PROSTAGLANDIN PRODUCTION BY NEUROBLASTOMA, GLIOMA AND FIBROBLAST CELL LINES; STIMULATION BY N^6, $O^{2'}$-DIBUTYRYL ADENOSINE 3':5'-CYCLIC MONOPHOSPHATE

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Received 28 June 1973
Revised version received 31 July 1973

1. Introduction

Prostaglandin E_1 (PGE_1) elevates the levels of intracellular adenosine 3':5'-cyclic monophosphate (cyclic AMP) in clonal lines of mouse neuroblastoma cells [1, 2], of fibroblasts [3, 4] and of hybrid cells involving cell lines derived from nervous tissues or fibroblasts [4]. If the cell lines preserve properties of the nervous tissue cells from which they were derived, their sensitivity to PGE_1 should reflect a physiological function. PGE compounds injected into an animal disappear very rapidly from the circulation [5], as they are oxidized to inactive derivatives [6]. Consequently, the PGE to which the cells would respond *in situ* would most probably have to be produced in the close neighbourhood of those cells. Therefore, it was of interest to investigate the capability of a neuroblastoma, a glioma and a fibroblast cell line to synthesize PGE. It has been shown that many actions of cyclic AMP can be mimicked by N^6,$O^{2'}$-dibutyryl adenosine 3':5'-cyclic monophosphate (dibutyryl cyclic AMP) [7–11]. The influence of this cyclic AMP analogue on the production of prostaglandins (PG) was measured in order to obtain information on the significance of cyclic AMP (and of neurohormones acting via cyclic AMP) in the regulation of PG synthesis.

All 3 cell lines essentially produce PGE; only minor amounts of PGF and PGA were detected. The amount of PGE synthesized per million cells was strongly increased in the presence of dibutyryl cyclic AMP. The drug markedly decreased the growth rates of the cell lines.

2. Methods

The three cell lines used were N4TG3, a 6-thioguanine resistant mutant [12] of mouse neuroblastoma clone N4 [13], C6-BU-1, a bromodeoxyuridine resistant mutant [12] of rat glioma clone C 6 [14] and B82, a bromodeoxyuridine resistant mutant L cell line [15]. The cells were grown at 37°C in Falcon plastic flasks (75 cm^2) using Dulbecco's Modified Eagle's Medium (Gibco), supplemented with 10% fetal bovine serum (Colorado Serum Co.), in an atmosphere of 90% air, 10% CO_2 and approximately 100% humidity. The medium for N4TG3 was supplemented with 0.1 mM 6-thioguanine (Sigma), that for C6-BU-1 and B82 with 0.1 mM bromodeoxyuridine (Sigma). The cells were subcultured by brief treatment with a 0.05% solution of trypsin (Nutritional Biochemicals Corp.) in medium D_1 adjusted to 340 mOsm by the addition of glucose and sucrose [16].

For measuring the PG production by a given cell line at different cell densities, two sets of flasks were inoculated with the same number of cells. The

medium in one of the sets was supplemented with 1 mM dibutyryl cyclic AMP (Sigma). Every day, starting the day after inoculation, one flask of each set was used for cell counting (hemocytometer) and determination of the viability (exclusion of nigrosin), another for quantitation of PG. The media in all other flasks were renewed daily. For determination of PG, the supernatant growth medium was extracted as described [17–19]. The cell layer was washed 3 times with 8 ml of medium D_1 [16]. As the washing fluids contained negligible amounts of PG, they were discarded. After breaking the flasks, the cells were scraped off and homogenized by sonication in medium D_1. One ml of cell homogenate was extracted with a mixture of 2 ml H_2O, 5 ml ethylacetate and 3 ml of a 3:3:1 mixture of ethylacetate:isopropanol:0.1 M HCl. The prostaglandins A, E and F were separated by chromatography on silicic acid and assayed by a radioimmunochemical method [19].

3. Results

Growth curves of the cell lines are shown in fig. 1 Dibutyryl cyclic AMP retards cell proliferation in all cases. The effect is most striking with the glioma line (fig. 1B), where the final cell density reached in the presence of the drug is only one seventh of that of the untreated controls. By the first day of incubation with the drug, the glioma cells start differentiating by extending long processes (fig. 2). No striking morphological changes were observed with lines N4TG3 and B82.

The cumulative PGE production per flask of cells is illustrated in fig. 3 (top). The amount of PGE per flask increases with time. It is only with line B82 (top, C) that the production per flask of PGE is far higher in the presence than in the absence of dibutyryl cyclic AMP. However, when the data are expressed as amount of PGE formed up to a given time per million cells, an entirely different picture

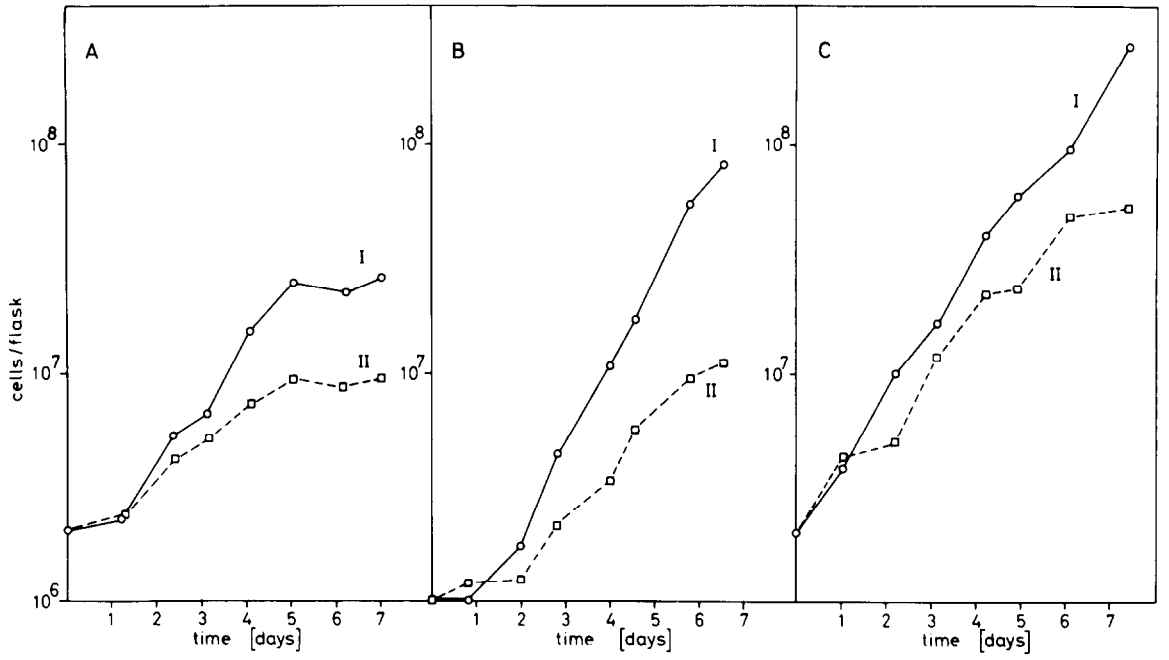


Fig. 1. Proliferation of cells of 3 clonal lines in the absence (curve I) and presence (curve II) of 1 mM dibutyryl cyclic AMP: A) neuroblastoma line N4TG3; B) glioma line C6-BU-1; C) fibroblast line B82. The ranges of cell viabilities were (data obtained in presence of dibutyryl cyclic AMP in parentheses): N4TG3, 87–95% (78–96%); C6-BU-1, 96–100% (91–100%); B82, 95–99% (95–99%). The total volumes of growth medium in the flask at the time the media were removed for assays of prostaglandins and for medium changes were 15 ml, except at the following times (rounded): N4TG3, days 5, 6, 7 (25 ml); C6-BU-1 and B82, day 7 (20 ml).

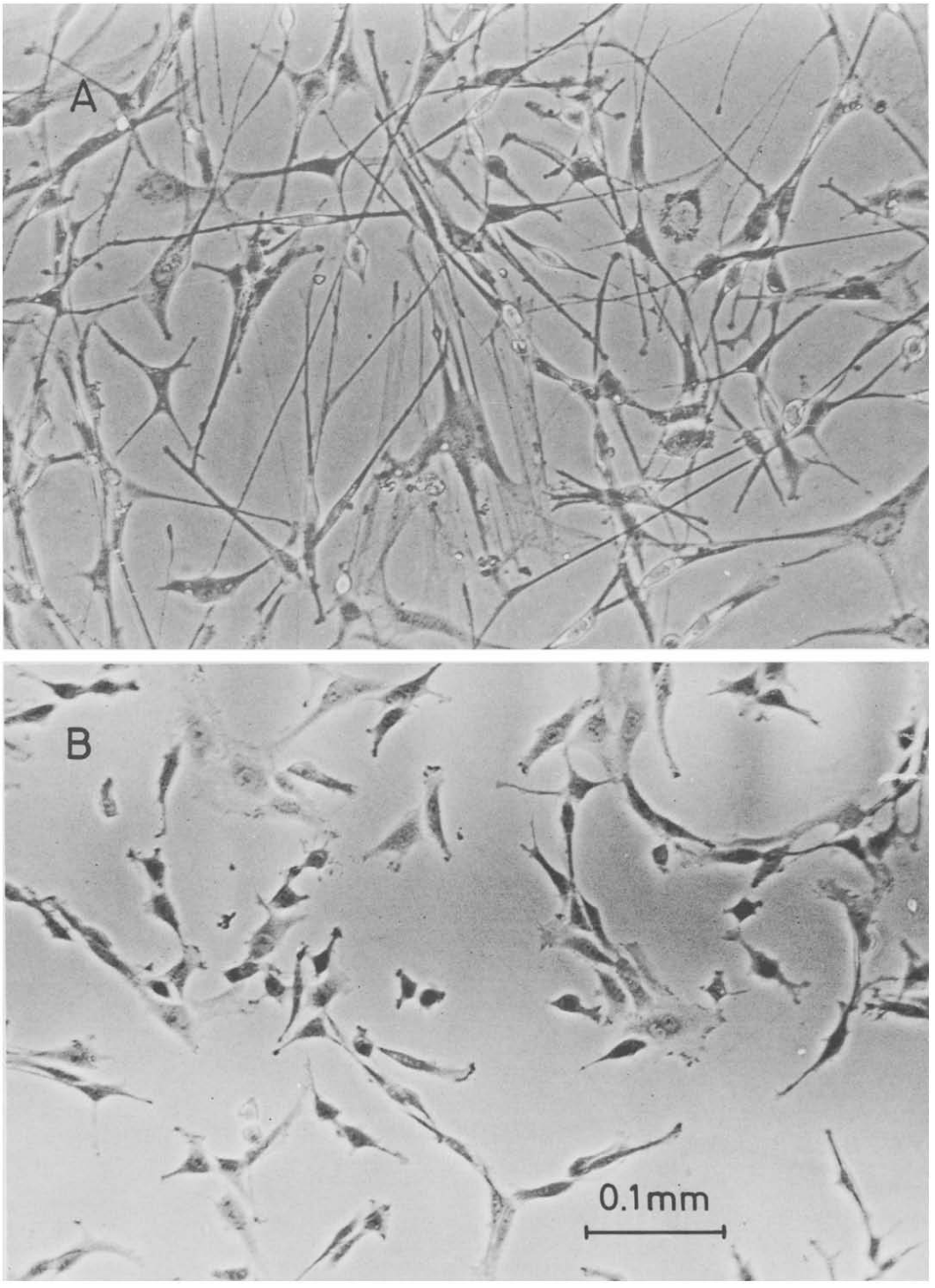


Fig. 2. Morphology of clonal glioma line C6-BU-1 grown in presence (A) and absence (B) of dibutyryl cyclic AMP.

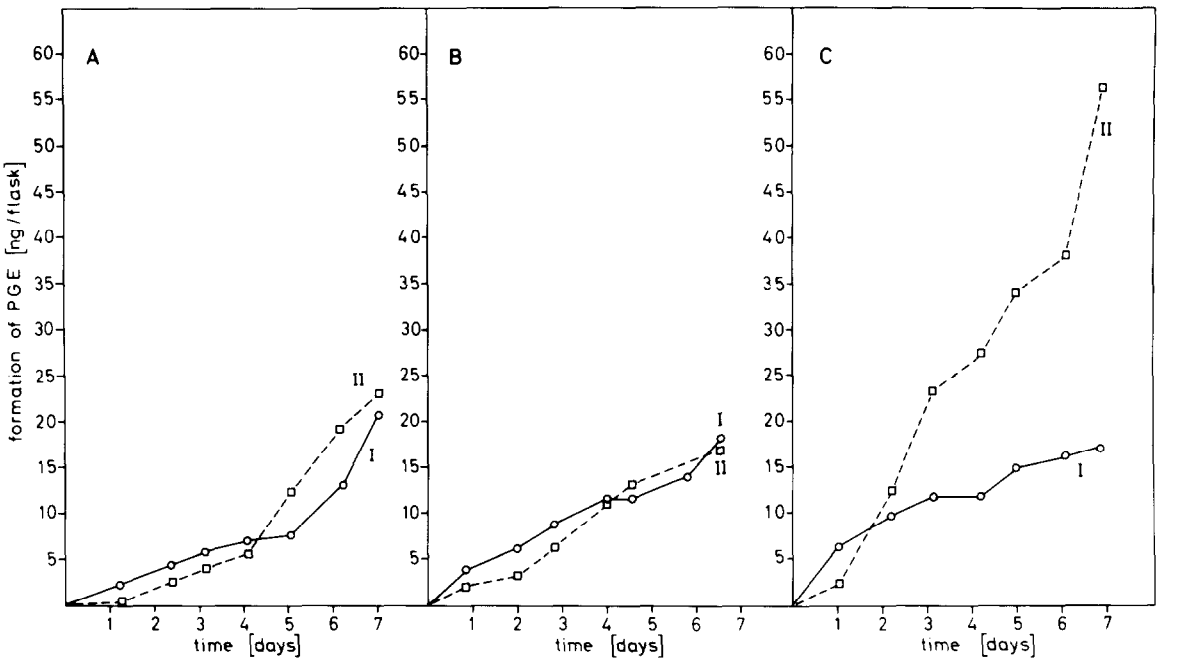
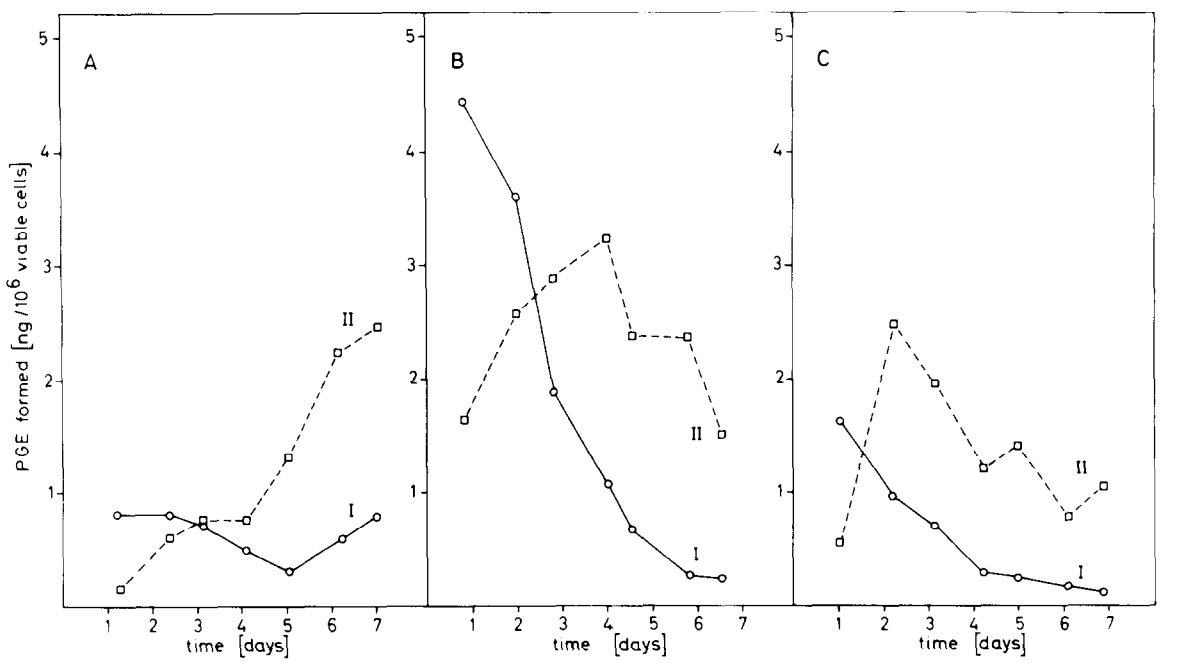


Fig. 3. Top: Cumulative production of PGE in absence (curve I) and presence (curve II) of dibutyryl cyclic AMP; A) neuroblastoma line N4TG3; B) glioma line C6-BU-1; C) fibroblast line B82. The flasks were replica flasks of those used for cell counts in fig. 1. For cell viabilities and for volumes of growth medium see fig. 1. The values for cumulative production of PGE up to a given time are obtained by addition of (i) the PGE content of the cells harvested at that time and (ii) the PGE contents of all the volumes of medium the cells had been in contact with up to that time.



Bottom: Cumulative production of PGE per million cells in absence (curve I) and presence (curve II) of dibutyryl cyclic AMP. The values for cumulative production of PGE up to a given time were divided by the number of viable cells per flask reached at that time

Table 1
Prostaglandin production per million cells per day by three different cell lines. The data were measured at day 3 of the growth curves shown in fig. 1.

Cell line	DBCA*	PG production (pg/10^6 cells/day)						
		PGE			PGA		PGF	
		total	found in cells	% of total PGE found in cells	found in cells	% of total PGE	found in cells	% of total PGE
N4TG3	–	713	58	8	100	14	14	2
	+	770	324	42	86	11	27	4
C6-BU-1	–	1940	148	8	53	3	24	1
	+	2880	167	6	195	7	41	1
B82	–	708	162	23	124	18	12	2
	+	1960	1252	64	60	3	11	1

* DBCA = dibutyryl cyclic AMP.

ensues (fig. 3 bottom). The production of PGE per million cells by N4TG3 (bottom, A) does not increase with the cell density in absence of dibutyryl cyclic AMP. In the presence of the drug (curve II), however, the values increase with the cell density to 3 times the control values (curve I).

The effect of dibutyryl cyclic AMP is even more impressive with the glioma line (fig. 3, bottom, B). In the absence of the drug, the values for PGE formed per million cells decrease strongly with increasing cell density (curve I). At low densities of C6-BU-1 the drug inhibits production of PGE per million cells (curve II). With increasing cell density the PGE formation per million cells increases and eventually exceeds that of the control cultures. When the cultures become more crowded, the production per unit number of cells falls again but stays always several times higher than that of the controls (curve I). A picture qualitatively similar to that for C6-BU-1 is found with the fibroblast line B82 (fig. 3, bottom, C).

PGA and PGF could not be detected in the medium that had been in contact with the cells for one day. However, low amounts of both PGA and PGF were found in the cells; at day three of the growth curves (fig. 1), e.g., the average content of the three cell lines was between 3 and 18% of the total PGE production per 10^6 cells per day for PGA and between 1 and 4% for PGF (table 1). Eight to 23% of the PGE produced by the 3 cell lines is retained in the cells, when they are grown in the absence of dibutyryl cyclic AMP. In the presence of the drug, this proportion is 6–64% of the total production (table 1).

4. Discussion

In experiments reported previously, carcinomas, sarcomas and normal fibroblasts grown in matrix organ culture [20, 21] as well as clonal lines derived from fibrosarcomas [22] were found to secrete different amounts of PGE into the medium. The experiments reported here show that the 3 cell lines investigated preferably synthesize PGE. Only minor amounts of PGA and PGF could be detected. In our experiments, PGA and PGF were only found within the cells. If they were excreted into the medium, the amounts must have been too low to be detected.

Levine et al. [22] reported that a mouse fibroblast and a mouse neuroblastoma line, both different from those used in this study, and the wild type rat glioma line of which C6-BU-1 is a mutant, did not produce significant amounts or contained measurable intracellular levels of PG. In view of the large quantities of PGE the fibrosarcoma cells, investigated by these authors, produced, this appears to be a fair statement. Nevertheless, the present study shows that the three cell lines used synthesize PG and that the rate of synthesis can be enhanced, when dibutyryl cyclic AMP is added to the growth medium. As mentioned, in the presence of dibutyryl cyclic AMP many effects

have been observed, which can also be produced by increasing the intracellular concentration of cyclic AMP [7–11]. Therefore, our results suggest that in the cells investigated cyclic AMP plays a positive regulatory function in the synthesis of PG. At day 5 of the growth curve (fig. 1), N4TG3 cells enter the stationary phase of apparent contact inhibition of proliferation. This phenomenon is accompanied by a spurt of PG production, especially in the presence of dibutyryl cyclic AMP. In all cases the retardation of cell proliferation goes along with an increase in production of PGE. In the case of C6-BU-1 striking morphological changes also take place. An extension of long cell processes is also observed in other cases, e.g., when mouse neuroblastoma clones [10, 11, 23] or mouse neuroblastoma x rat glioma hybrid clones [24] are treated with dibutyryl cyclic AMP [10, 23, 24], PG [11] or inhibitors of cyclic AMP phosphodiesterase [24, 25].

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