Vol. 126, No. 1, 1985 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 16, 1985 Pages 340-345

PROSTAGLANDIN E_2 STIMULATES COLLAGEN AND NON-COLLAGEN PROTEIN SYNTHESIS AND PROLYL HYDROXYLASE ACTIVITY IN OSTEOBLASTIC CLONE MC3T3-E1 CELLS[1]

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SUMMARY: We investigated the stimulative effect of prostaglandin E_2 (PGE_2) on an osteoblastic cell line, clone MC3T3-E1, in serum-free medium. PGE_2 elevated collagen and non-collagen protein syntheses in a dose-related fashion up to 2 μg/ml, the maximal increases being 2- and 3-fold, respectively, over that in the control. Its stimulative effect was evident as early as 12 h. PGE_2 slightly increased DNA content, but its effect was less than that on collagen and non-collagen protein syntheses. Moreover, PGE stimulated an increase in prolyl hydroxylase activity with a maximal effect at 1-2 μg/ml, the activity being 15-fold over that of the control. These results strongly indicate that PGE_2 directly enhances total protein synthesis including that of collagen in osteoblasts *in vitro*, suggesting its direct effect on bone formation *in vivo* as well.

Prostaglandins (PGs) are considered to be a potent stimulator of bone resorption, which has been demonstrated by numerous studies (1-3). Moreover, PGs have been reported to inhibit collagen synthesis and citrate decarboxylation in bone organ culture and osteoblast-enriched cell culture (4,5). In contrast, other evidence has shown that PGs stimulate collagen synthesis *in vitro* (6,7) and *in vivo* (8). Thus, the effect of PGs on bone formation is complicated: it remains unclear whether PGs affect bone formation directly, or indirectly *via* PG-induced bone resorption, or both.

[1] This work was supported by a grant from the Ministry of Education, Science, and Culture of Japan.

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0006-291X/85 $1.50

We have previously reported that PGs induced an increase in alkaline phosphatase in osteoblastic clone MC3T3-E1 cells cultured in media containing (9) or lacking (10) serum, suggesting PGs' direct stimulative effect on the functional activity of osteoblasts. However, it is necessary to examine the effect of PGs on the synthesis of collagen, a major component of bone matrix, to know the action of PGs on bone formation.

In this study, therefore, we investigated the effect of prostaglandin E_2 (PGE_2) on collagen and non-collagen protein syntheses and prolyl hydroxylase activity in osteoblastic clone MC3T3-E1 cells cultured in serum-free medium.

MATERIALS AND METHODS

Materials: Prostaglandin E_2 (PGE_2) and bovine serum albumin (BSA) were obtained from Sigma Chemical Co., St. Louis, MO. α-MEM was purchased from Flow Laboratories, Dublin VA. Fetal bovine serum was obtained from Irvine Scientific, Santa Ana, CA. Tritiated proline (L-[2,3-^{3}H] and [3,4-^{3}H], 30-50 Ci/m mol) was from New England Nuclear Corp., Boston, MA.
Cell culture: Clone MC3T3-E1 cells were isolated from the MC3T3-E cell line which had been derived from newborn C57BL/6 mouse calvaria (11). 5×10^4 cells were plated in 35 mm plastic dishes in 2 ml of α-MEM containing 10% fetal bovine serum, cultured for 3 days, and were then incubated with serum-free α-MEM supplemented with 0.1% BSA and one I.U./ml insulin (Nobo Industry, A/S, Denmark). Insulin was added into the serum-free medium in order to maintain the basal cellular activity. After one day, the cells were transferred to the media with or without various concentrations of PGE_2 and further incubated for appropriate periods.
Collagen and non-collagen protein synthesis assay: After appropriate periods of PGE_2 treatment, the cells were labeled for 3 h in one ml of the serum-free medium containing 2 μCi of [^{3}H] proline and 50 μg each of sodium ascorbate and β-aminopropionitrile with the PGE_2. At the end of the labeling, the medium was removed and the cells were scraped from the dishes and homogenized. Proteins in both medium and cell homogenate were precipitated with 10% trichloroacetic acid and 1% tannic acid (final concentrations). The precipitates were washed twice with trichloacetic acid and tannic acid and twice with ice-cold acetone. The washed precipitates were dissolved in 0.2 N NaOH, neutralized, and then used to determine amounts of collagenase-digestible and non-collagen protein according to the method of Peterkofsky and Diegelmann (12).
Assays of prolyl hydroxylase activity and DNA and protein contents: The cells treated with various concentrations of

PGE_2 for 24 h were washed three times with PBS(-), scraped into 1 ml of 50 mM Tris-HCl (pH 7.5) containing 1 mM dithiothreitol, 5% glycerol, and 0.5 mM phenylmethylsulfonyl fluoride, and then disrupted twice by sonication for 2 min. The sonicates were centrifuged for 15 min at 15,000×g, and the supernatant was used for the enzyme assay. Prolyl hydroxylase activity was assayed by the method of Peterkofsky and DiBlasio (13) with [3,4-^{3}H]proline-labeled unhydroxylated collagen from chick embryos as substrate. DNA content was determined fluorometrically by the method of Kissane and Robins (14). Protein content was estimated by the method of Bradford (15).

RESULTS AND DISCUSSION

Clone MC3T3-E1 cells, plated at a initial density of 5×10^4 cells in 35-mm plastic dishes containing α-MEM supplemented with 10% fetal bovine serum, were cultured for 3 days until nearly confluent and then were cultured in serum-free medium containing 0.1% BSA plus one unit/ml of insulin to remove unknown factors and to maintain basal cellular functions. First we investigated the time-dependent effect of PGE_2 (2 μg/ml) on collagen and non-collagen protein syntheses in the cells under these conditions. PGE_2 increased significantly both collagen and non-collagen protein syntheses as early as 12 h after its addition. The maximal stimulative effect of PGE_2 on collagen synthesis was observed at day one, at which time values of the synthesis in PGE_2-treated and control cultures were 5.29±0.20 and 1.78±0.19 dpm 10^{-4}/3h/dish, respectively. Similarly non-collagen protein synthesis was elevated by PGE_2, and the maximal increase occurred at 24-48 h. Thus the effects of various concentrations of PGE_2 on collagen and non-collagen protein syntheses were investigated at 24 h after its addition. PGE_2 raised collagen synthesis in a dose-related fashion: the synthesis was significantly increased by 0.5 μg/ml of PGE_2 and rose to a maximal 2-fold increase with 2 μg/ml (Table 1). While, at concentrations over 2 μg/ml, PGE_2 inhibited the synthesis. Non-collagen protein synthesis was also increased

Table 1. Effect of PGE_2 on collagen and non-collagen protein syntheses and DNA content in clone MC3T3-E1 cells

	DNA content* (μg/dish)	Collagen synthesis (10^{-4}×dpm/3h/dish)	Non-collagen protein synthesis (10^{-4} dpm/3h/dish)
Control	3.70 ± 0.59	1.46 ± 0.11	1.79 ± 0.06
PGE_2 (μg/ml)			
0.1	4.03 ± 0.70	1.69 ± 0.19	2.47 ± 0.30[b]
0.5	3.79 ± 0.72	1.96 ± 0.14[a]	2.61 ± 0.14[b]
1.0	4.25 ± 0.83	2.31 ± 0.14[b]	4.24 ± 0.43[b]
2.0	4.58 ± 0.36	3.03 ± 0.26[b]	5.03 ± 0.97[b]
4.0	3.84 ± 0.45	0.77 ± 0.10[b]	3.65 ± 0.63[b]

Clone MC3T3-E1 cells were incubated in serum-free medium containing or lacking various concentrations of PGE_2 for one day and then transferred to and incubated in the labeling medium for 3 h.
Values are means±SD from 5 dishes.
* DNA contents were determined from 4 dishes.
a, $p<0.05$; b, $p<0.01$ compared to control cultures.

by PGE_2 in a dose-dependent manner. A maximal rise in the synthesis of 3-fold was elicited by 2 μg/ml of PGE_2.

Recently, Chyun and Raisz (7) have demonstrated that at a low concentration (10^{-7} M), PGE_2 can increase collagen and non-collagen protein syntheses in organ cultures of rat calvaria, probably by a mechanism dependent upon cell replication. The stimulative effect of PGE_2 on these syntheses in their study required a longer period (96 h) of prostaglandin treatment. Our findings partly coincide with their obsevations; i.e., PGE_2 stimulated collagen and non-collagen protein syntheses in MC3T3-E1 cells over a narrow range of concentrations. However, the stimulative effect of PGE_2 became significant as early as 12 h after its addition, suggesting its direct effect on collagen synthesis rather than on cell proliferation. In fact, PGE_2 did not increase DNA content much as shown in Table 1. Moreover, MC3T3-E1 cells consists of a single cell

Table 2. Effect of PGE_2 on proly hydroxylase activity in clone MC3T3-E1 cells

	Prolyl hydroxylase activity	
	(dpm/min/mg protein)	(dpm/min/μg DNA)
Control	112 ± 18	10.6 ± 2.4
PGE_2 (μg/ml)		
0.1	158 ± 40	22.2 ± 3.0[a]
0.5	693 ± 103[a]	74.4 ± 13.0[a]
1.0	1471 ± 119[a]	152.4 ± 25.8[a]
2.0	1391 ± 282[a]	112.2 ± 16.4[a]
4.0	862 ± 216[a]	79.9 ± 15.5[a]

Values are means±SD for 5 dishes.

a, $p<0.01$ compared to control cultures.

population retaining some osteoblastic features (16). These results suggest that PGE_2 has a direct and immediate stimulative effect on osteoblast differentiation *in vitro*.

Finally we examined the effect of PGE_2 on the activity of prolyl hydroxylase which is involved in the collagen maturation process (Table 2). PGE_2 caused a marked increase in the enzyme activity in the cells: the maximal elevation in the activity was observed at 1-2 μg/ml of PGE_2, about 15-fold over that of control. This result also indicates that PGE_2 has a direct stimulative effect on collagen synthesis in the cells, reflecting on bone formation. Taken together, the stimulative effect of PGE_2 on bone formation appears to be a direct one rather than compensatory to PGE_2-stimulated bone resorption.

ACKNOWLEDGMENTS

Clone MC3T3-E1 cells were generously provided by Dr. H. Kodama (Tohoku Dental University, Koriyama, Japan).

We would like to thank Dr. L.D. Frye for reviewing the manuscript.

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