LYMPHOCYTE ACTIVATION: THE DUALISTIC EFFECT OF cAMP

by

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SUMMARY

The effects of exogenously added cyclic nucleotides on DNA synthesis have been investigated in human peripheral blood lymphocytes stimulated with phytohemagglutinin (PHA). At low doses of PHA the addition of exogenous cAMP resulted in an inhibition of DNA synthesis. At optimal or supraoptimal doses of PHA the addition of cAMP, db-cAMP, or 8-Br-cGMP resulted in enhancement of DNA synthesis. Measurement of cell associated cAMP and cGMP levels in lymphocytes exposed to PHA with or without exogenously added cAMP revealed a gradual increase in cAMP levels and a fluctuating decline in cGMP levels.

INTRODUCTION

A number of reports have been published which suggest that elevated cAMP levels in lymphocytes represent a repressive signal for intracellular metabolic pathways (1,2,3). In our laboratory, experiments performed using isolated human peripheral blood lymphocytes (PBL) exposed to a wide dose range of PHA-M (0-2000 µg) as well as exogenously administered cAMP (10^{-2}M to 10^{-7}M) revealed a dichotomous effect on DNA and RNA synthesis. At low to optimal mitogenic doses of PHA-M (1-100 µg) the addition of exogenous cAMP ($5x10^{-2}$M to 10^{-4}M) resulted in a suppression of RNA and DNA synthesis. However, at optimal to supraoptimal doses of PHA-M (100-2000 ug), which alone result in progressive decreases in synthesis, the addition of exogenous cAMP resulted in a 1-4 fold increase in DNA and RNA synthesis.

Subsequent experiments showed that this effect was probably not due to a reversal of possible toxic effects of the PHA, type of PHA used, a change in precursor pool size or the number of cells used (9,10).

The data reported here confirm this effect of cAMP with reference to enhancement of DNA synthesis in PBL exposed to supraoptimal doses of PHA-M, and further, show that the effect may also be obtained when either $N^6,0^2$-dibutyryl-cyclic adenosine monophosphate (db-cAMP) or 8Bromo-cyclic guanosine monophosphate (8Br-cGMP) is used. Most importantly, measurement of cell-associated cAMP and cGMP levels in PBL exposed to PHA-M and/or exogenous cAMP demonstrate that: 1) in situations where DNA or RNA synthesis is reduced or blocked (i.e., at very high doses of PHA), cAMP levels are very low; 2) it appears that large increases in cell-associated cAMP levels must occur before restoration of DNA synthesis can take place; and 3) a role for cGMP in this process could not be clearly delineated.

MATERIALS AND METHODS

Normal human peripheral blood lymphocytes (PBL) were isolated and cultured in microtiter trays using a serum free medium according to methods previously described (4,5,6). Following incubation the PBL were assayed for DNA synthesis by ^3H thymidine incorporation according to the method of Hartzman (6).

Cyclic AMP levels were assayed using the protein binding assay of Gilman (7) and cyclic GMP levels were measured using the radioimmunoassay technique of Steiner et al. (8).

RESULTS

The data presented in Table I show the results of a typical experiment depicting the enhanced DNA synthesis at high PHA dose obtained with cAMP, db-cAMP or 8Br-cGMP. Experiments with prostaglandin E_1 gave similar results while isoproterenol or acetylocholine yielded enhancement of DNA synthesis only at the highest dose of PHA (2000 μg),

TABLE I. Effect of cAMP and related compounds on PHA-induced DNA synthesis

COMPOUNDS ADDED	PHA-M µg / ml 0	1	10	100	250	500	1000	2000
cAMP 10^{-3}M	114[a] ± 8.0[b]	53 ± 3.0	36 ± 5.0	107 ± 1.3	145 ± 8.0	502 ± 0.8	237 ± 4.2	168 ± 3.7
8 Bromo-cGMP 10^{-9}M	92 ± 9.8	122 ± 12.6	96 ± 10.3	86 ± 11.5	142 ± 15.2	162 ± 0.5	133 ± 7.0	404 ± 3.0
dibutyryl cAMP 10^{-9}M	79 ± 3.4	153 ± 4.7	103 ± 4.4	130 ± 3.1	147 ± 8.0	160 ± 5.3	190 ± 4.6	738 ± 1.8
dibutryryl cAMP 10^{-8}M + 8 Bromo-cGMP 10^{-8}M	265 ± 20.9	111 ± 6.6	137 ± 6.1	106 ± 13.1	129 ± 3.4	158 ± 1.2	129 ± 2.8	170 ± 7.8

[a] Per cent stimulation of DNA synthesis representing the ratio of the mean of triplicate determinations of the compound-treated cultures/cultures exposed to PHA alone.

[b] Percent standard error of the mean.

Table II. Cell Associated cyclic nucleotide levels in PBL exposed to PHA-M and/or cAMP

TIME AFTER CULTURE INITIATION	TREATMENT: NONE		PHA 100 μ g		PHA 1000 μ g		cAMP 10^{-3}M		PHA 100 μ g + cAMP 10^{-3}M		PHA 1000 μ g + cAMP 10^{-3}M	
	cAMP	cGMP	cAMP	cGMP	cAMP	cGMP	cAMP	cGMP	cAMP	cGMP	cAMP	cGMP
0-5 Min.	3.44*	0.19*	2.98*	0.28*	13.14*	0.43*	3.80*	0.18*	3.12*	0.19*	6.89*	0.39*
1 hr.	-	-	51.3	89.2	37.6	30.2	104.6	89.0	109.9	76.3	215.0	42.0
4	-	-	125.3	92.8	23.7	87.2	75.0	96.7	158.8	68.4	121.7	67.9
24	56.7	134.2	49.8	105.4	11.0	47.7	176.7	96.7	178.2	88.9	162.4	92.3
46	45.8	84.2	51.3	64.3	9.2	39.5	458.9	91.7	74.5	92.1	22.6	15.4
48	47.1	92.1	41.1	58.9	13.9	62.8	164.0	116.7	479.2	60.5	258.6	21.7
50	45.5	84.2	61.7	91.1	11.2	32.6	488.2	52.7	392.9	47.4	259.3	39.7
70	36.5	58.0	42.8	50.8	9.7	67.4	156.6	63.8	293.4	50.0	153.4	-
72	35.9	81.0	51.5	37.5	0.08	19.8	258.8	47.2	80.0	-	173.9	29.5

PBL at a concentration of 1 x 10^7 were exposed for varying times to PHA or to PHA + cAMP in RPMI 1640 without serum supplement. Following incubation, the cultures were washed 4X in iced PBS. The cells were then treated according to the method of Whitfield et al. (12) and stored at -70°C until assay. The (*) values at the top of the table represent the mean of the 0-5 minute control values for the various groups reported in pMoles/10^6 lymphocytes. All other values (beginning with the 1-hr. sample) are reported as the percent of the 0-5 minute control for that group. All samples were assayed in duplicate.

inhibition or no effect being found at lower doses of PHA (D. Webb, unpublished observations).

The peak enhancing effect of cAMP or DNA synthesis occurs at the 500 μg dose of PHA, while the maximum enhancing effect with db-cAMP and 8Br-cGMP occurs at 2000 μg of PHA. It should be noted that at the doses reported here, neither db-cAMP nor 8Br-cGMP causes inhibition of DNA synthesis with low doses of PHA. Such inhibition occurs at all doses of PHA only when these compounds are used at higher concentrations (i.e., 10^{-6}M to 10^{-3}M). Since it was possible that the effect of exogenous cAMP on the intracellular cyclic nucleotide pool might involve elevations of both cyclic nucleotides, these analogues were added together. As illustrated in Table I, the two analogues in combination were mildly mitogenic in the absence of PHA. In the presence of the mitogen slight high dose enhancement occurred. However, it was not as great as when the analogues were added separately or when cAMP was used alone, except possibly at 2000 μg at PHA.

Because the high dose recovery effect obtained with cAMP could be mimicked by exogenous addition of db-cAMP and 8Br-cGMP, experiments were undertaken to ascertain cAMP and cGMP levels in PBL cultures exposed over a 72 hour period to PHA and/or cAMP. The results of such an experiment are depicted in Table II. In the cultures exposed to cAMP alone, there is a gradual increase in cell associated cAMP levels up to about 50 hours, followed by a decline with an increase occurring at the last point measured (72 hrs.). Cyclic GMP levels remain relatively stable during this time and then slowly drop from 50 to 72 hrs. In cultures exposed to 100 μg PHA, cAMP levels fluctuate over the first hours and then remain uniform beginning at 24 hours; cGMP levels fluctuate, finally dropping at 70 hours. In cultures exposed to 1000 μg of PHA, cAMP levels drop within the first hour and remain extremely low throughout the remainder of the experiment, whereas

cyclic GMP levels remain low but fluctuate throughout the period measured. On exposure to PHA + cAMP there is a gradual increase in cAMP levels until 46 hours, and then a second peak occurring from 48-72 hours. Cyclic GMP levels remain somewhat low in the cultures exposed to 100 μg PHA + cAMP, while in those exposed to 1000 μg PHA + cAMP the cGMP levels fluctuate over the first 24 hours and then remain below control values thereafter.

DISCUSSION

The experiments reported here demonstrate that: 1) exogenous cAMP may stimulate or inhibit DNA synthesis, the initial variable being the dose of PHA used; 2) both db-cAMP and 8Br-cGMP may mimic the stimulatory effect of cAMP; 3) the addition of a very high dose of PHA (1000 μg) which reduces DNA synthesis also reduces intracellular cAMP levels drastically over 72 hours compared to the levels in cells exposed to 100 μg of PHA or resting cells; 4) the addition of exogenous cAMP substantially raises, the intracellular cAMP levels but has little effect on cGMP levels; 5) the elevation of cAMP levels is prerequisite to a recovery of DNA synthesis in PBL exposed to high doses of PHA; 6) and lastly although cGMP has been implicated in lymphocyte activation by PHA (11) we could define no clear role for it in the experiments reported here.

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