

# Prostaglandins: Stimulation of Bone Resorption in Tissue Culture

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**ABSTRACT.** Prostaglandins (PG's) stimulated resorption of fetal rat bone in 48-96 hr tissue culture. The effects of several PG's were compared to the stimulation of bone resorption caused by parathyroid hormone (PTH). PGE<sub>1</sub> and PGE<sub>2</sub> caused increased release of previously incorporated radioactive calcium into the medium, losses of stable and labeled Ca from the bone and morphologic changes of osteoclastic resorption at 10<sup>-6</sup>-10<sup>-8</sup>M. The effects of PGE<sub>1</sub> and PGE<sub>2</sub> were similar to those of PTH (10<sup>-7</sup>-

10<sup>-9</sup>M) but in some experiments the effects of maximal doses of PTH were larger at 48 hr. Like PTH, the effects of PGE<sub>1</sub> were inhibited by thyrocalcitonin and cortisol. PGA<sub>1</sub> and PGF<sub>1α</sub> also stimulated bone resorption but only at higher doses (10<sup>-6</sup>M). *In vivo* studies demonstrated that injections of PGE<sub>1</sub> in parathyroidectomized rats did not increase serum calcium concentration, while parathyroid extract (4-40 U/rat) was effective. (*Endocrinology* 86: 1436, 1970)

**T**HE HYPOTHESIS that stimulation of bone resorption by parathyroid hormone (PTH) depends upon the activation of cellular adenylyl cyclase which results in an increase in the concentration of cyclic adenosine-3',5'-monophosphate (cAMP) is supported by a number of recent studies (1-8). Wells and Lloyd found that theophylline increased the serum calcium concentration in parathyroidectomized rats (1). Chase, Fedak and Aurbach (2) then showed that PTH activated adenylyl cyclase in homogenates of fetal rat calvaria, and later found that incubation of calvaria with PTH increased the concentration of cAMP in this tissue (3). Although no stimulatory effect of cAMP on bone resorption has been demonstrated, the 6-N,2'-O-dibutyryl derivative (DB-cAMP) has been observed to mobilize calcium in intact rats (4, 5) and to stimulate resorption of cultured fetal bone (6-8).

Recently, Aurbach and Chase found that several peptide hormones other than PTH did not affect the concentration of cAMP in fetal rat bone (3), but that prostaglandin

(PGE<sub>1</sub>) and epinephrine increased cAMP concentration. PGE<sub>1</sub> has similar effects on cAMP concentration in spleen, diaphragm and lung (9). These observations led us to study the effect of prostaglandins on bone in tissue culture. In the present study, the effects of several prostaglandins on bone resorption were found to be similar to those of PTH and, like PTH, the effects were inhibited by thyrocalcitonin and cortisol.

## Materials and Methods

**Tissue culture studies.** The culture technique used has been described previously (10, 11). The shafts of the radius or ulna of 19-day rat fetuses were prelabeled by injecting the pregnant rats with <sup>45</sup>Ca (0.5 mCi/rat) 24 hr prior to sacrifice. Bones were incubated in 0.5 ml of a chemically defined culture medium (BGJb, Grand Island Biological Company) supplemented with 1 mg/ml bovine serum albumin fraction V (Pentex). <sup>45</sup>Ca was counted by liquid scintillation and stable calcium measured by atomic absorption spectrophotometry. Prostaglandins<sup>2</sup> were either added to the culture medium directly at low concentrations or first dissolved in alcohol at 40 μg/ml and then diluted with the medium. Alcohol was added to the control media. Epinephrine (Sigma Com-

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pany), cortisol (Upjohn Company), porcine thyrocalcitonin (50 MRC U/mg, kindly provided by Dr. John Bastian of the Armour Pharmaceutical Company), partially purified rat thyrocalcitonin (5 MRC U/mg) and purified bovine PTH (1000-2000 U/mg, kindly provided by Dr. William Y. W. Au) were used.

*In vivo studies.* Male Sprague-Dawley rats weighing 200-220 g were parathyroidectomized by dissection. Ether anesthesia was used. Two days later control serum samples for calcium analysis were obtained by orbital puncture. Parathyroid extract (100 U/ml, Lilly) or PGE<sub>1</sub> (4 mg/ml dissolved in absolute alcohol) was diluted with saline and administered subcutaneously in a single 0.5 ml injection. The control animals received saline alone. A second serum sample was obtained 2 or 5 hr after injection. A similar second experiment was performed on these rats one week later.

## Results

*Tissue culture studies.* The ability of PGE<sub>1</sub> and PGE<sub>2</sub> to stimulate the release of previously incorporated <sup>45</sup>Ca from fetal bones in two-day cultures was similar to that of parathyroid hormone (Table 1). These prostaglandins showed near-maximal effects at 10<sup>-6</sup>M which did not increase or decrease at concentrations up to 10<sup>-5</sup>M. Low doses of PGE<sub>1</sub> were more effective on a weight basis and possibly on a molar basis than PTH, but large doses of PTH caused a somewhat greater maximal effect than large doses of prostaglandins. High concentrations of PGE<sub>1</sub> did not inhibit the effect of a maximal dose of PTH nor potentiate the effects of lower doses of PTH. PGA<sub>1</sub> was less effective than PGE<sub>2</sub> but did increase resorption at 10<sup>-6</sup>M. Epinephrine had no effect at 10<sup>-5</sup> or 10<sup>-7</sup>M.

The PTH and prostaglandins caused parallel losses of <sup>45</sup>Ca and total stable calcium from the bone (Table 2), indicating that these agents increased net resorption and not simply the rate of exchange of labeled calcium in the bone with stable calcium in the medium. In this experiment PGE<sub>1</sub> was slightly more effective than PGE<sub>2</sub> at 10<sup>-7</sup>M. PGF<sub>1α</sub> (10<sup>-6</sup>M) caused an increase in the release of <sup>45</sup>Ca which was ap-

TABLE 1. Effects of PTH, prostaglandins and epinephrine on <sup>45</sup>Ca release by embryonic bone during two-day organ culture

Addition	Treatment		% Increase in <sup>45</sup> Ca release relative to untreated control
	(μg/ml)	(M)	
<i>Experiment I</i>			
PTH	1.0	1 × 10 <sup>-7</sup>	52 ± 20*
PTH	0.3	3 × 10 <sup>-5</sup>	42 ± 21
PTH	0.1	1 × 10 <sup>-5</sup>	6 ± 16
PGE <sub>1</sub>	4.0	1 × 10 <sup>-5</sup>	45 ± 15*
PGE <sub>2</sub>	0.4	1 × 10 <sup>-6</sup>	30 ± 11*
PGE <sub>1</sub>	0.04	1 × 10 <sup>-7</sup>	41 ± 17*
PGE <sub>2</sub>	0.004	1 × 10 <sup>-5</sup>	32 ± 9*
PGE <sub>1</sub>	0.4	1 × 10 <sup>-5</sup>	
+PTH	1.0	1 × 10 <sup>-7</sup>	63 ± 4*
+PTH	0.3	3 × 10 <sup>-5</sup>	19 ± 26
+PTH	0.1	1 × 10 <sup>-5</sup>	21 ± 20
<i>Experiment II</i>			
PTH	3.0	3 × 10 <sup>-7</sup>	96 ± 17*
PTH	1.0	1 × 10 <sup>-7</sup>	44 ± 5*
PTH	0.3	3 × 10 <sup>-5</sup>	34 ± 11*
PGE <sub>1</sub>	0.4	1 × 10 <sup>-6</sup>	31 ± 8*
PGE <sub>1</sub>	0.004	1 × 10 <sup>-5</sup>	26 ± 7*
PGE <sub>1</sub>	0.0004	1 × 10 <sup>-5</sup>	11 ± 5
PGE <sub>1</sub>	0.00004	1 × 10 <sup>-10</sup>	9 ± 5
PGE <sub>2</sub>	0.4	1 × 10 <sup>-6</sup>	23 ± 12
PGE <sub>2</sub>	0.004	1 × 10 <sup>-5</sup>	21 ± 5*
PGA <sub>1</sub>	0.4	1 × 10 <sup>-6</sup>	19 ± 6*
PGA <sub>1</sub>	0.004	1 × 10 <sup>-5</sup>	-2 ± 3
EPI	1.8	1 × 10 <sup>-5</sup>	5 ± 2
EPI	.018	1 × 10 <sup>-7</sup>	-4 ± 3

Individual bones were incubated in 0.5 ml of media containing the indicated additions. Data are presented as the mean ± SE of the % difference in release of <sup>45</sup>Ca for 4 paired sets of treated and control bones. For these studies, PGE<sub>1</sub> and PGE<sub>2</sub>, supplied by the Bristol Laboratories, were used. Molar concentrations were based on the following approximated molecular weights: PTH 10,000; PGE 400; epinephrine (EPI) 180.

\* Significantly different from paired control cultures, p < 0.05.

parently too small to change Ca content of the bone significantly.

The time course of the response to maximally effective doses of PTH, PGE<sub>1</sub> and PGE<sub>2</sub> was examined (Table 3). Each compound caused the continuous resorption of bone and the cumulative results at four days were not significantly different. At two days, however, PTH treated bones had released significantly (p < .05) more <sup>45</sup>Ca than bones treated with PGE<sub>1</sub> or PGE<sub>2</sub>. The morphologic effects of these agents were similar (Fig. 1), consisting of patchy areas of bone loss in the center of the shaft.

TABLE 2. Effects of PTH and prostaglandins on  $^{45}\text{Ca}$  release, and final bone content of  $\text{Ca}^{++}$  and  $^{45}\text{Ca}$ 

Treatment	Media analysis % difference in $^{45}\text{Ca}$ release	Bone analysis		
		Final bone $\text{Ca}^{++}$ content $\mu\text{g}/\text{bone}$	% Difference in final bone $\text{Ca}^{++}$	% Difference in final $^{45}\text{Ca}$ in bone
PTH 3 $\mu\text{g}/\text{ml}$	53 $\pm$ 11*	7.4 $\pm$ .8	-17 $\pm$ 2*	-16 $\pm$ 7
Controls		8.9 $\pm$ .7		
PGE <sub>1</sub> 0.04 $\mu\text{g}/\text{ml}$	48 $\pm$ 11*	7.4 $\pm$ .2	-15 $\pm$ 5*	-17 $\pm$ 5*
Controls		8.8 $\pm$ .4		
PGE <sub>2</sub> 0.04 $\mu\text{g}/\text{ml}$	29 $\pm$ 14	8.2 $\pm$ .9	- 8 $\pm$ 5	-11 $\pm$ 5
Controls		9.1 $\pm$ .9		
PGE <sub>1a</sub> 0.4 $\mu\text{g}/\text{ml}$	20 $\pm$ 3*	8.4 $\pm$ .7	- 6 $\pm$ 3	- 4 $\pm$ 3
Controls		9.0 $\pm$ .7		

In this study each bone was incubated for 4 days. Data are the mean  $\pm$  SE of the % difference for pairs of bones, or for 4 individual bones for  $\text{Ca}^{++}$  content.

\* Significantly different from paired control cultures,  $p < .05$ .

Histologically, these areas showed osteoclastic resorption.

Both rat and porcine thyrocalcitonin (TCT) blocked the stimulatory effects of PGE<sub>1</sub> at concentrations similar to those which blocked PTH (Table 4) and, as observed previously (12), both had similar effects on a weight basis, although the porcine material was more potent by *in vivo* assay.

Addition of cortisol at  $10^{-7}$  and  $10^{-8}\text{M}$  also blocked the stimulatory effects of PGE<sub>1</sub> (Table 5). These concentrations were previously found to inhibit the stimulation of bone resorption by PTH (13, 14).

*In vivo studies.* Parathyroidectomized rats that were fasted overnight had an average serum calcium concentration of 4.7 mg/100 ml. Although PTE (4-40 U, rat) treatment increased the serum calcium con-

centration, PGE<sub>1</sub> (4 and 40  $\mu\text{g}$ ) treatment did not increase calcium at five hours. A small increase in calcium was observed two hours after treatment with 40  $\mu\text{g}$  of PGE<sub>1</sub>. In the second experiment rats were given 400  $\mu\text{g}$  of PGE<sub>1</sub> and serum was obtained two hours later. Serum calcium concentration decreased significantly. The treated animals were obviously ill, which might have been related to the hypotensive effects of PGE<sub>1</sub> at high doses (15).

### Discussion

The evidence presented shows that prostaglandins can stimulate the release of previously incorporated  $^{45}\text{Ca}$  from embryonic bones in tissue culture. These agents caused a parallel reduction of total and labeled calcium content of bones and produced morphologic changes similar to those caused by parathyroid hormone, indicat-

TABLE 3. Effects of PTH, prostaglandins E<sub>1</sub> and E<sub>2</sub> on the cumulative release of  $^{45}\text{Ca}$  during a four-day organ culture

Treatment	Cumulative % difference in $^{45}\text{Ca}$ release relative to controls			
	0-24 hr	0-48 hr	0-72 hr	0-96 hr
PTH 3 $\mu\text{g}/\text{ml}$ (4)	19 $\pm$ 1	41 $\pm$ 9	66 $\pm$ 18	79 $\pm$ 23
PGE <sub>1</sub> 0.4 $\mu\text{g}/\text{ml}$ (4)	14 $\pm$ 2	28 $\pm$ 2	58 $\pm$ 3	80 $\pm$ 3
PGE <sub>2</sub> 0.4 $\mu\text{g}/\text{ml}$ (4)	15 $\pm$ 6	24 $\pm$ 7	51 $\pm$ 9	72 $\pm$ 11

In this experiment 4 comparable sets of 2 bones were obtained by culturing the radius of one embryo with the ulna from another, both embryos being from the same mother. One set of 2 bones (radius + ulna) was used as a control for 3 treatment groups. Data represent the mean  $\pm$  SE of the % difference in cumulative  $^{45}\text{Ca}$  release from control during the period indicated. Bones were transferred to fresh media each day. Numbers in parentheses indicate number of sets of bones in each group. All values given are significantly different from controls,  $p < .05$ .

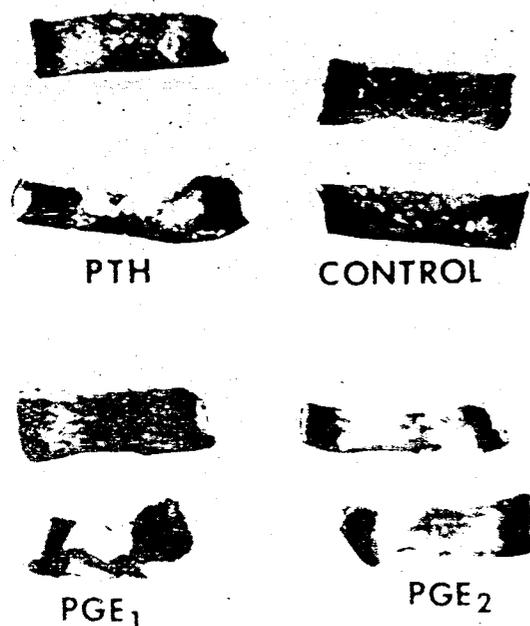


FIG. 1. Photograph of one set of bones from experiment detailed in Table 3. Bones were fixed in formaldehyde and photographed using transillumination. Density at ends is largely due to hypertrophic cartilage, which becomes opaque on fixation.

ing that they directly stimulated bone resorption *in vitro*. The responses to PTH and prostaglandins were similar, but maximal doses of PTH sometimes produced a larger effect in two days than maximal doses of prostaglandins. PGE<sub>1</sub> and PGE<sub>2</sub> were effective at concentrations as low as 10<sup>-8</sup>M. PGA<sub>1</sub> and PGF<sub>1</sub> were less

TABLE 4. Inhibition of PGE<sub>1</sub> and PTH stimulated bone resorption by rat and porcine thyrocalcitonin (TCT)

Group	% Increase in <sup>45</sup> Ca release relative to control values
PGE <sub>1</sub> (0.4 μg/ml)	42 ± 5*
-Rat TCT (1.6 μg/ml)	8 ± 3
-Porcine TCT (1.0 μg/ml)	14 ± 6
PTH (2 μg/ml)	48 ± 4*
-Rat TCT (1.6 μg/ml)	6 ± 3
-Porcine TCT (1.0 μg/ml)	10 ± 6

Bones were incubated for 48 hr. Data are means ± SE for 4 pairs of bones.

\* Significantly different from paired control cultures,  $p < .025$ , and from cultures with TCT,  $p < .05$ .

TABLE 5. Inhibition of PGE<sub>1</sub> stimulated bone resorption by cortisol

Group	% Increase in <sup>45</sup> Ca release relative to control values
PGE <sub>1</sub> (0.048 μg/ml) (8)	56 ± 25*
+Cortisol 10 <sup>-7</sup> (4)	6 ± 4
+Cortisol 10 <sup>-8</sup> (4)	18 ± 7

Bones were incubated for 48 hr. The data are means ± SE. Numbers in parentheses indicate the number of pairs of bones in each treatment group.

\* Significantly different from control,  $p < .05$ .

effective, showing small effects at 10<sup>-6</sup>M.

Prostaglandins have been shown to increase cAMP content of fetal rat bone as well as other tissues (3, 9), and it is reasonable to assume that the prostaglandins also increased cAMP concentration in our experiments. The failure of epinephrine to stimulate bone resorption, despite its ability to increase cAMP concentrations in other bone preparations, is unexplained. In the present experiments, epinephrine may have been ineffective because it was

TABLE 6. Comparison of the effects of parathyroid extract (PTE) and PGE<sub>1</sub> on serum calcium of parathyroidectomized rats

Treatment group	Effect of treatment on plasma calcium (Δ mg/100 ml)	
	2 hr	5 hr
<i>Experiment I</i>		
Control	-0.2 ± 0.2 (4)	-0.2 ± 0.2 (4)
PTE (40 U)	1.3 ± 0.1 (4)*	2.6 ± 0.3 (3)*
PTE (4 U)	—	1.1 ± 0.3 (6)*
PGE <sub>1</sub> (40 μg)	0.5 ± 0.1 (4)	0.0 ± 0.1 (5)
PGE <sub>1</sub> (4 μg)	0.2 ± 0.2 (4)	0.0 ± 0.2 (4)
<i>Experiment II</i>		
Control	-0.2 ± 0.1 (8)	
PTE (40 U)	1.0 ± 0.1 (7)*	
PGE <sub>1</sub> (400 μg)	-0.8 ± 0.2 (8)*	

Rats were parathyroidectomized 2 days prior to Experiment I and pretreatment serum calcium samples were obtained 1 hr prior to injection of saline, PTE or PGE<sub>1</sub>. The average pretreatment value was 4.7 mg/100 ml. Individual animals were bled only once post treatment. Rats from Experiment I were used 1 week later in Experiment II. At that time the average pretreatment serum calcium value was 7.1. Data are means ± SE of the difference between the pre- and post-treatment serum calcium values of individual animals. Number of animals/group is in parentheses.

\* Statistically significant change in serum calcium concentration compared to saline treated control,  $p < .05$ .

degraded during incubation, or because epinephrine in this system did not elevate cAMP concentrations.

The site of action of prostaglandins is unknown. The similar effect of inhibitors of bone resorption on bones treated with PGE<sub>1</sub> or PTH and the absence of any synergistic or inhibitory interaction are consistent with the hypothesis that they act by the same or closely related mechanisms to elevate cAMP concentrations in bone cells. In previous studies we found that DB-cAMP, which stimulated bone resorption in tissue culture, was ineffective at high concentrations (7, 8). Moreover, addition of stimulatory concentrations of DB-cAMP could inhibit the response to PTH (in preparation). One explanation of this interaction was that two antagonistic systems regulating bone resorption existed which were both stimulated by cAMP. This type of relationship was not found with PGE<sub>1</sub> and PTH. Unlike DB-cAMP, which stimulated resorption over a narrow dose range of 1 to  $3 \times 10^{-10}$  M and lost effectiveness at  $10^{-8}$  M (8), prostaglandins stimulated bone resorption over a wide range ( $10^{-9}$ – $10^{-7}$  M). If two cAMP-activated antagonistic systems regulating bone resorption exist, only one seems to be stimulated by PG's.

Aurbach and Chase found that TCT did not alter cyclic AMP content of bone or adenylyl cyclase activity in homogenates of bone (2, 3). TCT was antagonistic to the effects of PTH and prostaglandin on cultured fetal rat bones but it also inhibits resorption stimulated by vitamin D and other agents and probably acts on a separate system and not on adenylyl cyclase. The effects of cortisol on bones treated with PGE<sub>1</sub> and PTH were similar and this observation may be a useful tool in further analyzing the mechanism of action of this inhibitor of bone resorption.

The inability of PGE<sub>1</sub> to raise serum calcium concentration *in vivo*, despite its

great potency in tissue culture, is unexplained. Possibly PGE<sub>1</sub> is very rapidly degraded *in vivo* and reaches bone at concentrations which are insufficient to cause release of calcium. Massive doses were found to have toxic effects which may have prevented the observation of effects on serum calcium. On the basis of these findings, prostaglandins probably do not act as humoral regulators of bone resorption or of serum calcium concentration. However, prostaglandins could have important local or pathological effects on bone resorption. Prostaglandins can be produced by tumors, including medullary carcinomas of the thyroid, which also produce thyrocalcitonin, and the interrelation between these agents deserves further study (16).

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