Biochimica et Biophysica Acta, 972 (1988) 339–346
Elsevier

BBA 12371

Possible induction of fatty acid cyclooxygenase in mouse osteoblastic cells (MC3T3-E1) by cAMP

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(Received 20 June 1988)

Key words: Prostaglandin; Fatty acid cyclooxygenase; Epinephrine; Bone resorption; (Mouse osteoblast cells)

Prostaglandin E_2 (PGE_2), a bone-resorption factor, was essentially the sole arachidonate metabolite in an osteoblastic cell line cloned from mouse calvaria (MC3T3-E1). When the cells were cultured in the presence of 2% newborn bovine serum, 1 μM epinephrine markedly stimulated PGE_2 synthesis from endogenous arachidonic acid. The PGE_2 synthesis commenced after a lag phase of 1–2 h, and reached a maximum at about 3 h after the addition of epinephrine. The effect of epinephrine was inhibited by propranolol, and epinephrine could be replaced by isoproterenol, suggesting β-adrenergic stimulation of PGE_2 production. A rapid increase in intracellular cAMP was observed upon the addition of epinephrine. When the intracellular cAMP level was raised using cholera toxin or forskolin, the PGE_2 synthesis was also stimulated. The enhanced PGE_2 synthesis was attributed to an increased level of cyclooxygenase, which was shown by immunoprecipitation of the enzyme using anti-cyclooxygenase antibody. Inhibitors of transcription and translation suppressed the epinephrine-dependent increase in cyclooxygenase activity. These findings suggest induction of cyclooxygenase involving cAMP via an as yet unclarified mechanism.

Introduction

A clonal osteogenic cell line, MC3T3-E1, was established from newborn mouse calvaria by Kodama et al. [1]. This cell line differentiates into an osteoblast, and mineralizes in vitro [1,2]. The parameters of bone formation (alkaline phosphatase activity, collagen and DNA synthesis) changed in response to a variety of hormones and growth factors [3–5]. We have utilized this unique and useful cell line to study the regulatory mechanism of the biosynthesis of prostaglandin E_2, a bone-resorption factor. As previously reported [6], our studies at the enzyme level demonstrated a stimulatory effect of epidermal growth factor (EGF) on the PGE_2 synthesis presumably by the induction of fatty acid cyclooxygenase, a key enzyme for PGE_2 biosynthesis.

Experimental procedures

Materials. Authentic prostaglandins and thromboxane B_2 were kindly provided by the Ono Re-

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Abbreviations: IBMX, 3-isobutyl-1-methylxanthine; PG, prostaglandin; EGF, epidermal growth factor; α-MEM, α-modified Eagle's minimum essential medium; HPLC, high-performance liquid chromatography; GC-SIM, gas chromatography-selected ion monitoring.

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search Institute, and the radioimmunoassay kits for cAMP were provided by Yamasa Shoyu Co. (Chiba). Epinephrine bitartrate, isoproterenol bitartrate, propranolol hydrochloride, forskolin, dibutyryl cAMP, dibutyryl cGMP, dibutyryl cCMP, 8-bromo-cAMP, cAMP, 3-isobutyl-1-methylxanthine (IBMX), actinomycin D, α-amanitin, puromycin and penicillin G were obtained from Sigma (St. Louis). Phenylephrine hydrochloride, cycloheximide, tryptophan and GSH were from Wako (Osaka), while norepinephrine bitartrate and phenoxybenzamine hydrochloride were from Tokyo Chemical Industry (Tokyo). Tween 20 was from Nakarai Chemical (Kyoto). Cholera toxin was supplied by List Biological Laboratories (Campbell). [5,6,8,11,12,14,15(n)-^{3}H]PGE_2 (160 Ci/mmol) and [1-^{14}C]arachidonic acid (59.6 mCi/mmol) were purchased from Amersham International (Amersham). Arachidonic acid was from Nu-Chek-Prep (Elysian), 9-anthryldiazomethane was from Funakoshi (Tokyo), α-modified Eagle's minimum essential medium (α-MEM) was from Flow Laboratories (Irvine), fetal bovine serum was from M.A. Bioproducts (Walkersville, Maryland), newborn bovine serum was from Flow Laboratories (North Ryde), actinase E was from Kaken Chemical (Tokyo), plastic dishes (100 mm and 35 mm) for tissue culture from Corning Glass Works (Corning), silica gel 60, F_{254} thin-layer plates were from Merck (Darmstadt), silicic acid for column chromatography was from Mallinckrodt (Paris, KY), and Sep-Pak C_{18} and Sep-Pak silica cartridges were from Waters (Milford). All other reagents were of analytical grade.

[1-^{14}C]PGH_2 was prepared by the method described previously [7] using sheep seminal vesicles instead of bovine seminal vesicles. PES-5 (monoclonal anti-bovine cyclooxygenase antibody) [8], was prepared in the peritoneal cavity of mouse and purified by ammonium sulfate precipitation and chromatography using protein A-Sepharose (Pharmacia). Protein A-bearing Cowan I strain of *Staphylococcus aureus* (NCTC 8530) was a gift from Dr. W.L. Smith of Michigan State University. The bacterial cells were fixed in 1.5% formaldehyde and heat-inactivated at 80°C for 5 min [9].

Cell culture. The MC3T3-E1 cell line was generously provided by Dr. H. Kodama of Nippon Zen-yaku Kogyo Co. The cell culture was performed according to the procedures described previously [6]. Briefly, $5 \cdot 10^4$ cells were plated in 35-mm dishes containing 2 ml of α-MEM supplemented with 10% fetal bovine serum and 100 U/ml of penicillin G (medium A). After 4 days, the cells were transferred to medium A containing 2% newborn bovine serum instead of 10% fetal bovine serum (medium B).

Radioimmunoassay of PGE_2. PGE_2 was determined by radioimmunoassay using monoclonal anti-PGE_2 antibody [10] according to the procedures described previously [6].

High-performance liquid chromatography of PGE_2. Culture medium (2 ml) was acidified to pH 3–4 with 1 M HCl, and applied onto a Sep-Pak C_{18} cartridge. Prostaglandins were eluted by the method of Powell [11], but with the substitution of methanol for methyl formate. The methanol of the prostaglandin fraction was evaporated, and the residue was dissolved in 600 μl of methanol/water/acetic acid (65 : 35 : 0.01, solvent A). A 200-μl aliquot was applied to reverse-phase high-performance liquid chromatography (HPLC) with Nova-Pak C_{18} Radial-Pak cartridge (8 × 100 mm, Waters) connected to a Waters dual pump model 510 and a Waters injector model U6K. Elution was carried out with solvent A for 30 min and with methanol for the following 20 min. Eluate was collected every 1 min. The solvent of a 20-μl aliquot was evaporated, and the residue was subjected to radioimmunoassay for PGE_2. For conversion of PGE_2 to PGB_2, the dried residue of the fraction containing PGE_2 was subjected to alkali treatment [12]. For fluorometric detection of prostaglandins, the Sep-Pak C_{18} extract from the culture medium was treated with 9-anthryldiazomethane by the previously described method [6] and analyzed by HPLC using a solvent mixture of acetonitrile/water/acetic acid (9 : 5 : 0.01).

Radioimmunoassay of cAMP. Cells were incubated with various drugs in the presence of 0.1 mM IBMX at 37°C for 5 min. Then, the medium was removed and cold 6% trichloroacetic acid (0.5 ml) was added. Cells were scraped off, and dispersed by sonication, followed by centrifugation at 1700 × *g* for 15 min at 4°C. Trichloroacetic acid in the supernatant was extracted three times with 3 ml of diethyl ether saturated with distilled water

and the water phase was subjected to radioimmunoassay for cAMP according to the method of Honma et al. [13].

Assays of cyclooxygenase and PGE synthase. The cyclooxygenase assay mixture (100 μl) contained 0.1 M Tris-HCl (pH 8.0), 2 μM hematin, 5 mM tryptophan and 20 μM [1-^{14}C]arachidonic acid. The PGE synthase assay mixture contained 0.1 M Tris-HCl (pH 8.0), 2 mM GSH and 20 μM [1-^{14}C]PGH_2. The reactions were carried out at 24° C. Extraction, thin-layer chromatography and radioactivity determination were performed as described previously [14]. Whole cells cultured for 4 days were incubated for 3 h in α-MEM containing 2% newborn bovine serum with or without 1 μM epinephrine, and then suspended in phosphate-buffered saline containing 0.001% actinase E and 0.2% EDTA. An appropriate number of cells were subjected to the cyclooxygenase assay for 1 min and to the PGE synthase assay for 20 s. Microsomes were prepared as described previously [6] and incubated for 1 min for the assay of both enzyme activities.

Immunoprecipitation of cyclooxygenase. The cells were incubated for 3 h in the absence or presence of epinephrine (1 μM), and then harvested with phosphate-buffered saline containing 1 mM EDTA with the aid of a rubber policeman. The cells were precipitated by centrifugation and suspended in 1.2 ml of 0.2 M Tris-HCl (pH 8.0). The cell suspension was sonicated in four bursts each of 20 kHz for 15 s, and mixed with Tween 20 at 0.5% final concentration. After 30 min on ice with occasional shaking, the mixture was centrifuged at 200000 × *g* for 40 min. The supernatant was used as the solubilized enzyme. A 10% suspension of *S. aureus* (25 μl) and a solution of PES-5 (10 μg IgG in 200 μl) were incubated at room temperature for 5 min, followed by the addition of 2 ml of phosphate-buffered saline. After centrifugation at 1500 × *g* for 10 min, the precipitate was suspended in 50 μl of phosphate-buffered saline, and the suspension was mixed with 100 μl of the solubilized enzyme (336 μg protein of the epinephrine-treated preparation and 233 μg protein of the control preparation). After incubation at room temperature for 30 min, the mixture was centrifuged at 1500 × *g* for 5 min. The precipitate was washed twice with 2 ml of phosphate-buffered saline containing 0.05% Tween 20 and the cyclooxygenase activity of the immunoprecipitate was determined.

Gas chromatography-mass spectrometry. PGE_2 was extracted from 6 ml of the culture medium using a Sep-Pak C_{18} cartridge. The extract was purified by silicic acid column chromatography and HPLC using a μBondapak C_{18} column. PGE_2 was derivatized with diazomethane, methoxyamine and dimethylisopropylsilyl imidazole as described for 6-keto-$PGF_{1\alpha}$ [15]. Gas chromatography-selected ion monitoring (GC-SIM) was performed using a Hitachi M-80B gas chromatograph-mass spectrometer essentially as described previously [6].

Results

Stimulation of endogenous PGE_2 synthesis by epinephrine

MC3T3-E1 cells were incubated with 1 μM epinephrine in medium B for 3 h. Then, arachidonate metabolites released into the culture medium were subjected to fluorometric analysis by reverse-phase HPLC. In the extract from the medium of epinephrine-treated cells, a prominent fluorescent peak co-chromatographed with authentic PGE_2. Furthermore, the extract from the culture medium was applied to reverse-phase HPLC, and the fractions were monitored by radioimmunoassay for PGE_2. The only immunoreactive peak was found at the retention time of authentic PGE_2. The conversion of the immunoreactive compound to PGB_2 by alkali treatment, suggested its identity with PGE_2. Identity of the immunoreactive material with PGE_2 was also confirmed by GC-SIM.

Arachidonate metabolites other than PGE_2 were not detected in significant amounts by fluorometric analysis. Epinephrine-stimulated cells or the microsomes prepapred from the cells transformed neither [1-^{14}C]arachidonic acid nor [1-^{14}C]PGH_2 to metabolites other than PGE_2.

As shown in Fig. 1, in the presence of 1 μM epinephrine an increase in the PGE_2 level in the medium started at 1–2 h and reached a maximum by 3 h. The basal level of PGE_2 (in the absence of epinephrine) remained unchanged even after 24 and 48 h. Fig. 2 shows the effects of various concentrations of epinephrine on the release of

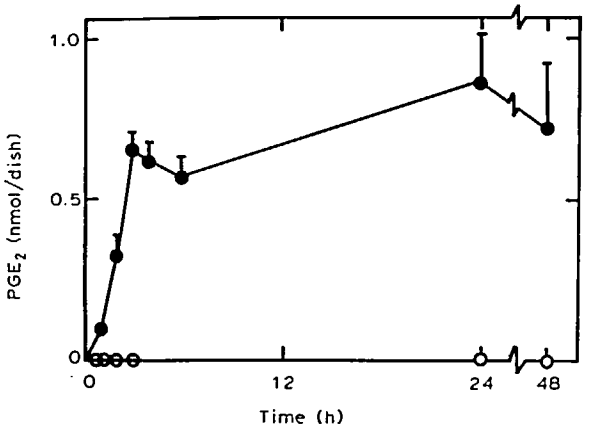

Fig. 1. Time course of epinephrine-dependent PGE_2 synthesis by MC3T3-E1 cells. Cells were precultured to confluency and then the culture medium was replaced by medium B in the presence (●) or absence (○) of 1 μM epinephrine. Aliquots of the culture medium were removed at intervals for radioimmunoassay of PGE_2. Data are means ± S.D. of quadruplicate determinations.

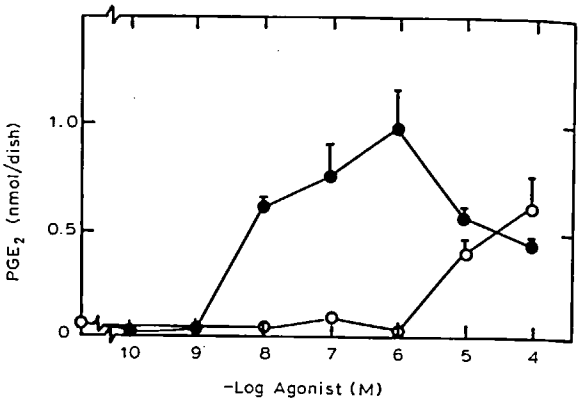

Fig. 3. Effects of α- and β-adrenergic agonists on PGE_2 synthesis by MC3T3-E1 cells. Cells were precultured to confluency and then the culture medium was replaced by medium B containing phenylephrine (○) or isoproterenol (●) at the indicated concentrations. After incubation for 3 h, aliquots of the culture medium were subjected to radioimmunoassay for PGE_2. Data are means ± S.D. of quadruplicate determinations.

PGE_2 for 3 h. The optimal concentration of epinephrine was 0.1–1 μM.

Effect of adrenergic agonists and antagonists on PGE_2 synthesis

When 1 μM epinephrine was replaced by norepinephrine, much higher concentrations (10–100 μM) of the latter compound were necessary for stimulation of PGE_2 synthesis. As illustrated in Fig. 3, isoproterenol, a β-agonist, was as active as epinephrine in stimulating PGE_2 synthesis. Its optimal concentration was about 1 μM. On the other hand, phenylephrine, an α-agonist, was almost inactive at 1 μM. Furthermore, 1 μM propranolol, a β-antagonist, abolished the stimulatory effect of 1 μM epinephrine, but 10 μM phenoxybenzamine, an α-antagonist, had no effect.

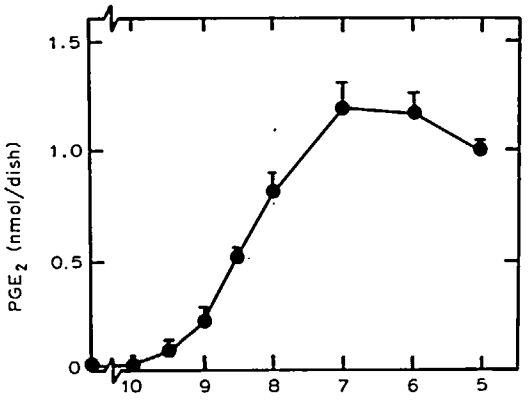

Fig. 2. Effect of epinephrine concentrations on PGE_2 synthesis by MC3T3-E1 cells. Cells were precultured to confluency and then the culture medium was replaced by medium B containing various amounts of epinephrine as indicated. After a 3-h incubation, aliquots of the culture medium were subjected to radioimmunoassay for PGE_2. Data shown are means ± S.D. of quadruplicate determinations.

Relationship between PGE_2 synthesis and cAMP production

Epinephrine markedly stimulated cAMP production by MC3T3-E1 cells. As shown in Fig. 4, cAMP levels within the cells reached a maximum 5 min after the addition of epinephrine. Then, cAMP within the cells decreased and was released gradually into the medium. Fig. 5 shows the effect of the epinephrine concentration on cAMP production, which almost reached a maximum with 1 μM epinephrine.

Compounds known to increase intracellular cAMP, were tested with MC3T3-E1 cells. Forskolin and cholera toxin, which actually increased the cAMP level within the MC3T3-E1 cells, stimulated PGE_2 synthesis in a dose-dependent

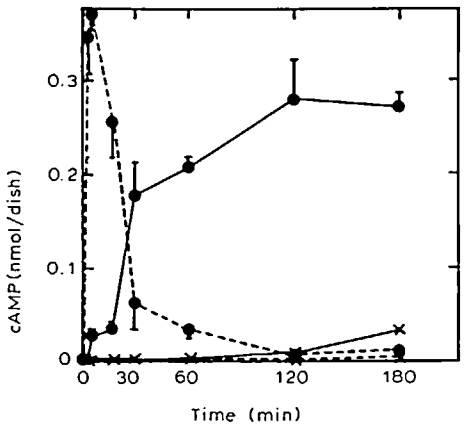

Fig. 4. Time course of epinephrine-dependent cAMP synthesis by MC3T3-E1 cells. Cells were precultured to confluency and then the culture medium was replaced by medium B containing 0.1 mM IBMX with (●) or without (×) 1 μM epinephrine. After incubation for the indicated period of time, the mediuum (———) and the cell extracts with trichloroacetic acid (- - - - - -) were subjected to radioimmunoassay for cAMP. Data are means ± S.D. of quadruplicate determinations.

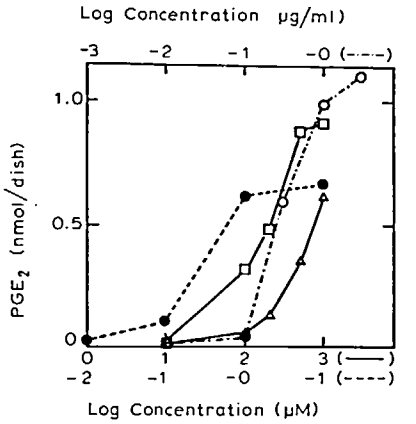

Fig. 6. Effect of increased endogenous cAMP on PGE_2 synthesis by MC3T3-E1 cells. Cells were precultured to confluency and then the medium was replaced by medium B containing various compounds as indicated. After a 3-h incubation, aliquots of the medium were subjected to radioimmunoassay for PGE_2. Data are mean values of quadruplicate determinations. Cholera toxin (○); forskolin (●); dibutyryl cAMP (△); 8-bromo-cAMP (□).

manner (Fig. 6). Dibutyryl cAMP and 8-bromo-cAMP were also stimulatory while dibutyryl cGMP and dibutyryl cCMP (0.01–2 mM) had no effect. cAMP added as such to the culture medium did not stimulate PGE_2 production.

Possible induction of cyclooxygenase by intracellular cAMP

MC3T3-E1 cells were incubated with 0.2 mM aspirin (an irreversible cyclooxygenase inhibitor) for 18 h and then aspirin was washed out. The

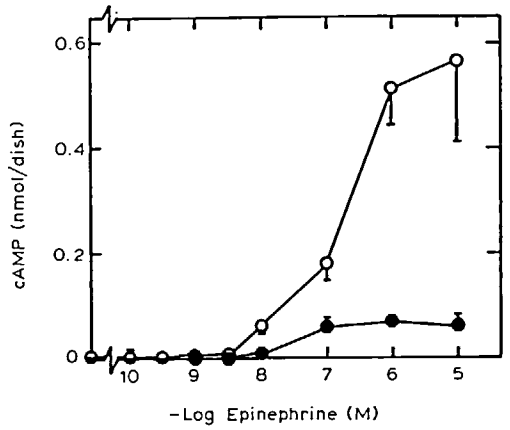

Fig. 5. Effect of epinephrine concentration on cAMP production by MC3T3-E1 cells. Cells were precultured to confluency and then the culture medium was replaced by medium B containing 0.1 mM IBMX and epinephrine at the indicated concentrations. After incubation for 5 min, the medium (●) and the cell extracts with trichloroacetic acid (○) were subjected to radioimmunoassay for cAMP. Results are means ± S.D. of quadruplicate determinations.

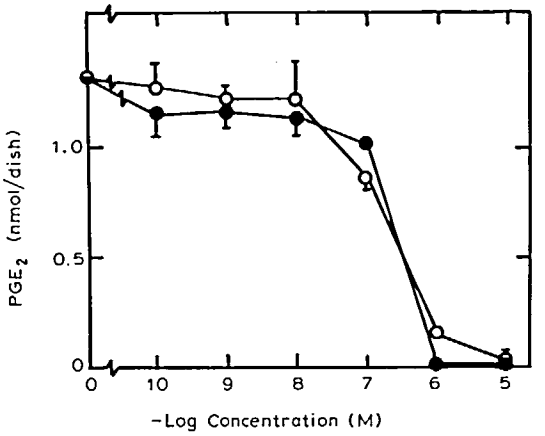

Fig. 7. Effect of transcription and translation inhibitors on epinephrine-dependent PGE_2 synthesis by MC3T3-E1 cells. Cells were precultured to confluency and then the culture medium was replaced by medium B containing 1 μM epinephrine in the presence of various amounts of actinomycin D (●) or cycloheximide (○). After a 3-h incubation, PGE_2 in the culture medium was determined by radioimmunoassay. Results are expressed as mean ± S.D. of quadruplicate determinations.

aspirin-treated cells started to synthesize PGE_2 by the addition of epinephrine (1 μM) with a time course similar to that of the non-treated cells. As shown in Fig. 7, both cycloheximide (a translation inhibitor) and actinomycin D (a transcription inhibitor) at a concentration of about 1 μM inhibited the PGE_2 synthesis stimulated by epinephrine. Puromycin (a translation inhibitor) and α-amanitin (a transcription inhibitor) also had inhibitory effects on the PGE_2 synthesis. Furthermore, PGE_2 synthesis stimulated by 2 mM dibutyryl cAMP was abolished by cycloheximide (1 μM) and actinomycin D (0.3 μM). On the other hand, cycloheximide and actinomycin D did not inhibit cAMP production stimulated by 1 μM epinephrine.

MC3T3-E1 cells were incubated with or without 1 μM epinephrine for 3 h. The cells were harvested with the aid of actinase E, and incubated with arachidonic acid or PGH_2 for the assay of cyclooxygenase (arachidonic acid $\rightarrow$ PGH_2) or PGE synthase ($PGH_2 \rightarrow PGE_2$), respectively. As shown in Table I, epinephrine stimulated the activity of cyclooxygenase, but not of PGE synthase. The effect of epinephrine on cyclooxygenase activity was completely abolished by the simultaneous presence of actinomycin D, cycloheximide or propranolol. The microsomes prepared from the epinephrine-treated cells showed a much higher activity of cyclooxygenase than the preparation from the control cells. The microsomal PGE synthase activity was not affected by epinephrine treatment.

Furthermore, we measured the activity of precipitable cyclooxygenase using an anti-cyclooxygenase antibody, results are shown in Table II. Epinephrine increased the activity of cyclooxygenase, which was solubilized by the use of a non-ionic detergent. Almost all the solubilized cyclooxygenase was precipitated with the aid of antibody.

TABLE I

ACTIVITIES OF CYCLOOXYGENASE AND PGE SYNTHASE IN MC3T3-E1 CELLS

Cells were precultured to confluency and then the culture was continued with medium B in the presence or absence of 1 μM epinephrine. Actinomycin D, cycloheximide or propranolol was added, each at a concentration of 1 μM. After a 3-h incubation, the whole cells or the microsomes were incubated with 20 μM [1-^{14}C]arachidonic acid or [1-^{14}C]PGH_2 for cyclooxygenase or PGE synthase assay, respectively. Data shown are means $\pm$ S.D. of quadruplicate determinations (whole cells) or duplicate determinations (microsomes).

Enzyme preparation	Cyclooxygenase activity (nmol PGH_2/min per 10^6 cells)	PGE synthase activity (nmol PGE_2/20 s per 10^5 cells)
Whole cells		
Control	0.22±0.03	0.69±0.06
Epinephrine	1.70±0.24	0.62±0.04
Epinephrine+ actinomycin D	0.24±0.06	0.66±0.06
Epinephrine+ cycloheximide	0.30±0.05	0.63±0.08
Epinephrine+ propranolol	0.22±0.07	0.64±0.16
Microsomes from control cells		
	(nmol PGH_2/min)	(nmol PGE_2/min)
40 μg protein	0.03	
80 μg protein	0.06	
4 μg protein		0.18
8 μg protein		0.33
Microsomes from epinephrine-treated cells		
40 μg protein	0.13	
80 μg protein	0.28	
4 μg protein		0.20
8 μg protein		0.37

TABLE II

IMMUNOPRECIPITATION OF CYCLOOXYGENASE IN MC3T3-E1 CELLS

Cells were precultured to confluency and then the medium was replaced by medium B. After a 3-h incubation in the presence or absence of 1 μM epinephrine, the cells were harvested and washed with phosphate-buffered saline containing 1 mM EDTA. A suspension of 1.4×10^8 cells in 1.2 ml of 0.2 M Tris-HCl buffer at pH 8.0 was treated with 0.5% Tween 20 followed by centrifugation at 200000$\times g$ for 40 min. The solubilized enzyme (100 μl) was mixed with a monoclonal anti-cyclooxygenase antibody (PES-5) bound to *S. aureus* as described in Experimental procedures. After centrifugation, the precipitate was assayed for cyclooxygenase activity.

Treatment	Cyclooxygenase activity (nmol/2 min)	
	control	epinephrine
Solubilized	0.06	1.00
Precipitate	0.00	0.86
Supernatant	0.04	0.13

Discussion

The function of epinephrine in the cellular signal transduction is generally understood in terms of a specific receptor in the cell membrane and intracellular cAMP as a second messenger. We carried out several experiments to find out which type of adrenergic receptor is involved in the stimulation of PGE_2 production in MC3T3-E1 cells and the results suggest that the epinephrine-dependent PGE_2 production is mediated by a β-receptor. (i) As a stimulant of PGE_2 synthesis, norepinephrine with higher α-activity and lower β-activity was inferior to epinephrine with higher β-activity and lower α-activity; (ii) isoproterenol (a β-agonist) was almost as active as epinephrine, while phenylephrine (an α-agonist) was much less active; (iii) phenoxybenzamine (an α-antagonist) was inactive as an inhibitor of the epinephrine-stimulated PGE_2 synthesis, whereas propranolol (a β-antagonist) was a potent inhibitor.

As usually occurs with other cells, the intracellular cAMP increased to a maximum level within 5 min of the addition of epinephrine to MC3T3-E1 cells. Thus, cAMP production preceded PGE_2 synthesis, which started after 1–2 h. This raised the question of whether the increased PGE_2 synthesis was a consequence of the enhanced cAMP production. To answer this question we utilized 8-bromo-cAMP and dibutyryl cAMP (membrane-permeable analogs of cAMP) and cholera toxin and forskolin (cAMP-increasing agents). These compounds did stimulate PGE_2 synthesis as well as epinephrine. These findings suggest that the binding of epinephrine to a β-receptor activates adenylate cyclase and the cAMP produced stimulates the PGE_2 synthesis.

As is well known for platelets [16] and leukocytes [17], the release of arachidonic acid followed by its further conversion to bioactive oxyeicosanoids is a rapid event occurring within several minutes after a certain physiological stimulus is given to the cell. In this case, the activation of phospholipase triggers the release of arachidonic acid, which is immediately metabolized to prostaglandins and other bioactive oxyeicosanoids. However, the PGE_2 synthesis by MC3T3-E1 cells commenced 1–2 h after the addition of epinephrine to the culture medium. Therefore, we examined the possibility of induction of an enzyme at a step in the PGE_2 synthesis. The experiment with aspirin, which inhibited cyclooxygenase irreversibly, ruled out the possibility that the constitutive latent cyclooxygenase was activated by epinephrine or cAMP. Additionally, inhibitors of both transcription and translation decreased the epinephrine- or cAMP-dependent synthesis of PGE_2. Furthermore, in the series of experiments in which cyclooxygenase was solubilized from MC3T3-E1 cells and incubated with anti-cyclooxygenase antibody, the immunoprecipitate showed almost all the activity of cyclooxygenase which was increased by the epinephrine treatment of the cells. By western blot analysis, the epinephrine-stimulated cells showed a larger amount of cyclooxygenase protein than the control cells (Oshima, T., et al., unpublished results). Thus several lines of evidence suggest the induction of cyclooxygenase by a mechanism involving cAMP, with a role for cAMP in the activation and expression of the cyclooxygenase gene. A similar stimulatory effect of cAMP on PGE_2 synthesis has been reported for human amnion cells, but increased cyclooxygenase activity was not mentioned [18,19].

A well-known function of cAMP as the second messenger is the activation of protein kinase leading to phosphorylation of various proteins and enzymes. Another function of cAMP was found earlier in bacterial cells, in which cAMP plays a role in positive transcriptional control with the aid of a specific receptor protein [20]. The cAMP-mediated induction has recently been reported for mammalian enzymes and bioactive proteins: phospho*enol*pyruvate carboxykinase of rat liver cytosol [21–23], bovine and human vasoactive intestinal polypeptide [24,25], rat somatostatin [26], human chorionic gonadotropin-α [27,28] and proenkephalin [29]. Using deletion mutants, the region of the gene regulated by cAMP has been clarified and the homology among these polypeptides has been described [26,30–34]. These are attractive examples for us to elucidate the mechanism involved in the cAMP-mediated induction of cyclooxygenase in MC3T3-E1 cells. Our previous study on the same cell line demonstrated a possible induction of cyclooxygenase by EGF [6]. Since EGF did not increase the intracellular cAMP

(Kusaka, M., et al., unpublished data), separate mechanisms may be working in the cyclo-oxygenase induction by EGF and epinephrine.

A stimulatory effect of epinephrine on the production of PGE_2, well-known as a bone-resorption factor [35,36], was demonstrated by our in vitro experiments using MC3T3-E1 cells. However, its physiological significance awaits further investigations because, to the best of our knowledge, little is known concerning the direct involvement of epinephrine in the physiological control of bone formation and resorption.

Acknowledgments

We are grateful to Drs. H. Miyazaki and K. Yamashita for their expert assistance in selected ion monitoring of PGE_2, and to Dr. Michael McCabe for reading this manuscript. S.Y. was supported by grants-in-aid for scientific research from the Ministry of Education, Science and Culture and the Ministry of Health and Welfare of Japan, and grants from the Japanese Foundation of Metabolism and Diseases, Takeda Science Foundation, the Mochida Memorial Foundation, the Tokyo Biochemical Research Foundation and Pfizer Central Research (Nagoya).

References

1 Kodama, H., Amagai, Y., Sudo, H., Kasai, S. and Yamamoto, S. (1981) Jpn. J. Oral Biol. 23, 899–901.
2 Sudo, H., Kodama, H., Amagai, Y., Yamamoto, S. and Kasai, S. (1983) J. Cell Biol. 96, 191–198.
3 Kumegawa, M., Hiramatsu, M., Hatakeyama, K., Yajima, T., Kodama, H., Osaki, T. and Kurisu, K. (1983) Calcif. Tissue Int. 35, 542–548.
4 Kumegawa, M., Ikeda, E., Tanaka, S., Haneji, T., Yora, T., Sakagishi, Y., Minami, N. and Hiramatsu, M. (1984) Calcif. Tissue Int. 36, 72–76.
5 Hakeda, Y., Nakatani, Y., Kurihara, N., Ikeda, E., Maeda, N. and Kumegawa, M. (1985) Biochem. Biophys. Res. Commun. 126, 340–345.
6 Yokota, K., Kusaka, M., Ohshima, T., Yamamoto, S., Kurihara, N., Yoshino, T. and Kumegawa, M. (1986) J. Biol. Chem. 261, 15410–15415.
7 Yoshimoto, T., Yamamoto, S., Okuma, M. and Hayaishi, O. (1977) J. Biol. Chem. 252, 5871–5874.
8 Yoshimoto, T., Magata, K., Ehara, H., Mizuno, K. and Yamamoto, S. (1986) Biochim. Biophys. Acta 877, 141–150.
9 Kessler, S.W. (1976) J. Immunol. 117, 1482–1490.
10 Shono, F., Yokota, K., Horie, K., Yamamoto, S., Yamashita, K., Watanabe, K. and Miyazaki, H. (1988) Anal. Biochem. 168, 284–291.
11 Powell, W.S. (1980) Prostaglandins 20, 947–957.
12 Pike, J.E., Lincoln, F.H. and Schneider, W.P. (1969) J. Org. Chem. 34, 3552–3557.
13 Honma, M., Satoh, T., Takegawa, J. and Ui, M. (1977) Biochem. Med. 18, 257–273.
14 Miyamoto, T., Ogino, N., Yamamoto, S. and Hayaishi, O. (1976) J. Biol. Chem. 251, 2629–2636.
15 Tonai, T., Yokota, K., Yano, T., Hayashi, Y., Yamamoto, S., Yamashita, K. and Miyazaki, H. (1985) Biochim. Biophys. Acta 836, 335–343.
16 Rittenhouse-Simmons, S. and Deykin, D. (1981) in Platelets in Biology and Pathology (Gordon, J.L., ed.) pp. 349–372, Elsevier, Amsterdam.
17 Samuelsson, B. and Hammarström, S. (1982) Vitamins and Hormones 39, 1–30.
18 DiRenzo, G.C., Anceschi, M.M. and Bleasdale, J.E. (1984) Prostaglandins 27, 37–49.
19 Warrick, C., Skinner, K., Mitchell, B.F. and Challis, J.R.G. (1985) Am. J. Obstet. Gynecol. 153, 66–71.
20 Watson, J.D., Hopkins, N.H., Roberts, J.W., Steitz, J.A. and Weiner, A.M. (1987) Molecular Biology of the Gene, 4th Ed, pp. 478–479, Benjamin/Cummings Publishing Company, Menlo Park.
21 Cimbala, M.A., Lamers, W.H., Nelson, K., Monahan, J.E., Yoo-Warren, H. and Hanson, R.W. (1982) J. Biol. Chem. 257, 7629–7636.
22 Chrapkiewicz, N.B., Beale, E.G. and Granner, D.K. (1982) J. Biol. Chem. 257, 14428–14432.
23 Lamers, W., Meisner, H. and Hanson, R.W. (1982) Proc. Natl. Acad. Sci. USA 79, 5137–5141.
24 Eiden, L.E. and Hotchkiss, A.J. (1983) Neuropeptides 4, 1–9.
25 Hayakawa, Y., Obata, K., Itoh, N., Yanaihara, N. and Okamoto, H. (1984) J. Biol. Chem. 259, 9207–9211.
26 Montminy, M.R., Sevarino, K.A., Wagner, J.A., Mandel, G. and Goodman, R.H. (1986) Proc. Natl. Acad. Sci. USA 83, 6682–6686.
27 Darnell, R.B. and Boime I. (1985) Mol. Cell. Biol. 5, 3157–3167.
28 Burnside, J., Nagelberg, S.B., Lippman, S.S. and Weintraub, B.D. (1985) J. Biol. Chem. 260, 12705–12709.
29 Eiden, L.E., Giraud, P., Affolter, H.-U., Herbert, E. and Hotchkiss, A.J. (1984) Proc. Natl. Acad. Sci. USA 81, 3949–3953.
30 Short, J.M., Wynshaw-Boris, A., Short, H.P. and Hanson, R.W. (1986) J. Biol. Chem. 261, 9721–9726.
31 Tsukada, T., Fink, J.S., Mandel, G. and Goodmann, R.H. (1987) J. Biol. Chem. 262, 8743–8747.
32 Silver, B.J., Bokar, J.A., Virgin, J.B., Vallen, E.A., Milsted, A. and Nilson, J.H. (1987) Proc. Natl. Acad. Sci. USA 84, 2198–2202.
33 Deutsh, P.J., Jameson, J.L. and Habener, J.F. (1987) J. Biol. Chem. 262, 12169–12174.
34 Comb, M., Birnberg, N.C., Seasholtz, A., Herbert, E. and Goodmann, H.M. (1986) Nature 323, 353–356.
35 Raisz, L.G., and Kream, B.E. (1983) N. Engl. J. Med. 309, 83–89.
36 Harvey, W. and Meghji, S. (1986) Agents and Actions 19, 73–79.