Calcif Tissue Int (1986) 38:130–134

Calcified Tissue International
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Laboratory Investigations

Effects of Prostacyclin and Prostaglandin E_1 (PGE_1) on Bone Resorption in the Presence and Absence of Parathyroid Hormone

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Summary. Prostaglandins have been shown to stimulate osteoclastic bone resorption in organ culture but morphologic studies of isolated osteoclasts have shown a transient calcitonin-like inhibiting effect of these agents. We looked for a dual effect on bone resorption by comparing the early and late effects of prostaglandin E_1 (PGE_1), prostacyclin (PGI_2), 6α-carbaprostaglandin I_2 (C-PGI_2), a carbon substituted analog of PGI_2, and salmon calcitonin (CT) on the release of previously incorporated ^{45}Ca from fetal rat long bones cultured in the presence of an inhibitor of cyclooxygenase, RO-20-5720. Experiments were performed in both the presence and absence of PTH (400 ng/ml), which was administered 24 hours before addition of prostaglandins or CT. In control cultures not stimulated by PTH, CT (100 mU/ml) produced significant decreases in ^{45}Ca release at 48, 72, and 96 hours while PGE_1 (10^{-6} M), PGI_2 (10^{-5}), and C-PGI_2 (10^{-6} M) each produced significant increases in resorption at 24 through 96 hours. PGE_1 at 10^{-5} M, but not 10^{-6} M, caused a significant decrease in medium ^{45}Ca of 21% at 1 and 2 hours. Medium calcium measurements suggest that the change in ^{45}Ca was due to inhibition of release and not to increased uptake. PGI_2 (10^{-5} M) and C-PGI_2 (10^{-6} M) caused no significant inhibitory effect. In cultures stimulated by PTH, CT produced significant inhibition of bone resorption of 6 through 96 hours, but no inhibition of bone resorption was noted at either early or late time points with PGE_1, PGI_2, or C-PGI_2. Moreover, the addition of PGI_2 (10^{-5} M) to PTH-treated cultures actually enhanced ^{45}Ca release beginning to 6 hours, when PGI_2 alone had no effect upon bone resorption. These results confirm that high concentration of PGI_2 can stimulate bone resorption and show a similar response to a stable analog, C-PGI_2. Moreover, PGI_2 was found to enhance PTH-stimulated bone resorption. A small transient inhibition of ^{45}Ca release was observed with a high concentration of PGE_1 (10^{-5} M) in the absence of PTH, which could be due to a transient direct inhibitory action upon osteoclasts.

Key words: Fetal bone cultures — Prostaglandins — Bone resorption.

The prostaglandins represent an important group of cyclic fatty acids which affect virtually every organ in the body. In skeletal tissue, there is increasing evidence for a major local regulatory role for prostaglandins in bone remodeling [1]. Prostaglandins, particularly those of the E series, are potent bone resorbing agents [2]. The resorptive effects of prostaglandin E_2 and E_1 (PGE_2 + PGE_1) are similar, although PGE_2 is somewhat more potent. Other stable prostanoids and their metabolites are less active [2–5]. Stimulation of bone resorption is also observed with repeated or continuous administration of prostacyclin (PGI_2), although the highly unstable nature of this compound makes it difficult to determine its potency precisely [6, 7]. While stimulation of bone resorption by prostaglandins might be due to a direct effect of these agents on osteoclasts, the response could depend on an initial interaction with osteoblasts, leading to production of an intermediary humoral factor [8].

More recent studies have indicated that certain prostaglandins, the most potent being PGI_2 and PGE_1, as well as a stable analog of PGI_2, 6α-carbaprostaglandin I_2 (C-PGI_2), will cause a transient decrease in the cytoplasmic motility of isolated osteoclasts [9, 10]. This effect differs only in duration

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from the more prolonged morphological change observed when isolated osteoclasts are treated with calcitonin (CT), a well-known inhibitor of bone resorption [11]. This similarity between the effects of prostaglandins and CT on isolated osteoclasts has led to the suggestion that an early and perhaps direct regulatory action of prostaglandins might be inhibition of bone resorption.

The present study was undertaken to compare the early and long-term effects of PGI_2, PGE_1, and C-PGI_2 on the release of previously incorporated ^{45}Ca from cultured fetal rat long bones. Experiments were performed with CT for comparison and in the presence and absence of parathyroid hormone (PTH). All studies were carried out in the presence of RO-20-5720 (carprofen), an inhibitor of prostaglandin cyclo-oxygenase, which was added to minimize the possibility that endogenous prostaglandins had already engaged receptors involved in early prostaglandin effects or produced down-regulation of the response to exogenous prostaglandins.

Materials and Methods

Experimental Protocol

The culture procedures used in the study have been described previously [12]. Fetal rat bones were labeled by subcutaneously injecting 100–200 μCi of ^{45}Ca (New England Nuclear Corporation, Boston, MA) into pregnant rats on the 18th day of gestation. On the 19th day the fetuses were removed and the radius and ulna were dissected free of muscle; cartilage and the four bones from each fetus were cultured for 18–24 hours in 0.5 ml BGJ medium (Fitton-Jackson Modification; Grand Island Biochemical Co., Grand Island, NY) containing 1 mg/ml bovine serum albumin (Sigma Chemical Co., St. Louis, MO) at 37°C in an atmosphere of 5% CO_2. Bones were then transferred to fresh culture medium which contained either 10^{-5} M RO-20-5720 (Hoffman-LaRoche, Nutley, NJ) or 10^{-5} M RO-20-5720 plus 400 ng/ml PTH (Bovine, 1-34; Boehringer Mannheim Biochemicals, Indianapolis, IN) for a 24 hour period. This was followed by subsequent medium changes for up to 96 hours into culture medium which contained the same agents as in the initial 24 hour treatment period plus additions of either of the other prostaglandins—10^{-6} or 10^{-5} M PGE_1, 10^{-5} M PGI_2, 10^{-6} M C-PGI_2 (kindly supplied by Dr. John Pike of the Upjohn Company), or 100 mU/ml Salmon CT (Armour Pharmaceuticals, Kankakee, IL). Due to its instability (a chemical half-life of 2–3 minutes at neutral pH), PGI_2 was added to individual culture dishes at the time of each exchange in 5 μl 0.1 M Na_2CO_3. Vehicle alone was added to control cultures. ^{45}Ca was measured in 0.1 ml medium and in bones following extraction with 5% TCA. Release of ^{45}Ca was expressed as the cumulative % of total bone radioactivity released into culture medium beginning at the time of prostaglandin or CT addition. Total medium calcium was measured fluorometrically using a Corning calcium analyzer, Model 940.

Statistical Analysis

Statistical analysis of multiple treatment groups was performed using a two-way analysis of variance after logarithmic, transformation of data [13]. Where a significant F was observed, the least significant difference was used to determine significance between treatments and control. For two group analysis, the Student's t test was used to determine significance between treatment and control.

Results

Tables 1 and 2 show the cumulative % of total bone ^{45}Ca released when PGI_2 (10^{-5} M), C-PGI_2 (10^{-6} M), PGE_1 (10^{-6}), and CT (100 mU/ml) are added to fetal rat long bones treated with RO-20-5720 (10^{-5} M) or RO-20-5720 (10^{-5} M) plus PTH (400 ng/ml).

In the absence of PTH, no significant changes in ^{45}Ca release were noted in prostaglandin-treated groups after 30 minutes, 1 hour or 6 hours of culture (Table 1). In contrast, significant increases in ^{45}Ca release were observed at 24 through 96 hours with each of the prostaglandins tested—PGI_2 (10^{-5} M), C-PGI_2 (10^{-6} M), and PGE_1 (10^{-6} M). The responses observed with PGE_1 (10^{-6} M) and PGI_2 (10^{-5} M) are in close agreement with ^{45}Ca release data reported for these agents in previous studies [6]. Bone resorption with C-PGI_2 (10^{-6} M) was 70% of that elicited by an equal concentration of PGE_1 (10^{-6} M). Salmon CT (100 mU/ml) treatment produced significant decreases in resorption at 48, 72, and 96 hours in control cultures, but not at earlier time points.

When PTH (400 ng/ml) had been added during the previous 24 hours (Table 2), ^{45}Ca release was substantially greater, and no inhibition of ^{45}Ca release was observed in any treatment groups at 30 minutes or 1 hour. Moreover, no significant effects on ^{45}Ca release were observed at 6 through 96 hours with PGE_1 (10^{-6} M) or C-PGI_2 (10^{-6} M). However, the addition of PGI_2 (10^{-5} M) to PTH-(400 ng/ml) treated cultures led to an enhancement of the rate of ^{45}Ca release beginning at 6 hours. This difference was maximal to 48 hours and persisted, although by 96 hours the bones treated with PTH alone had approached the rate with PTH plus PGI_2. In contrast, the addition of CT (100 mU/ml) to PTH-(400 ng/ml) treated fetal long bones produced significant decreases in bone resorption beginning at 6 hours of culture. After 24 hours the rate of resorption began to increase, indicating the onset of escape, although cumulative ^{45}Ca release remained less with CT than with PTH alone.

Because of the small decrease in ^{45}Ca release observed after 1 hour of treatment with PGI_2 (10^{-5} M)

Table 1. Effects of PGI_2, C-PGI_2, PGE_1, and CT on ^{45}Ca release from fetal rat long bones treated with R0-20-5720 (10^{-5} M)

Treatment	Cumulative % ^{45}Ca released						
	1/2 h	1 h	6 h	24 h	48 h	72 h	96 h
Control	.32 ± .03	.57 ± .04	2.08 ± .13	5.94 ± .40	10.64 ± 0.77	15.51 ± 1.37	19.81 ± 2.10
PGI_2 (10^{-5} M)	.28 ± .03	.48 ± .03	1.99 ± .12	8.07 ± .51[b]	22.50 ± 1.80[b]	40.50 ± 3.35[b]	51.42 ± 4.29[b]
C-PGI_2 (10^{-6} M)	.30 ± .03	.55 ± .04	1.92 ± .16	7.96 ± .87[a]	19.43 ± 2.72[b]	35.31 ± 5.29[b]	47.92 ± 6.85[b]
PGE_1 (10^{-6} M)	.31 ± .03	.54 ± .03	1.96 ± .14	12.36 ± .29[b]	31.53 ± 1.15[b]	53.54 ± 2.64[b]	68.51 ± 3.30[b]
CT (100 mU/ml)	.30 ± .04	.60 ± .04	2.22 ± .14	4.93 ± .46	6.96 ± 0.68[b]	8.33 ± 0.80[b]	9.82 ± 0.90[b]

Values are mean ± SE for 8–16 cultures
[a] Significantly different from control, $P < .05$; [b] $P < .01$

Table 2. Effects of PGI_2, C-PGI_2, PGE_1, and CT on ^{45}Ca release from fetal rat long bones treated with R0-20-5720 (10^{-5} M) and PTH (400 ng/ml)

Treatment	Cumulative % ^{45}Ca released						
	1/2h	1 h	6 h	24 h	48 h	72 h	96 h
PTH alone	1.05 ± .10	1.95 ± .20	9.27 ± .87	36.80 ± 3.05	62.01 ± 4.01	77.41 ± 3.99	82.87 ± 3.95
PTH +							
PGI_2 (10^{-5} M)	1.26 ± .06	2.24 ± .07	12.17 ± .46[b]	49.70 ± 2.65[b]	79.10 ± 3.35[a]	90.89 ± 2.56	94.00 ± 2.12
C-PGI_2 (10^{-6} M)	1.05 ± .11	1.84 ± .17	9.33 ± .95	37.51 ± 3.45	62.09 ± 4.22	76.13 ± 4.41	81.87 ± 4.35
PGE_1 (10^{-6} M)	1.06 ± .06	1.88 ± .12	10.05 ± .87	40.35 ± 3.34	67.60 ± 4.77	81.90 ± 4.69	87.45 ± 4.24
CT (100 mU/ml)	0.91 ± .08	1.61 ± .11	5.13 ± .39[b]	12.79 ± 1.15[b]	27.11 ± 2.52[b]	47.16 ± 4.30[b]	63.36 ± 5.22[a]

Values are mean ± SE for 8–16 cultures
[a] Significantly different from control, $P < .05$; [b] $P < .01$

in initial studies, additional experiments were performed to investigate the early effects of prostaglandin administration. Table 3 shows the cumulative % of ^{45}Ca released at 1 and 2 hours when high concentrations of PGI_2 (10^{-5} M) or PGE_1 (10^{-5} M) were added to fetal long bones treated with RO-20-5720 (10^{-5} M). The decrease in ^{45}Ca release after 1 or 2 hours of exposure to PGI_2 (10^{-5} M) was less than 10% and was not significant. The decrease in ^{45}Ca release observed at both 1 and 2 hours with PGE_1 (10^{-5} M) was 21% and was statistically significant when compared to control resorption ($P < .01$). Moreover, inhibition was seen in each of four experiments. PGE_1 did not produce any early inhibition in PTH-treated cultures even at 10^{-5} M (data not shown).

To test whether these small decreases in ^{45}Ca release reflect an inhibition of calcium release and not an increase in calcium uptake, total medium calcium was measured after 2 hours of culture in this second series of experiments. Mean values were 5.36 ± .03 mg/dl for controls and 5.33 ± .04 mg/dl for cultures treated with PGE_1 (10^{-5} M).

Table 3. Effects of PGI_2 and PGE_1 on ^{45}Ca release from fetal rat long bones treated with R0-20-5720 (10^{-5} M)

Treatment	Cumulative % ^{45}Ca released	
	1 h	2 h
Control	.57 ± .03	1.31 ± .07
PGI_2 (10^{-5} M)	.52 ± .03	1.22 ± .05
Control	.62 ± .03	1.18 ± .05
PGE_1 (10^{-5} M)	.48 ± .02[b]	0.93 ± .03[b]

Values are means ± SE for 23–30 cultures
[b] Significantly different from control, $P < .01$

Discussion

The present studies were undertaken to test the hypothesis that prostanoids produce an initial inhibitory effect on bone resorption, based on the report of morphologic changes in isolated osteoclasts which resemble those in response to calcitonin [9–11]. We compared direct addition of PGI_2 with addition of PGE_1, which is a known potent stimulator of bone resorption and structurally similar to PGI_2. We also tested C-PGI_2, a stable analog in which the oxygen bridge is replaced by a carbon bridge. Bones were pretreated with an inhibitor of prostaglandin cyclooxygenase in order to minimize the effects of endogenous products. We had observed previously that fetal rat long bone can produce substantial amounts of PGE_2 and 6-keto

$PGF_{1\alpha}$, the stable metabolite of PGI_2, from labeled arachidonate [6]. Studies with calvaria and isolated bone cells have confirmed this and have indicated that PGI_2 may be one of the most important products of arachidonic acid metabolism in bone [14, 15].

The inhibitory effects of prostanoids on bone resorption in this model system were minimal. The only significant effect was that of a high concentration of PGE_1 (10^{-5} M) which produced a transient 21% inhibition of ^{45}Ca release at 1 and 2 hours from bones pretreated only with a prostaglandin cyclooxygenase inhibitor and not with PTH. None of the compounds tested inhibited ^{45}Ca release from bones that had been pretreated with PTH and were undergoing rapid resorption. Calcitonin produced a more marked and prolonged inhibition of resorption in both systems, although in the PTH-stimulated system there was evidence of escape after 12 hours. The decrease in ^{45}Ca release seen with PGE_1 appears to be due to decreased bone resorption rather than increased redeposition of ^{45}Ca since there was a 21% decrease in radioactivity with no decrease in medium calcium concentration. A parallel decrease should have occurred if the effect was due solely to an increase in calcium movement from medium to bone.

Although slightly lower mean values of ^{45}Ca release were observed at 1 and 2 hours with PGI_2, significance was not observed at either time interval. Increased calcium uptake has been reported in rat calvaria discs after a 30 minute exposure to PGI_2 [16]. Such redeposition of calcium should have enhanced an inhibitory effect of PGI_2 upon ^{45}Ca release.

It is difficult to assess the physiologic importance of the small inhibitory effect that was observed with PGE_1 in unstimulated cultures. Concentrations employed were high and no inhibition was observed at a concentration (10^{-6} M) that produced near maximal resorption responses to PGE_1 in more prolonged culture. The discrepancy between these studies and observations with isolated osteoclasts could be due to the effects of other bone cells present in the organ culture system. It has been suggested that products of osteoblasts or other bone cells can overcome these inhibitory effects and may in fact be the mediators of stimulation of bone resorption not only by prostanoids, but also by PTH. Moreover, the small inhibition of ^{45}Ca release observed with PGE_1 may not be due to an effect upon osteoclasts, but to an effect upon other bone cells, occurring perhaps as a result of a change in calcium flux. Regardless of the mechanism, this effect could represent a transient local regulatory action of prostaglandins, and might play a significant role in bone remodeling and calcium homeostasis. Other *in vitro* and *in vivo* systems, which are more sensitive for the detection of early effects on osteoclastic bone resorption, should be used to assess this role.

The results of this study confirm previous studies indicating that PGI_2 can stimulate bone resorption [6, 7]. Moreover, in addition to the stable sulfur analog previously tested, a carbon analog of PGI_2 can affect the system. Of particular interest was the enhancement of the bone resorption rate obtained with high concentrations of PGI_2 in bones which had been already treated with PTH for 24 hours. This effect appeared to be more than additive at 6 and 24 hours. The enhancement could be the results of the involvement of different cyclic AMP pathways by the two compounds. In isolated bone cells and osteosarcoma cells, PGE_2 and PTH have different effects on cyclic AMP-dependent protein kinase I and II [17]. Interaction of prostanoids and PTH on osteoblasts might result in the production of an increased amount of mediator of bone resorption. In previous studies we demonstrated a synergistic enhancement of resorption of fetal rat long bones using low concentrations of endotoxins and either PGE_2 or PTH [18]. However, in these studies submaximal concentrations of PGE_2 and PTH were not synergistic. Further studies are needed to determine whether the apparent synergism observed in the present study is limited to high concentration of PGI_2 or can be produced by other prostanoids.

References

1. Raisz LG, Martin TJ (1984) Prostaglandins in bone and mineral metabolism. In: Peck WA (ed) Bone and mineral annual review. II. Exerpta Medica, Amsterdam
2. Dietrich JW, Goodson JM, Raisz LG (1975) Stimulation of bone resorption by various prostaglandins in organ culture. Prostaglandins 10:231–240
3. Raisz LG, Dietrich JW, Simmons HA, Seyberth HW, Hubbard W, Oates JA (1977) Effect of prostaglandin endoperoxides and metabolites on bone resorption in vitro. Nature (London) 267:532–534
4. Tashjian AH Jr, Tice JE, Sides K (1977) Biological activities of prostaglandin analogues and metabolites on bone in organ culture. Nature (London) 266:645–646
5. Neuman SD, Raisz LG (1984) Effects of the prostacyclin products, 6-keto prostaglandins E_1 and 6-keto prostaglandin $F_{1\alpha}$, on bone resorption in vitro. Prostaglandin Leuk Med 15:103–108
6. Raisz LG, Vanderhoek JY, Simmons HA, Kream BE, Nicolaou KC (1979) Prostaglandin synthesis by fetal rat bone in vitro: evidence for a role of prostacyclin. Prostaglandins 17:905–914
7. Ali NN, Auger DW, Bennett A, Edwards DA, Harris M

(1979) Effect of prostacyclin and its breakdown product 6-Oxo-$PGF_{1\alpha}$ on bone resorption in vitro. In: Vane JR, Bergstrom S (eds) Prostacyclin. Raven Press, New York, p 179

8. Rodan GA, Martin TJ (1981) Role of osteoblasts in hormonal control of bone resorption—a hypothesis. Calcif Tissue Int 33:349–351
9. Chambers TJ, Ali NN (1983) Inhibition of osteoclastic motility by prostaglandins I_2, E_1, E_2 and 6-Oxo-E_1. J Pathol 139:383
10. Ali NN, Chambers TJ (1983) The effect of prostaglandin I_2 and 6α-carba-PGI_2 on the motility of isolated osteoclasts. Prostaglandins 25:603–614.
11. Chambers TJ, Magnus CJ (1982) Calcitonin alters behavior of isolated osteoclasts. J Pathol 136:27–29
12. Stern PH, Raisz LG (1979) Organ culture of bone. In: Simmons DJ, Kunin AS (eds) Skeletal research: an experimental approach. Academic Press, New York, p 21
13. Steel RGD, Torrie JH (1960) Principles and procedures of statistics. McGraw-Hill, New York, pp 67, 106, 111, 132
14. Nolan RD, Partridge NC, Godfrey HM, Martin TJ (1983) Cyclo-oxygenase products of arachidonic acid metabolism in rat osteoblasts in culture. Calcif Tissue Int 35:294–297
15. Voelkel EF, Tashjian AH Jr, Levine L (1980) Cyclooxygenase products of arachidonic acid metabolism by mouse bone in organ culture. Biochim Biophys Acta 620:418–428
16. Walenga RW, Bergstrom W (1985) Stimulation of calcium uptake in rat calvaria by prostacyclin. Prostaglandins 29:191–202
17. Livesey SA, Kemp BE, Re CA, Partidge NC, Martin TJ (1982) Selective hormonal activation of cyclic AMP-dependent protein kinase isoenzymes in normal and malignant osteoblasts. J Biol Chem 257:14983–14987
18. Raisz LG, Nuki K, Alander CB, Craig RG (1981) Interactions between bacterial endotoxin and other stimulators of bone resorption in organ culture. J Period Res 16:1–7

Received July 9, 1985, and in revised form October 8, 1985