CONTROL OF ORNITHINE DECARBOXYLASE ACTIVITY BY CYCLIC NUCLEOTIDES IN THE PHYTOHEMAGGLUTININ INDUCED LYMPHOCYTE TRANSFORMATION

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SUMMARY: $N^{2},O^{2'}$-Dibutyryl guanosine 3':5'-cyclic monophosphate enhanced the ornithine decarboxylase activity which was induced during lymphocyte transformation by phytohemagglutinin, while $N^{6},O^{2'}$-dibutyryl adenosine 3':5'-cyclic monophosphate tended to inhibit it. The increased ornithine decarboxylase activity was not observed when $N^{2},O^{2'}$-dibutyryl guanosine 3':5'-cyclic monophosphate alone was added to the non-stimulated lymphocytes. In contrast to the dibutyryl derivative, guanosine 3':5'-cyclic monophosphate did not show any effect on enzyme induction following phytohemagglutinin stimulation.

Mitogenic agents, such as phytohemagglutinin or concanavalin A, may interact with ligands on the cell membranes to initiate the differentiation process and the intracellular mediator of stimulation has been postulated to be either cAMP(1) or cGMP(2) or combination of both nucleotides(3). However, the inhibitory effects of cAMP on cell proliferation have been frequently reported in cultured cells(4-9) and much evidence suggests that the intracellular level of cAMP is higher in resting cells than in growing cells(10-14). In contrast to cAMP, cGMP stimulates the incorporation of [^{3}H]thymidine into DNA of splenic lymphocytes(15) and of fibroblasts(16). cGMP level in cells shows an early transient increase in growth induction of lymphocytes by PHA(2) and of

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Abbreviations: cAMP, adenosine 3':5'-cyclic monophosphate: cGMP, guanosine 3':5'-cyclic monophosphate: Bu_2cAMP, $N^{6},O^{2'}$-dibutyryl adenosine 3':5'-cyclic monophosphate: Bu_2cGMP, $N^{2},O^{2'}$-dibutyryl guanosine 3':5'-cyclic monophosphate: PHA, phytohemagglutinin: ODC, ornithine decarboxylase

fibroblasts stimulated by fibroblast growth factor or serum(17). These observations raise the interesting possibility that endogenous cGMP may act as the intracellular mediator of the effects of stimulative agents.

On the one hand, polyamine biosynthesis is one of the earliest events occuring during lymphocyte transformation and ornithine decarboxylase[E.C. 4.1.1.17], the initial enzyme in the polyamine biosynthetic pathway, elevates its activity dramatically in the early stage. There is accumulating evidence which indicates that polyamine may play an important role in the cellular process when cells are transformed from the resting to the growing state; in such cells, polyamine accumulation and following RNA and DNA synthesis(18) are observed and the inhibitor of polyamine formation reduces the protein and DNA synthesis(19,20). Therefore, it would be interesting to investigate the relationship between the level of intracellular cyclic nucleotides and the induction of ODC in mitogen-induced lymphocyte transformation.

The present article mainly concerns the effects of exogenous cyclic nucleotides on the induction of ODC during the lymphocyte transformation by PHA.

MATERIALS AND METHODS

Bu_2cAMP, Bu_2cGMP, cAMP, cGMP and pyridoxal phosphate were purchased from Sigma Chemical Co., St.Leuis, Mo.. DL-[1-^{14}C]Ornithine(specific activity 61 mCi/mmole) was the product of The Radiochemical Centre, England. L-Ornithine was from Kyowa Hakko Chemical Co., Tokyo. PHA-P was from Difco Laboratories, Detroit, Mich..

Preparation of lymphocyte culture: Lymph node cell suspensions from guinea pigs sensitized with the killed tubercle bacilli was prepared as previously described(21). The cells suspended at a concentration of 1×10^7 cells/ml in Eagle's MEM solution containing 20 % calf fetal serum were incubated in a CO_2 incubator by airation of 5 % CO_2 in air at 37°C for 15 hrs. After addition of PHA and cyclic nucleotides, a further 6 hrs incubation was carried out to obtain maximal ODC induction.

Preparation of enzyme solution: Harvested cells(4×10^7 cells) were suspended in 1 ml of 50 mM Tris-HCl(pH 7.5) containing 0.1 mM of EDTA, 0.05 mM of pyridoxal phosphate and 10 mM of dithiothreitol and were disrupted by three times freezing and thawing. The disrupted cell suspensions were centrifuged

at 1000 x g for 10 min and the supernatant was used as the enzyme solution.

Measurement of ODC activity: The enzyme activity was measured by the release of $^{14}CO_2$ from [1-^{14}C]ornithine according to the method of Kay and Lindsay(22). The reaction mixture contained 1 ml of enzyme solution, DL-[1-^{14}C]ornithine (0.5 uCi) and 80 nmoles of L-ornithine in final volume of 1.02 ml. Incubation was for 60 min at 37°C in tightly-capped conical flasks containing 0.3 ml of 1 M hyamine hydroxide in the center well. The reaction was terminated by the addition of 0.5 ml of 50 % trichloroacetic acid solution. The $^{14}CO_2$ released was absorbed into hyamine hydroxide during additional 60 min incubation at 37°C. The radioactivity contained in hyamine hydroxide was counted in a Packard Tricarb liquid scintillation counting system.

RESULTS

The effects of Bu_2-cyclic nucleotides on ODC activity of PHA-stimulated lymphocytes were studied(Fig. 1). PHA-stimulated cells had a several-fold

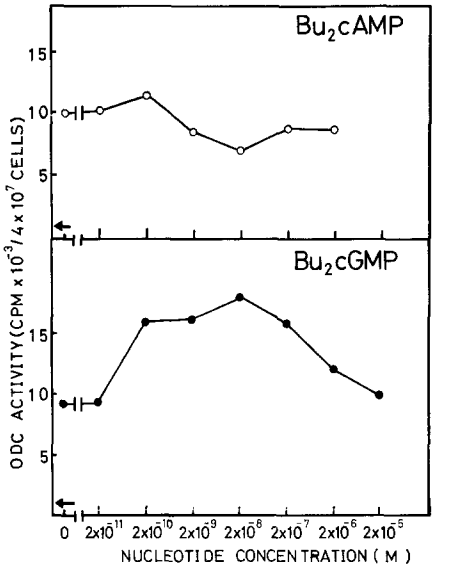

Fig. 1 Effects of dibutyryl cyclic nucleotides on ODC activity during lymphocyte transformation by PHA.

Lymphocytes were incubated for 6 hrs in the presence of PHA(3 μg/ml) and various concentrations of Bu_2cAMP or Bu_2cGMP. ODC activity was measured as described in the text. The values are the means of three different experiments. The arrow indicates ODC activity of non-stimulated lymphocytes.

enzyme activity, compared to that of non-stimulated lymphocytes. The increase in enzyme activity reduced to 70 % when the cells were incubated with 20 nM of Bu_2cAMP. The inhibitory effect of Bu_2cAMP was less evident at a concentration of more and less than 20 nM. In contrast to Bu_2cAMP, the addition of Bu_2cGMP into cell culture caused 100 % enhancement of enzyme activity in the PHA-stimulated lymphocytes. The facilitatory effect of Bu_2cGMP was dependent on the nucleotide concentration added and 20 nM was found to maximally stimulate activity.

The cyclic nucleotides were added at various times of cell culture between 0 and 5 hrs, and then ODC activity of the cells was measured at 6 hrs of cell culture(Fig. 2). The rate of stimulating and inhibiting effects of the nucleotides were dependent on the time of their additions. The rate of effect was gradually reduced, as the time of the nucleotide addition was delayed after PHA addition.

Unlike Bu_2cGMP, cGMP(20 nM) and sodium butyrate(100 nM) were not effective in stimulating ODC activity(Table Ia, Ib). Various concentration of sodium butyrate(1 nM-10 mM) had no effect on the activity(data are not shown).

It was of considerable interest to find whether Bu_2cGMP alone is active in the induction of ODC activity without PHA stimulation, since elevation of cellular concentration of cGMP by PHA was reported(2). Fig. 3 showed that Bu_2cGMP was inactive in stimulating ODC activity in the absence of PHA.

DISCUSSION

Our results presented in this paper have shown that Bu_2cGMP enhanced PHA-induced stimulation of ODC activity, while Bu_2cAMP inhibited the stimulation. Considered together with recent reports that cAMP may be involved in induction of ODC activity of rat liver(23) and adrenal gland(24), these results strongly suggest that intracellular cyclic nucleotides participate in the regulation of polyamine synthesis which may be concerned

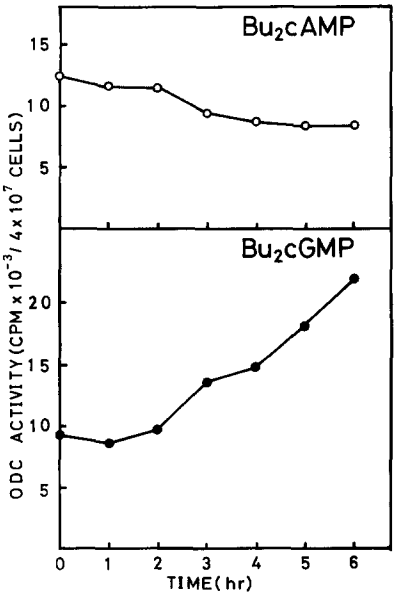

Fig. 2 Effect of time of cyclic nucleotide addition into cell culture medium on ODC activity.

PHA-stimulated lymphocytes were incubated for the time indicated before harvest with Bu_2cAMP(20 nM) or Bu_2cGMP(20 nM). PHA(3 μg/ml) were added at 0 time of the cell culture. The time of dibutyryl cyclic nucleotide addition was delayed after PHA addition. All cells were harvested at 6 hr and ODC activity was measured. The values are the means of two different experiments.

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with growth stimulation of mammalian cells, although the effect of cAMP on enzyme activity in the lymphocyte is inhibitory rather than stimulatory.

Since PHA increased the cGMP concentration of lymphocytes(2), it is considered that PHA-induced stimulation of ODC activity is due to a rising cGMP level. However, exogenous Bu_2cGMP could not replace PHA in respect of enzyme induction, providing the evidence that Bu_2cGMP cannot induce ODC, but can enhance its activity which has been induced by PHA. In contrast to dibutyryl derivative, cGMP at a concentration of 20 nM did not affect the activity, although it was assumed that cGMP could penetrate into cells much more easily than cAMP(25). When the concentration of exogenous cGMP was

Ia		Ib	
Additions	ODC activity (cpm/4 x 10^7cells)	Additions	ODC activity (cpm/4 x 10^7cells)
PHA	17820	PHA	15453
PHA, Bu_2cGMP	31337	PHA, Bu_2cGMP	25357
PHA, cGMP	19926	PHA, Sodium butyrate	13237

Table I Effect of cGMP and sodium butyrate on ODC activity of PHA-stimulated lymphocytes.

The cells were cultivated for 6 hrs with PHA(3 μg/ml) and cGMP(20 nM)(Table Ia) or with PHA(3 μg/ml) and sodium butyrate(100 nM)(Table Ib). The values are the means of duplicate experiments.

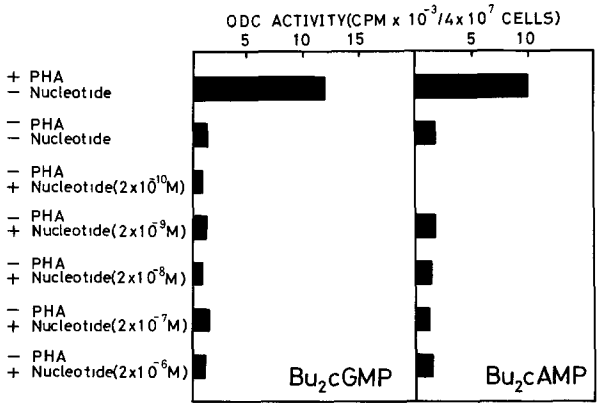

Fig. 3 Effect of cyclic nucleotides on ODC activity of non-stimulated lymphocytes.

ODC activity of the lymphocytes was measured after 6 hrs incubation with cyclic nucleotide alone at the indicated concentrations. The values are the means of duplicate experiments.

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increased(data are not shown), no effect was observed, indicating that the difference between the effects of cGMP and its dibutyryl derivative may not be due to the different penetration rate into cells, but may be due to other unknown factors.

Although the data are not shown, the addition of nucleotides, GTP, GDP, GMP, ATP, ADP and AMP which may be derived from cGMP and cAMP in the cells, into the enzyme assay mixture had no effect on the enzyme activity, suggesting that the mechanism of Bu_2cGMP stimulation is unlikely to be that of stimulation of Escherichia coli ODC by GTP(26). Another regulatory mechanism for ODC was reported by Hogan and Murden(27) and Kay and Lindsay(28) who found that degradation of the enzyme in hepatoma cells and human lymphocytes was prevented by the addition of nonessential amino acids and glutamine into the cell culture. Since cGMP and cAMP have a reciprocal effect on cell transport of leucine in mouse fibroblasts(29), it is possible that Bu_2cGMP may prolong the half-life of the enzyme due to the stimulation of membrane permeability, creating an elevation of intracellular concentration of amino acids. However, the mechanism of stimulation of ODC activity by Bu_2cGMP is not clear from our results and is under investigation.

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