

# Effects of bovine parathyroid hormone and 1,25-dihydroxyvitamin D<sub>3</sub> on the production of prostaglandins by cells derived from human bone

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Local production of prostaglandins by osteoblasts may be important in controlling the bone resorbing activity of some hormones which have receptors on osteoblasts. We have demonstrated that osteoblast-like cells derived from human bone can incorporate [<sup>14</sup>C]arachidonic acid into phospholipids and synthesise immunoreactive PGE. Parathyroid hormone increases both the release of incorporated arachidonic acid and the synthesis of PGE. This is the first demonstration of modulation of bone cell prostaglandin synthesis by a bone resorbing hormone.

*Human bone cell      Prostaglandin      PTH      1,25-Dihydroxyvitamin D<sub>3</sub>*

## 1. INTRODUCTION

Both parathyroid hormone (PTH) and 1,25-dihydroxycholecalciferol [1,25(OH)<sub>2</sub>D<sub>3</sub>] stimulate bone resorption in organ culture. However the mechanisms involved in this process remain unclear. It has been suggested that the activity of some bone resorbing agents may be mediated by the osteoblast [1]. One hypothesis is that the osteoblast is stimulated to release factors which then act directly on the osteoclast. One group of such factors could be prostaglandins, some of which stimulate bone resorption and can be synthesised by osteoblasts in vitro [2,3]. We have developed a system for culturing non-transformed cells from adult human bone. These cells display the characteristics expected of osteoblasts and respond to a wide range of hormones and drugs [4]. We have investigated the incorporation and release of [<sup>14</sup>C]arachidonic acid, the production of immunoreactive prostaglandin E (PGE) by human bone cells and the effects of PTH and 1,25(OH)<sub>2</sub>D<sub>3</sub> on these activities.

## 2. MATERIALS AND METHODS

### 2.1. Human bone cell culture

Specimens of human trabecular bone were extensively washed in phosphate-buffered saline (PBS) and dissected into fragments 0.3–0.5 cm in diameter. Explants were plated out into 9 cm tissue culture dishes (5–8 explants/dish) and cultured in Eagles minimum essential medium, containing 10% fetal calf serum (EMEM + 10% FCS), and 1% penicillin/streptomycin solution (10000 units/ml). All cultures were maintained at 37°C in a humidified atmosphere of 95% air/5% CO<sub>2</sub>. Cells migrating from the explants formed a confluent monolayer after 3–5 weeks. All cell cultures used in these experiments synthesised osteocalcin in response to 1,25(OH)<sub>2</sub>D<sub>3</sub> ( $5 \times 10^{-9}$  M), and all experiments were conducted at first subculture.

### 2.2. Measurement of prostaglandins

Cells were passaged using 0.5% trypsin/0.02% EDTA into 3.5 or 1.6 cm multiwells at varying cell densities (fig.1) and allowed to settle for 24 h.

After 24 h incubation with or without PTH the medium was assayed for PGE content by radioimmunoassay using antiserum to PGE<sub>2</sub> which also recognised PGE<sub>1</sub>. Cross-reactivities with 6-keto PGF<sub>1 $\alpha$</sub> , PGF<sub>2 $\alpha$</sub> , TxB<sub>2</sub> and PGD<sub>2</sub> were all less than 0.05% at 50% displacement of the [<sup>3</sup>H]PGE<sub>2</sub> tracer. In some wells bovine PTH (0.1–8.1 IU/ml) or 1,25(OH)<sub>2</sub>D<sub>3</sub> (10<sup>-10</sup>–10<sup>-8</sup> M) was added. Neither of these agents cross-reacted with the antibody.

### 2.3. Incorporation and release of [<sup>14</sup>C]arachidonic acid

#### 2.3.1. Incorporation

Cells were passaged into 3.5 cm multiwells (10<sup>5</sup> cells/well) and allowed to settle for 24 h. The cells were incubated in EMEM + 0.5% bovine serum albumin (BSA) containing 1.6  $\mu$ Ci/ml [<sup>14</sup>C]arachidonic acid (58 mCi/mmol) for 4 h. Cells were removed from the plate with trypsin/EDTA and frozen to -20°C. Phospholipids were extracted and separated by thin-layer chromatography (TLC) as in [5]. Radioactivity areas were visualised by autoradiography and the relative amounts of radioactivity in each fraction were determined by densitometry using an LKB Ultrascan laser densitometer. A standard quantity of each phospholipid was run as standard.

#### 2.3.2. Release

Cells were passaged into 9 cm tissue culture plates (10<sup>6</sup> cells/plate) and after 24 h were incubated in EMEM + 0.5% BSA containing 1.6  $\mu$ Ci/ml [<sup>14</sup>C]arachidonic acid for 4 h. This medium was removed and stored and the cells washed 4 times with PBS to remove any residual unincorporated radioactivity. Cells were then incubated in 5 ml BGJ medium without serum, containing in some cases PTH (1–84) (2.0 IU/ml). Radioactivity released was assessed by liquid scintillation counting.

### 2.4. Materials

Penicillin/streptomycin solution, 0.5% trypsin/0.02% EDTA, EMEM, FCS and BGJ medium were purchased from Gibco (Paisley, Scotland). All tissue culture vessels purchased from Falcon (Oxford). [<sup>3</sup>H]PGE<sub>2</sub> and [<sup>14</sup>C]arachidonic acid were purchased from Amersham International plc.

Solvents and plates for TLC were purchased from E. Merck (Darmstadt). Purified bovine PTH (1–84) was obtained from National Institutes for Biological Standards, Mill Hill, England. 1,25(OH)<sub>2</sub>D<sub>3</sub> was a gift from Hoffman La Roche. Antiserum to PGE<sub>2</sub> was a kind gift from R.A. Forder, ICI Pharmaceuticals.

## 3. RESULTS AND DISCUSSION

Results from two separate bone cell cultures indicated that, after a 4 h incubation with [<sup>14</sup>C]arachidonic acid, most of the label is incorporated into the neutral lipid fraction (table 1). However appreciable amounts may also be found in the phosphatidylcholine (PC) and phosphatidylinositol (PI) fractions (table 1). Basal release of previously incorporated arachidonic acid represented 9.5% of the incorporated radioactivity. Addition of PTH (2.0 IU/ml) stimulated this release to 16.3%. PTH-stimulated release of arachidonic acid was inhibited by 1,25(OH)<sub>2</sub>D<sub>3</sub> (10<sup>-8</sup> M) to 7.2% of incorporated radioactivity.

These results demonstrate that cells derived from human bone can incorporate [<sup>14</sup>C]arachidonic acid into phospholipids from which it may be released, presumably by the action of phospholipase A<sub>2</sub>. This enzyme is activated by

Table 1  
Incorporation of [<sup>14</sup>C]arachidonic acid into the lipid fractions of cells derived from human bone

	[ <sup>14</sup> C]Arachidonic acid (%)	
	Culture 1	Culture 2
TG	55.3	53.0
PC	30.8	27.0
PE	2.2	2.1
PI	8.9	10.8
PS	1	1.2

Results show the percentage of a 2000 cpm aliquot of the cell layer radioactivity incorporated into the triglyceride and each of the major phospholipid fractions after TLC (see section 2). Culture 1, normal trabecular bone/2 yr old; culture 2, normal trabecular bone/19 yr old. TG, triglyceride; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine

agents which increase influx of calcium. The presence of calcium-dependent phospholipase  $A_2$  may explain the increased release of arachidonic acid in response to PTH since this hormone stimulates the influx of calcium into rodent bone cells [6]. The liberated arachidonic acid may then act as substrate for the cyclo-oxygenase and lipoxygenase enzymes. Thus PTH is able to stimulate the production of immunoreactive PGE by cells derived from human bone.

Production of PGE by human bone cells was stimulated by bovine PTH 1-84 in 6 separate experiments. Data from 3 of these are presented in fig.1. In contrast,  $10^{-8}$  M  $1,25(OH)_2D_3$  decreased basal production of PGE. Data from 3 separate experiments are shown in fig.1. Prostaglandins are potent stimulators of bone resorption in vitro [7]. Previous studies have demonstrated that osteoblast-like cells are capable of synthesising prostaglandins [2,3] and also that several bone resorbing agents including epidermal growth factor (EGF) [8] and platelet derived growth factor

(PDGF) [9] require the production of prostaglandins by bone in organ culture to mediate their activity. This has led to the concept of mediation of bone resorbing activity by osteoblastic production of prostaglandins.

Osteoblasts have receptors for PTH [10], and it has been proposed that the bone resorbing activity of this hormone may be modulated through the osteoblast. This hypothesis is consistent with our present demonstration that PTH stimulates production of PGE by human bone cells in culture. The concentrations of PTH capable of stimulating prostaglandin synthesis (0.9 IU/ml) are within the range used in previous bone resorption studies [11]. This is the first demonstration that production of prostaglandins by osteoblast-like cells may be affected by hormonal factors.

Previous investigation of PTH-stimulated bone resorption has produced conflicting data on the effect of inhibition of cyclo-oxygenase. Authors in [12] demonstrated a partial inhibition of bone resorption in the mouse calvarial system using aspirin whereas those in [13] did not show any effect of indomethacin. However, it is now recognised that the cyclo-oxygenases of different tissues vary in sensitivity to inhibitors such as indomethacin [14].

Preliminary results from separation of [ $^{14}C$ ]arachidonic acid metabolites by TLC suggest that human bone cells possess both cyclo-oxygenase and lipoxygenase pathways and are capable of metabolising arachidonic acid into a number of identifiable products and one unidentified metabolite. The fact that not all bone resorbing agents are likely to mediate their activity via increased osteoblastic production of prostaglandins is highlighted by the inhibitory effect of  $1,25(OH)_2D_3$  on synthesis of PGE.

In conclusion we have obtained evidence that PTH increases, whereas  $1,25(OH)_2D_3$  decreases, the production of prostaglandins by cells derived from human bone. This effect of PTH may mediate some or all of its bone resorbing activity. However the opposing effects of these agents on the synthesis of prostaglandins may also be important in the regulation of osteoblastic function.

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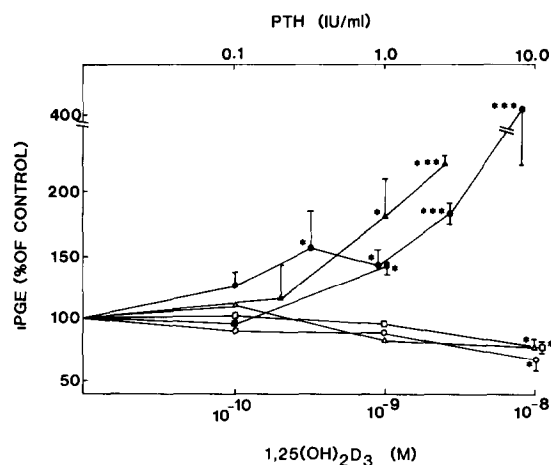


Fig.1. Synthesis of prostaglandins by various human bone cell cultures. (●) Normal bone, bovine PTH (1-84),  $1 \times 10^4$  cells/cm<sup>2</sup>, 5% FCS; (▲) normal bone, bovine PTH (1-84),  $0.25 \times 10^4$  cells/cm<sup>2</sup>, 10% FCS; (■) normal bone, bovine PTH (1-84),  $0.15 \times 10^4$  cells/cm<sup>2</sup>, 10% FCS; (○) normal bone,  $1,25(OH)_2D_3$ ,  $0.5 \times 10^4$  cells/cm<sup>2</sup>, 1% FCS; (Δ) normal bone,  $1,25(OH)_2D_3$ ,  $0.2 \times 10^4$  cells/cm<sup>2</sup>, 5% FCS; (□) normal bone,  $1,25(OH)_2D_3$ ,  $4 \times 10^4$  cells/cm<sup>2</sup>, 10% FCS. All points represent the percentage difference from control, as assessed by the means  $\pm$  SE of 6 separate incubations. Values significantly different from control: \*  $p < 0.05$ , \*\*\*  $p < 0.001$ .

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