the changes in phospholipid metabolism may be at least one of the mechanisms by which $1,25(\text{OH})_2\text{D}_3$ and other calcitropic

hormones regulate the bone mineralization process. The present study was undertaken to examine whether or not PTH also affects phospholipid metabolism in UMR 106 cells. The results indicate that PTH markedly inhibits PE synthesis in a dose- and time-dependent manner, but does not affect PS synthesis in these cells. A possible relationship between the change in PE synthesis and the desensitization phenomenon is discussed.

MATERIALS AND METHODS

Cell culture

UMR 106 cells were a gift from Dr. T. J. Martin, University of Melbourne, Melbourne, Vic., Australia. Cells were cultured in Eagle's minimum essential medium with 5% (w/v) heat-inactivated foetal-bovine serum and kanamycin (60 $\mu\text{g}/\text{ml}$) at 37 °C under CO_2/air (1:19). Experiments were carried out on cells between passage 11 and 24.

Incorporation of labelled substrates

After treatment of the cells with synthetic human PTH-(1–34)-peptide for the indicated period of time, the cells were washed and incubated for 1 h in the medium containing 0.1 μCi of [^3H]ethanolamine and 0.2 μCi of [^{14}C]serine. At the end of incubation, cells were washed twice with phosphate-buffered saline (137 mM- NaCl /2.6 mM- KCl /8.1 mM- Na_2HPO_4 /1.5 mM- KH_2PO_4 , pH 7.4) and scraped into distilled water. Lipids from the cell suspensions were extracted by the method of Bligh & Dyer (1959).

Analysis of labelled phospholipids

As reported previously (Matsumoto *et al.*, 1985a), when the lipid extracts were separated by two-step t.l.c.

Abbreviations used: PTH, parathyroid hormone; $1,25(\text{OH})_2\text{D}_3$, 1,25-dihydroxycholecalciferol, calcitriol, '1,25-hydroxyvitamin D_3 '; PS, phosphatidylserine; PE, phosphatidylethanolamine.

and the radioactivity in each phospholipid fraction was counted, approx. 95% of [^3H]ethanolamine was incorporated into PE fraction and about 60% of [^{14}C]serine into PS fraction. In addition, PTH-(1-34)-peptide treatment did not affect the relative percentages of the incorporation of the substrates into respective phospholipids. Thus, for the experiments reported in the present paper, radioactivity in total lipid extract was directly determined by double-channel counting of ^3H and ^{14}C .

Measurement of cyclic AMP content

The cells were preincubated in serum-free Eagle's medium containing 20 mM-Hepes (pH 7.4)/1 mM-isobutylmethylxanthine for 15 min at 25 °C. After the cells had been incubated with PTH-(1-34)-peptide for 5 min at 25 °C, 50% trichloroacetic acid was added to a final concentration of 5%. The cells and medium were collected, freeze-thawed and centrifuged. The cyclic AMP concentration in the supernatant was measured with a radioimmunoassay kit (Yamasa Shoyu Co., Choshi, Japan) after extraction of trichloroacetic acid with water-saturated diethyl ether (Matsumoto *et al.*, 1985b).

Materials

L-[U- ^{14}C]Serine and [1- ^3H]ethan-1-ol-2-amine hydrochloride were purchased from The Radiochemical Centre (Amersham, Bucks., U.K.). Synthetic human PTH-(1-34)-peptide was kindly donated by Toyo Jozo Co. (Shizuoka, Japan) and 1,25(OH) $_2\text{D}_3$ by Chugai Pharmaceutical Co. (Tokyo, Japan).

RESULTS

When the cells were treated with PTH-(1-34)-peptide, the increase in cell number was inhibited in a dose-dependent manner. The suppressive effect of PTH-(1-34)-peptide on proliferation of UMR 106 cells was maximum with 24 nM-PTH-(1-34)-peptide [$(1.95 \pm 0.12) \times 10^6$ cells/well in control versus $(1.16 \pm 0.08) \times 10^6$ cells/well in PTH-(1-34)-treated group; means $\pm$ S.E.M.]. Therefore all the data were corrected for by the number of cells in the subsequent experiments. Fig. 1 shows the dose response of the effect of PTH-(1-34)-peptide on [^3H]ethanolamine and [^{14}C]serine incorporation into lipid fraction of UMR 106 cells. Treatment of the cells for 48 h with 0.24 nM-PTH-(1-34)-peptide caused a marked suppression of [^3H]ethanolamine incorporation, and the suppressive effect reached a maximum with 2.4 nM-PTH-(1-34)-peptide treatment. In contrast, the incorporation of [^{14}C]serine into lipid fraction of UMR 106 cells was not significantly affected by PTH-(1-34)-peptide. The time course of the effect of PTH-(1-34)-peptide on [^3H]ethanolamine incorporation is illustrated in Fig. 2. Treatment with 24 nM-PTH-(1-34)-peptide for 2 h significantly reduced [^3H]ethanolamine incorporation, and the effect reached maximum after 48 h with the treatment. Because most of the [^3H]ethanolamine in lipid extract was in the PE fraction and the relative percentage of the radioactivity among phospholipid fractions was not affected by the PTH-(1-34)-peptide treatment (results not shown), the observed effect of PTH-(1-34)-peptide is mainly due to an inhibition of the incorporation of [^3H]ethanolamine into PE. In addition, analyses of phospholipid phosphorus content revealed that PTH-

Fig. 1. Dose-response curves for the effect of human PTH-(1-34)-peptide on [^3H]ethanolamine and [^{14}C]serine incorporation into the lipid fraction of UMR 106 cells

After cells were treated with the indicated amount of PTH-(1-34)-peptide for 48 h, they were incubated with 0.1 μCi of [^3H]ethanolamine and 0.2 μCi of [^{14}C]serine for 1 h. Radioactivity in lipid extract of the cells was determined by double-channel counting. Results are means $\pm$ S.E.M. for four experiments.

Fig. 2. Time course of the effect of human PTH-(1-34)-peptide on [^3H]ethanolamine incorporation into lipid fraction of UMR 106 cells

After cells were treated with 24 nM-PTH-(1-34)-peptide for the indicated period of time, [^3H]ethanolamine incorporation into lipid extract was determined as described in the legend to Fig. 1. Results are means $\pm$ S.E.M. for four experiments.

(1-34)-peptide treatment significantly decreased the content of PE as well [0.22 ± 0.01 μg of phosphorus/ 10^6 cells in control versus 0.18 ± 0.01 μg of phosphorus/ 10^6 cells treated with 24 nM-PTH-(1-34)-peptide; means $\pm$ S.E.M., $P < 0.05$]. These results indicate that PTH-(1-34)-peptide suppresses the synthesis of PE without

Fig. 3. Dose-response curve for the effect of human PTH-(1-34)-peptide on cyclic AMP production in UMR 106 cells

The cells were incubated in serum-free Eagle's minimum essential medium containing 20 mM-Hepes, pH 7.4, 1 mM-isobutylmethylxanthine and the indicated amount of PTH-(1-34)-peptide for 5 min at 25 °C. The incubation was terminated by adding trichloroacetic acid at a final concentration of 5%, and the cyclic AMP concentration in the trichloroacetic acid extract was measured by radio-immunoassay. Results are means ± S.E.M. for four experiments.

affecting PS metabolism. As shown in Fig. 3, PTH-(1-34)-peptide stimulated cyclic AMP production in UMR 106 cells, but the dose of PTH-(1-34)-peptide required to stimulate cyclic AMP production was higher compared with that required to inhibit PE synthesis. In order to examine if the effect of PTH-(1-34)-peptide on PE synthesis is mediated by cyclic AMP production, the effect of dibutyryl cyclic AMP on [³H]ethanolamine and [¹⁴C]serine incorporation was examined. As shown in Table 1, dibutyryl cyclic AMP suppressed the incorporation of [³H]ethanolamine dose-dependently without

Table 1. Effect of dibutyryl cyclic AMP on the incorporation of [³H]ethanolamine and [¹⁴C]serine into lipid fraction of UMR 106 cells

Results are means ± S.E.M. for four experiments. Abbreviation used: DBcAMP, dibutyryl cyclic AMP.

Treatment	Incorporation (d.p.m./10 ⁶ cells) of:	
	[³ H]Ethanolamine	[¹⁴ C]Serine
None	15 300 ± 130	1840 ± 75
100 mM-DBcAMP	12 800 ± 390*	2010 ± 91
10 mM-DBcAMP	5 100 ± 240*	2100 ± 55

* Significantly different from the control (*P* < 0.001).

affecting the incorporation of [¹⁴C]serine into lipid fraction of UMR 106 cells. Thus the effect of PTH-(1-34)-peptide on PE synthesis appears to be mediated by the production of cyclic AMP. Finally, interaction of PTH-(1-34)-peptide with 1,25(OH)₂D₃ on phospholipid metabolism of UMR 106 cells was examined. As noted previously (Matsumoto *et al.*, 1985a), treatment with 1,25(OH)₂D₃ caused an increase in [¹⁴C]serine incorporation and a decrease in [³H]ethanolamine incorporation into lipid extract (Table 2) which reflects changes in the synthesis of PS and PE. When PTH-(1-34)-peptide was added together with 1,25(OH)₂D₃, there was a further decline in [³H]ethanolamine incorporation, whereas the effect of 1,25(OH)₂D₃ on [¹⁴C]serine incorporation was not affected (Table 2).

DISCUSSION

The present results using an osteoblast-like cell line, UMR 106, demonstrate that PTH-(1-34)-peptide suppresses PE synthesis without affecting PS metabolism in a dose- and time-dependent manner, that dibutyryl cyclic AMP causes similar changes in phospholipid metabolism, that the suppressive effects of PTH-(1-34)-peptide and 1,25(OH)₂D₃ on PE synthesis is additive, and that the stimulatory effect of 1,25(OH)₂D₃ on PS synthesis is not influenced by PTH-(1-34)-peptide.

Table 2. Effect of 1,25(OH)₂D₃ and human PTH-(1-34)-peptide on the incorporation of [³C]serine into lipid fraction of UMR 106 cells

Results are means ± S.E.M. for four experiments.

Treatment	Incorporation (d.p.m./10 ⁶ cells) of:	
	[³ H]Ethanolamine	[¹⁴ C]Serine
None	12 700 ± 430	880 ± 48
100 nM-1,25(OH) ₂ D ₃	5 200 ± 300*	1400 ± 58†
24 nM-PTH-(1-34)-peptide	3 500 ± 130*	940 ± 61
100 nM-1,25(OH) ₂ D ₃ and 24 nM-PTH-(1-34)-peptide	2 900 ± 88*‡	1470 ± 91†

* Significantly different from the control (*P* < 0.001).
† Significantly different from the control (*P* < 0.01).
‡ Significantly different from 1,25(OH)₂D₃-treated group (*P* < 0.001) and from the PTH-(1-34)-peptide-treated group (*P* < 0.02).

Osteoblast and matrix vesicle membranes are rich in PS (Majeska *et al.*, 1979). PS avidly binds Ca^{2+} , and the binding of Ca^{2+} is facilitated by phosphate to form Ca^{2+} -PS-phosphate complexes (Wuthier, 1982). The Ca^{2+} -PS-phosphate complexes are present in newly mineralizing tissues and are shown to nucleate apatite formation *in vitro* (Boskey & Posner, 1977). Therefore these phospholipids may play an important role in the bone mineralization process. In addition, our previous results demonstrate (Matsumoto *et al.*, 1985a) that $1,25(\text{OH})_2\text{D}_3$ increases PS synthesis while inhibiting the synthesis of PE and other phospholipids. However, in the present study, PTH-(1-34)-peptide did not affect the synthesis or turnover of PS in UMR 106 cells, whereas it markedly inhibited the synthesis of PE. We have demonstrated (Matsumoto *et al.*, 1981; Rasmussen & Matsumoto, 1982) that alterations in phospholipid composition can affect activities or functions of membrane proteins, and it has been shown that PE may be involved in hormone receptor-adenylate cyclase coupling through methylation into phosphatidylcholine (Hirata & Axelrod, 1980). Therefore there is a possibility that the decrease in PE synthesis may lead to a suppression of adenylate cyclase stimulation by PTH. Prolonged exposure of osteoblasts to PTH results in desensitization to the subsequent stimulation by PTH (Wong, 1979). Furthermore, $1,25(\text{OH})_2\text{D}_3$ and dibutyryl cyclic AMP have been shown to cause heterologous desensitization to PTH in stimulating cyclic AMP production of osteoblastic cells (Peck & Kohler, 1980; Catherwood, 1985). In addition, present studies revealed that all of these hormones or agents cause a decrease in PE synthesis in UMR 106 cells. Thus the decrease in PE synthesis by PTH and other agents may be, at least in part, involved in the development of desensitization phenomenon to PTH in these cells. However, further studies are needed before the functional role of the changes in phospholipid metabolism of osteoblast-like cells by PTH and other calcitropic hormones can fully be explored.

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