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EFFECTS OF PROSTAGLANDINS E_1, E_2, AND $F_2\alpha$ ON THE GROWTH OF LEUKAEMIA CELLS IN CULTURE

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SUMMARY

Prostaglandins E_1, E_2, and $F_2\alpha$ (PGE_1, PGE_2, and $PGF_2\alpha$) were shown to inhibit the growth of mouse leukaemia lymphoblasts L5178Y in culture. The effects of PGE_1 and PGE_2 were greater than that of $PGF_2\alpha$. PGE_1 and PGE_2, at the concentration of 100 μg per ml showed significant inhibitory effects on the rates of incorporation of tritiated thymidine, uridine and leucine. At concentrations of 50 and 25 μg per ml, there was significant inhibition of thymidine and uridine incorporation, but not of leucine. $PGF_2\alpha$ showed significant inhibition of thymidine and uridine incorporation but not leucine incorporation, in all 3 concentrations studied (100, 50, and 25 μg/ml). The ability of the cells to form colonies in soft agar was significantly inhibited by PGE_1 and PGE_2 at concentrations as low as 1·8 μg/ml. For $F_2\alpha$, however, a concentration as high as 56 μg/ml was required to show inhibitory effect, but at 1·8 μg/ml it was found to be stimulatory.

INTRODUCTION

Prostaglandins have been shown to mimic many of the responses usually attributed to cyclic adenosine monophosphate (cAMP). Depending on the tissue involved, prostaglandins either increase or decrease the synthesis of cAMP by adenyl cyclase or the degradation of cAMP by phosphodiesterase. As an oversimplified generalization, prostaglandins decrease cAMP in adipose tissue and toad bladder and increase cAMP in most other tissues, such as corpus luteum, spleen, and leukocytes (Hinman, 1972). Prostaglandins also increase cAMP levels in cell cultures, such as neuroblastoma cells (Gilman & Nirenberg, 1971) and fibroblasts (D'Armiento, Johnson & Pastan, 1972; Manganiello & Vaughan, 1972).

Recently, cyclic AMP has been shown to inhibit the growth of tumour cells *in vitro* (Ryan & Heidrick, 1968; Yang & Vas, 1971; Van Wijk, Wicks & Clay, 1972) and *in vivo* (Gericke & Chandra, 1969; Cho-Chung, 1974). This and other evidence (MacManus & Whitfield, 1969) indicates that cAMP, along with cyclic guanosine monophosphate (cGMP), has a central role in the regulation of cell metabolism and growth (Sutherland, 1972; Johnson & Pastan, 1972; Hsie, Jones & Puck,

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1971). Furthermore, prostaglandins have been shown to interfere with the division of HeLa cells and to slow the growth of L-929 cells, mouse embryo fibroblasts and plasma cell tumours (Adolphe, Giroud, Timsit & Lechat, 1973; Johnson & Pastan, 1971; Naseem & Hollander, 1973). The interrelationship of cAMP and prostaglandins in cell growth regulation needs to be elucidated further. The present study was performed to determine the effects of prostaglandins on the growth of cAMP-sensitive mouse leukaemia lymphoblasts.

MATERIALS AND METHODS

Leukaemia lymphoblasts

The L5178Y mouse leukaemia lymphoblast cell line, which was originally provided by Dr Bruce Bosmann of the University of Rochester, was used throughout the experiments. This line has been maintained for the past 2 years in our laboratory in Fischer's medium supplemented with 10% heat-inactivated horse serum, penicillin and streptomycin. Details of the culture conditions have been described previously (Yang & Vas, 1970).

Prostaglandins

Prostaglandins E_1, E_2, and $F_2\alpha$ (tromethamine salt) (PGE_1, PGE_2 and $PGF_2\alpha$) were kindly supplied by Dr John E. Pike of the Upjohn Company (Kalamazoo, Mich.). Stock solutions containing 1 mg/ml of prostaglandin as the sodium salt in 10% ethanol were prepared as recommended by Dr Pike and sterilized by sintered glass filters.

Dose/response study

Beginning with the stock solutions, doubling dilutions of the prostaglandins were made with the culture medium. Two tenths of a millilitre of these dilutions were added to triplicate tubes of 2-ml cultures containing 2.5×10^5 viable cells/ml to give final concentrations of 100, 50 and 25 μg/ml (i.e. for PGE_1: 0.282, 0.141 and 0.071 mM; for PGE_2: 0.284, 0.142 and 0.071 mM; for $PGF_2\alpha$ [tromethamine salt]: 0.210, 0.105 and 0.053 mM). After 23 h the cultures were labelled with [^{3}H]thymidine (sp. act. 6.7 Ci/mmol; New England Nuclear Corp. Boston, Mass.), 5-[^{3}H]uridine (sp. act. 27.1 Ci/mmol) or [^{3}H]leucine (sp. act. 40.7 Ci/mmol) at a final concentration of 1 μCi/ml. Incorporation allowed to proceed for 60 min in a 37 °C water bath. At the end of this period, the tubes were chilled in an ice bath, and the cells were processed as described previously. Briefly, the cells were poured on to glass-fibre filters (Reeve-Angel, Clifton, N.J.) in an H. Holzel manifold (Bernöderwez, Germany). The culture tubes were then rinsed with 5 ml physiological saline solution. The cells were fixed with two 5-ml washes of cold 5% trichloroacetic acid (TCA) followed by one 5-ml rinse of 95% ethanol to clear the filter disks. Subsequently, the disks were placed in scintillation vials and dried in an oven at 80 °C for 60–90 min. After the addition of 15 ml toluene scintillation fluid, the samples were counted, using a Packard Tri-Carb-scintillation spectrometer (Yang, Dale & Jones, 1975).

Viable cell counts

For studying the effects of the prostaglandins on viable cell numbers, 2-ml cultures were treated with 50 μg/ml of prostaglandins (E_1, E_2, and $F_2\alpha$). Viable cell counts were made after 1 and 24 h of incubation. The dye exclusion test was employed using trypan blue and a haemacytometer.

Growth inhibition in soft agar

The soft agar technique previously used for studying growth inhibition of leukaemia cells by antiserum was adapted for the present experiments (Yang & Vas, 1971). Soft agar medium was prepared by mixing equal portions of 1% purified agar (Difco Laboratories, Detroit,

Table 1. *Effects of prostaglandins E_1, E_2, and $F_2\alpha$ on tritiated thymidine, uridine and leucine incorporation by* L5178Y *cells*

	Prostaglandin conc., μg/ml	cpm/culture		
		[³H]Thymidine	[³H]Uridine	[³H]Leucine
Prostaglandin				
PGE₁	(*a*) 100	8423 ± 735 (91·2)†	12801 ± 899 (75·9)	6072 ± 743 (42·3)
	(*b*) 50	42348 ± 6820 (61·6)	34393 ± 1905 (47·4)	7689 ± 320 (3·8)*
	(*c*) 25	65777 ± 8404 (43·6)	48239 ± 844 (29·9)	9880 ± 559 (−10·6)*
PGE₂	(*a*) 100	12142 ± 1063 (83·7)	18367 ± 461 (65·4)	6167 ± 383 (41·4)
	(*b*) 50	51635 ± 1820 (53·2)	44712 ± 689 (31·6)	7031 ± 475 (12·0)*
	(*c*) 25	69678 ± 3533 (40·3)	55731 ± 816 (19·0)	9959 ± 719 (−11·5)*
PGF₂α	(*a*) 100	69591 ± 4749 (27·0)	40596 ± 1791 (23·5)	9937 ± 524 (5·6)*
	(*b*) 50	62445 ± 3367 (43·4)	47371 ± 2278 (27·5)	8886 ± 382 (−11·2)*
	(*c*) 25	69326 ± 38c5 (40·6)	5c128 ± 147 (27·1)	7965 ± 903 (10·8)*
Control, % ethanol‡				
(*a*) 1·00		95323 ± 1054	53071 ± 891	10528 ± 1037
(*b*) 0·50		110263 ± 1958	65323 ± 1371	7989 ± 980
(*c*) 0·25		116697 ± 1209	68796 ± 2419	8929 ± 983

* Data are not significant (based on *t*-statistic derived from difference between means test). All other data are significant at levels of $P < 0.05$ to $P < 0.001$.

† Represent % inhibition based on control values. Negative values indicate stimulation.

‡ Controls (*a*), (*b*) and (*c*) contained the same concentrations of ethanol as in prostaglandin solutions (*a*), (*b*) and (*c*) respectively.

Michigan) in distilled water (kept at 45 °C) and double strength Fischer's medium containing 20% heat-inactivated normal horse serum (kept at 37 °C). A basal layer of 7 ml was poured into 60 × 15 mm plastic tissue culture dishes (Falcon Plastics, Oxnard, Calif.), allowed to harden, and then overlaid with 1·5 ml of the cell suspension in soft agar medium. For the preparation of the cell suspension in soft agar medium, L5178Y cells during the log phase of growth were counted and suspended in soft agar medium to contain 1×10^5 viable cells/ml. After the agar solidified, 0·5 ml dilutions of prostaglandins in Fischer's medium were spread evenly on the top of the agar. The plates were incubated in a humidified atmosphere of 5% CO_2 in air at 37 °C and examined daily under an inverted microscope. Colonies with a cell number greater than 20 cells were counted after 10 days. Four replicate plates were set up for each prostaglandin dilution.

Statistics

The data were analysed using a computer programme for statistical analysis (STATPACK) on the University of Tennessee Research Center MUMPS time-sharing system. The significance levels for experimental data are based on the *t*-statistic derived from the difference between means test.

RESULTS

Dose/response study of the effects of prostaglandins E_1, E_2, and $F_2\alpha$

The results of the dose/response study are shown in Table 1; 100 μg/ml of PGE_1 or PGE_2 showed significant inhibitory effects on the rates of incorporation of tritiated thymidine, uridine and leucine. In the 2 lesser concentrations shown (50 and 25 μg/ml) there was significant inhibition of thymidine and uridine incorporation but not of leucine. $PGF_2\alpha$ showed significant inhibition of thymidine and uridine incorporation in all 3 concentrations, but leucine incorporation was not significantly affected by $PGF_2\alpha$ at any of the dilutions.

Table 2. *Effects of prostaglandins on the viable numbers of L5178Y cells in culture*

	Viable cell numbers/ml (% inhibition)†	
	1 h	24 h
Prostaglandin		
E_1	$1·74 \times 10^5$ (21·9)	$3·08 \times 10^5$ (53·6)
E_2	$1·65 \times 10^5$ (26·9)	$2·76 \times 10^5$ (58·4)
$F_2\alpha$	$2·03 \times 10^5$ (9·0)*	$4·41 \times 10^5$ (33·6)
Control, 0·50% ethanol	$2·23 \times 10^5$	$6·64 \times 10^5$

* Value is not significant. All other data are significant at levels of $P < 0·05$ to $P < 0·001$.

† $\% \text{ inhibition} = \dfrac{\text{cell number of control} - \text{cell number of experimental}}{\text{cell number of control}} \times 100.$

Effects of prostaglandins on viable cell numbers

The results of the viable cell counts are shown in Table 2. After 1 h of incubation with PGE_1 and PGE_2, there was significant inhibition in the ability of the cells to exclude trypan blue. This inhibition was not significant with $PGF_2\alpha$. After 24 h of incubation, all 3 prostaglandins showed significant inhibitory effects on viable cell numbers when compared to the controls.

Effects of prostaglandins on soft agar colony formation

Table 3 shows the results of the effects of the three prostaglandins studied on mouse lymphoblast cells in soft agar culture. The prostaglandins E_1 and E_2 significantly inhibited colony formation compared to the controls in all of the dilutions used. $PGF_2\alpha$ again showed effects which were not originally hypothesized. The highest concentration of the prostaglandin showed significant inhibitory effects, the intermediate concentrations produced no significant changes in colony growth, and the lowest concentration produced a significant stimulatory response. There were 4 controls used: one for each alcohol dilution used in the experimental plates.

Table 3. *Inhibitory effect of prostaglandins on colony formation of L5178Y cells in soft agar*

	Concentration, μg/ml	Mean colonies/plate ± S.E.	% Inhibition*
Prostaglandin			
E_1	(*a*) 56·0	0	100·00
	(*b*) 28·0	0	100·00
	(*c*) 7·0	77·71 ± 12·33	83·57
	(*d*) 1·8	219·21 ± 25·35	39·49
E_2	(*a*) 56·0	0	100·00
	(*b*) 28·0	0·74 ± 0·74	99·81
	(*c*) 7·0	95·42 ± 14·16	79·82
	(*d*) 1·8	241·09 ± 6·05	33·44
$F_2\alpha$	(*a*) 56·0	249·92 ± 27·74	37·11
	(*b*) 28·0	330·44 ± 38·36	14·16 (N.S.)
	(*c*) 7·0	354·04 ± 24·71	25·15 (N.S.)
	(*d*) 1·8	516·70 ± 54·18	−42·64†
Control, % ethanol‡			
(*a*) 0·560	—	397·41 ± 53·43	
(*b*) 0·280	—	384·95 ± 25·93	
(*c*) 0·070	—	473·01 ± 62·41	
(*d*) 0·018	—	362·25 ± 20·71	

$$* \ \% \text{ inhibition} = \frac{\text{colony number of control} - \text{colony number of experimental}}{\text{colony number of control}} \times 100.$$

Significant at levels of $P < 0.05 - P < 0.001$ except those marked N.S. (not significant).

† Stimulation.

‡ Controls (*a*), (*b*), (*c*) and (*d*) contained the same concentrations of ethanol present in prostaglandin solutions (*a*), (*b*), (*c*) and (*d*), respectively.

DISCUSSION

In this experiment, we have demonstrated that prostaglandins inhibit growth of mouse leukaemia lymphoblasts L5178Y in culture. The rates of synthesis of DNA and RNA were inhibited significantly by 100, 50 and 25 μg/ml of prostaglandins E_1, E_2, and $F_2\alpha$. The inhibitory effect of $PGF_2\alpha$, however, was less than those of PGE_1 and PGE_2. Unexpectedly, the rate of protein synthesis was not as sensitive to prostaglandins. It was inhibited only by 100 μg/ml of PGE_1 and PGE_2 but not by 100 μg/ml

of $PGF_2\alpha$ or 50 and 25 μg/ml of any of the prostaglandins studied. This and the following studies by others suggest that there may be some tissue specificity and site specificity to the observed effects of prostaglandins. Eisenbarth, Wellman & Lebovitz (1974) found that PGA_1 markedly inhibited the synthesis of DNA, RNA and protein in rat and murine chrondrosarcomas. PGE_1 and $PGF_1\alpha$ had no effects on the synthesis of macromolecules by either tumour. Adolphe *et al.* (1973) reported that prostaglandins E_1, E_2 and A_2, but not $F_1\alpha$ and $F_2\alpha$, interfered with division of HeLa cells. Also, prostaglandins E_1, B and $F_2\alpha$ slowed the growth of L-929 cells and mouse embryo fibroblasts.

The mechanism of greater inhibition of DNA and RNA synthesis than protein synthesis is unknown. The viable cell counts made from the cultures treated with 50 μg/ml of prostaglandins for 24 h indicated that the rates of protein synthesis per cell actually doubled because the number of viable cells in cultures treated with prostaglandins E_1, E_2, and $F_2\alpha$ were 53·6, 58·4 and 33·6% of the control cultures, respectively. Furthermore, the cells in the cultures treated with PGE_1 and PGE_2, but not $PGF_2\alpha$, were much larger than those of controls. Probably due to inhibition of cell division, many of them had twice or more of the control cell volume. Similar increase in cell volume and rate of protein synthesis per cell has been observed previously in the cultures inhibited by heat-inactivated anti-L5178Y antibodies in the absence of complement (Yang & Vas, 1970, 1972). It has been suggested that agents which inhibit cell division (DNA synthesis), but not protein synthesis, may cause an increased cell mass due to accumulation of protein (Curtis, Elliott, Wilson & Ryan, 1973). For example, Cohen & Studzinski (1967) noted a dissociation of the synthesis of RNA and protein from the synthesis of DNA in HeLa cells treated with inhibitors of DNA synthesis. In such cells with unbalanced growth, there were approximately 100% increases in RNA and protein content and cell volume. In the present study, however, RNA synthesis, but not protein synthesis, was inhibited along with the inhibition of DNA synthesis. The reason for the apparent dual mechanism of action of prostaglandins remains obscure. Kinetic analysis may give additional insight into the sequence of events. A possible explanation may lie in the speculation of Ruddon & Johnson (1967) that certain concentrations of prostaglandins might inhibit the enzymic degradation of messenger RNA.

In soft agar culture, the ability of the leukaemia cells to form colonies was also inhibited by prostaglandins, especially PGE_1 and PGE_2. The inhibitory effect of $PGF_2\alpha$ was again much less than those of PGE_1 and PGE_2 and higher concentration, 56 μg/ml, was required to see an effect compared to PGE_1 and PGE_2 at 1·8 μg/ml. Because 0·07% of ethanol was found to have a slight stimulatory effect of undetermined cause, the significance of the stimulatory effect produced by 1·8 μg/ml of $PGF_2\alpha$ in 0·018% ethanol solution cannot be evaluated at the moment. Although the inhibitory concentrations of prostaglandins used in this study are admittedly higher than that shown to be present in human serum (316 $\pm$ 36 pg/ml) (Hertelendy, Woods & Jafee, 1973), some of them fall in the range of haemopoietic stem cell stimulatory dose (35 μg/ml, highest dose tested) reported by Fehér & Gidali (1974). The ability of a cell to grow in soft agar is considered to be one of the characteristics of

its *in vivo* tumorigenicity. The effect of prostaglandins on tumour growth *in vivo* needs further elucidation.

The prostaglandins may affect the cellular levels of cAMP, but there are unexplained discrepancies. For example, cAMP inhibits protein synthesis as well as DNA and RNA synthesis (Yang & Vas, 1971). The quantitative effects of prostaglandins on cAMP have to be determined.

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