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Cyclo-oxygenase Products of Arachidonic Acid Metabolism in Rat Osteoblasts in Culture

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Summary. The metabolism of arachidonic acid to its cyclo-oxygenase products was studied in monolayer cultures of osteoblast-rich rat calvarial cells and of clonal cell lines from a rat osteogenic sarcoma, enriched in the osteoblast phenotype. Prostanoids were measured by radioimmunoassay after extraction of media and fractionation by high pressure liquid chromatography. In both normal and malignant osteoblasts the major cyclo-oxygenase product was 6-oxo-prostaglandin $F_{1\alpha}$, the hydration product of prostacyclin, with lesser amounts of prostaglandin E_2 and prostaglandin $F_{2\alpha}$. No significant thromboxane B_2 was detected. Prostaglandins are thought to have a local role in the regulation of bone resorption. These results point to the possible importance of prostacyclin either in bone resorption or in some other local function, e.g., regulation of bone blood flow.

Key words: Prostaglandins — Osteoblasts — Bone resorption.

Prostaglandins stimulate bone resorption in organ culture [1] by increasing the numbers and activity of osteoclasts [2]. The actions of certain bone resorbing factors, notably phorbol esters [3], epidermal growth factor [4], and complement-replete serum [5], appear to require the synthesis within bone of prostanoids. Rat bone can metabolize arachidonic acid to several products [6] and the concept has developed that locally produced prostaglandins might be important in regulating bone resorption [4, 5, 7, 8].

We have noted that the relative potencies of various prostanoids upon cyclic AMP generation in cultured malignant and normal osteoblasts closely parallel their relative potencies as stimulators of bone resorption [8–10]. This observation contributes to our argument that the osteoblast has a role in the bone resorptive process [10–12]. In the present work, we have analyzed the cyclo-oxygenase products of arachidonic acid metabolism in osteoblast-rich rat bone cell cultures and in four clonal lines of rat osteogenic sarcoma cells, enriched in the osteoblast phenotype [13].

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Materials and Methods

Cell Culture

Cultures of osteoblast-rich rat calvarial cells were established by a method described in detail by Partridge et al [10], based on the method developed for mouse bone cells [14]. Four clonal lines, designated UMR 104, UMR 105, UMR 106, and UMR 108 were cultured as previously described [13]. These clones, which are enriched in the osteoblast phenotype, are derived from a transplantable rat osteogenic sarcoma [15] the properties of which have been described elsewhere [9, 16]. Cell cultures were maintained in Eagle's minimal essential medium (MEM) with nonessential amino acids, Hepes buffer (20 mM), and 80 mg gentamicin/l (Hepes-MEM) containing 10% fetal calf serum. Experiments on osteoblast-rich normal rat calvarial cells were carried out on cultures no later than the second subculture.

Assay of Arachidonic Acid Metabolites

After medium change, cells were incubated in 25 cm^2 Costar culture flasks for 4 hr in Hepes-MEM containing 0.1% bovine serum albumin and sodium arachidonate (10 μg per ml). Arachidonic acid (sigma grade 1) was stored in n-hexane under nitrogen at −20°C. Immediately before use, the solvent was evaporated under nitrogen and the arachidonic acid dissolved in 0.1 M sodium carbonate, making the sodium salt. At the end of incubation periods, medium (5 ml) was acidified to pH 3.5 with 0.1 M formic acid, extracted twice with 3 volumes ethyl acetate, the extracts pooled and dried under a stream of nitrogen, then resuspended in 0.5 ml of column solvent for high pressure liquid

chromatography. A Waters instrument was used with a 30 cm × 3.9 mm ID reverse phase fatty acid analysis column (Waters Associates), and arachidonic acid metabolites were eluted isocratically with a solvent system consisting of acetonitrile-water-benzene-acetic acid (230:767:2:1) [17]. The flow rate was 2 ml/min and fractions of 2 ml were collected, lyophilized, and assayed by radioimmunoassays for 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$), thromboxane B_2 (TxB_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and prostaglandin E_2 (PGE_2). Recoveries of added [^{3}H] PGE_2 [^{3}H] $PGF_{2\alpha}$, [^{3}H] 6-oxo-$PGF_{1\alpha}$, and [^{3}H] TxB_2 were monitored and data were corrected for the average loss. Yields were 56.6 ± 2.1%, 71.6 ± 7%, 59.7 ± 5.6%, and 53.2 ± 1.7% (mean ± SEM) respectively, for 6-oxo- $PGF_{1\alpha}$, TxB_2, $PGF_{2\alpha}$, and PGE_2. Details of chromatography procedures, radioimmunoassay, and cross reactivities of the antisera have been reported [18].

Materials

Arachidonic acid (sigma grade 1) was stored in n-hexane (10 mg/ml) under nitrogen at −20°C. Prostanoids were gifts from Dr. J. E. Pike, Upjohn Co., Kalamazoo, MI, U.S.A. Antibodies used were diluted rabbit antisera directed against PGE_2, $PGF_{2\alpha}$, and 6-oxo-$PGF_{1\alpha}$. The antibody for TxB_2, the hydration product of TxA_2, was obtained as purified anti-TxB_2 rabbit IgG (a gift from Dr. J. Bryan Smith). Anti-PGE_2 antiserum was a gift from Dr. Lawrence Levine and anti-6-oxo-$PGF_{1\alpha}$ from Dr. L.C. Best. Anti-$PGF_{2\alpha}$ antiserum was purchased from ONO Pharmaceutical Co., Japan. ^{3}H-labeled arachidonic acid metabolites were obtained from New England Nuclear Corp., Boston, MA, USA or the Radiochemical Centre, Amersham, Bucks., UK.

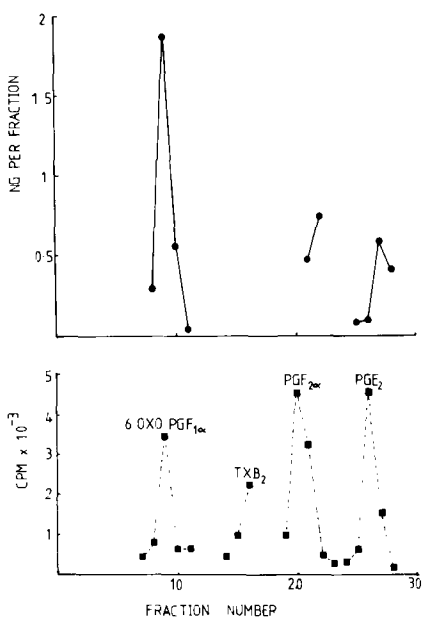

Fig. 1. Arachidonic metabolism in monolayer cultures of clonal osteogenic sarcoma cells (UMR 108). High pressure liquid chromatography of extracts of culture media after incubation of cells (see Materials and Methods); every fraction was assayed by radioimmunoassay for each metabolite (6-oxo-$PFG_{1\alpha}$, TxB_2, $PGF_{2\alpha}$, and PGE_2); immunologic activity with each assay was detected only in the region of column effluent corresponding to the appropriate tracer. Upper panel: radioimmunoassay data. Lower panel: separation of radioactive metabolites.

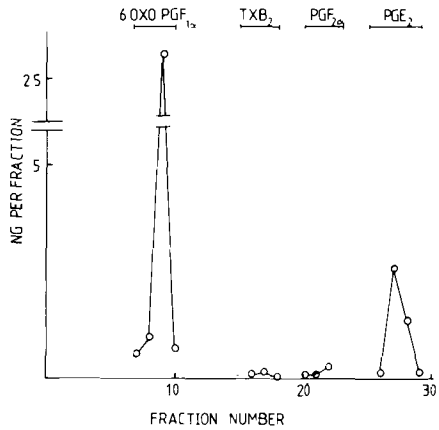

Fig. 2. Arachidonate metabolism in monolayer cultures of osteoblast-rich rat calvarial cells. Experimental method was as for Fig. 1 and in Materials and Methods.

Results

The major cyclo-oxygenase product of arachidonic acid metabolism in the clonal malignant osteoblasts and in the osteoblast-rich calvarial cells was 6-oxo-$PGF_{1\alpha}$, the hydration product of prostacyclin (PGI_2). Figures 1 and 2 illustrate the chromatographic profiles obtained with clone UMR 106 and with osteoblast-rich normal rat calvarial cells after radioimmunoassay of column fractions with each of the four assays. Data from an experiment with all four clonal lines and the normal osteoblast-rich cells are shown in Table 1, indicating that PGI_2 (as measured by 6-oxo-$PGF_{1\alpha}$), PGE_2, and $PGF_{2\alpha}$ were produced by these several cell strains, and furthermore that 6-oxo-$PGF_{1\alpha}$ was the predominant product. Thromboxane B_2 (TxB_2), the stable hydration product of thromboxane A_2, was not detected in significant amounts in any extracts of media whether or not arachidonic acid was included in the incubations. Although clone UMR 105 produced less 6-oxo-$PGF_{1\alpha}$ than PGE_2 in the experiment shown in Table 1, this was not so in repeated experiments in which the prostaglandin products resembled those from the other clonal lines. Extractions of fresh media with and without added arachidonate were carried out with each separate experiment, and no prostanoids were detected.

Other controls included experiments which showed that acidification and extraction of medium did not in any way modify the chromatographic behavior of the ^{3}H-labeled prostanoids. Moreover, no evidence for degradation of PGE_2 under the conditions of these experiments was found, since incubation of [^{3}H]-PGE_2 with either calvarial cells or

Table 1. Cyclo-oxygenase products of arachidonic acid metabolism[a] in osteoblast-like cells

Cells	Prostanoid production (ng per 10^6 cells in 4 h)			
	6-oxo-$PGF_{1\alpha}$	PGE_2	$PGF_{2\alpha}$	TxB_2
UMR 104	1.68	0.56	0.41	<0.05
UMR 105	0.95	1.39	0.68	<0.05
UMR 106	1.56	0.78	0.76	<0.05
UMR 108	2.11	0.63	0.11	<0.05
Osteoblast-rich calvarial cells	29.5	4.9	0.34	<0.05

[a] Cells were incubated for 4 h in medium containing arachidonic acid (10 μg per ml) without fetal calf serum.

Table 2. Cyclo-oxygenase products of arachidonic acid metabolism in cloned osteogenic sarcoma cells[a], incubated with fetal calf serum

Cells	Prostanoid production (ng per 10^6 cells in 4 h)		
	6-oxo-$PGF_{1\alpha}$	PGE_2	$PGF_{2\alpha}$
UMR 104	96.6	2.6	16.6
UMR 105	39.2	5.8	17.1
UMR 106	98.8	1.3	14.5
UMR 108	43.1	6.3	32.4

[a] Cells were incubated for 4 h in medium containing arachidonic acid (10 μg per ml) with 10% fetal calf serum.

cloned osteosarcoma cells, with or without fetal calf serum, did not yield any PGE_2 breakdown products. Finally, incubation of osteoblast-rich calvarial cells in culture for 24 hr with [^{3}H]-6-oxo-$PGF_{1\alpha}$ did not result in the generation of any labeled species other than 6-oxo-$PGF_{1\alpha}$ upon high pressure liquid chromatography of acidified, extracted medium (data not shown).

When cells were incubated in media including fetal calf serum, prostanoid production was very much greater (Table 2), but the predominance of 6-oxo-$PGF_{1\alpha}$ as a product was maintained. Under these conditions, the amounts of $PGF_{2\alpha}$ released into media were greater than those of PGE_2. In those experiments, data from TxB_2 was not included because it was present in large amounts in fetal calf serum.

Discussion

It has been shown in cultured fetal rat long bones [6] and in cultured mouse calvaria [19] that both PGE_2 and 6-oxo-$PGF_{1\alpha}$ are released from resorbing bones. In resorbing mouse calvaria, small amounts of TxB_2 were also found in the media after stimulation of bones with epidermal growth factor. In the present work with cloned malignant osteoblasts and with osteoblast-rich normal rat bone cells, no significant thromboxane was detected in media after incubation with or without arachidonic acid. It is possible that thromboxane production by osteoblasts might require other stimuli. Alternatively, thromboxane production in bone might arise from osteoclasts. There would be no osteoclasts in our clonal lines and if there were any in the osteoblast-rich cultures it would be at an extremely low level of contamination.

PGE_2 is the most potent of the prostanoids in resorbing bone [20–22]. In most hands, $PGF_{2\alpha}$ is of fairly low potency, although it has been suggested by one group to be almost as effective as PGE_2 [23]. $PGF_{2\alpha}$ production by osteoblasts was shown in the present experiments, with greater amounts produced in media containing fetal calf serum. In resorbing mouse bone, less $PGF_{2\alpha}$ than PGE_2 was formed [19]. PGI_2 has been shown to resorb bone [6], and although it is difficult from those experiments to ascribe a potency to it, it seems likely that it is less potent than PGE_2. Certainly, in short incubations with normal or malignant osteoblasts, PGI_2 is less potent than PGE_2 in stimulating cyclic adenosine monophosphate (AMP) formation [10, 24, 25], and on this basis we predicted it would be a less potent bone resorber [24].

EGF stimulates resorption in mouse calvaria by a process requiring prostanoid synthesis within bone [4], although this requirement was not found in experiments with cultured rat long bones [26]. Moreover, antibody-mediated bone resorption requires prostanoid synthesis [5]. It is possible that prostanoids generated within bone are important mediators of the action of some bone resorbing agents, and they may even have a role in normal bone remodeling processes. The present work demonstrates that osteoblasts produce predominantly PGI_2 (as measured by its stable hydration product) with lesser amounts of PGE_2 and $PGF_{2\alpha}$, but no evidence was obtained for production of significant amounts of thromboxane. These generated prostanoids may act on other cells within bone (e.g., osteoclasts) or upon osteoblasts themselves, in the manner proposed for endothelial cell prostacyclin [27]. We have found PGE_2 and prostacyclin to have effects on cyclic AMP metabolism in osteoblasts very similar to those of parathyroid hormone (PTH) [10, 25], and this might suggest that the prostanoids and PTH share a common pathway of action on bone resorption. The osteoblast produces more PGI_2 than it does PGE_2, but it does seem likely that PGI_2 is a less potent bone resorber than PGE_2. It is possible that PGE_2 is the more important bone resorbing prostanoid, and any effect of PGI_2 is simply

through a PGE_2-like action. This would fit with the fact that PGI_2 is 5–10% as effective as PGE_2 in stimulating cyclic AMP formation in bone cells [10, 24, 25], whereas in a genuinely PGI_2-responsive tissue (e.g., platelets), PGI_2 is far more potent than other prostanoids [28].

If any other local function of PGI_2 is needed to explain its formation in significant amounts by osteoblasts, it might have a role in the regulation of blood flow in vessels and capillaries in bone, especially since it has such effects in several other tissues.

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