*Acta Medica Okayama*

*Volume* 28, *Issue* 2 1974 *Article* 2

APRIL 1974

# Enhancement of DNA synthesis of mouse myelogenous cells by cyclic adenosine 3',5'-monophosphate (cAMP)

Shigeru Okada* Atsuko Okamoto† Michiyasu Awai‡

Mitsuko Naito** Satimaru Seno††

*Okayama University,

†Okayama University,

‡Washington University,

**Okayama University,

††Okayama University,

# Enhancement of DNA synthesis of mouse myelogenous cells by cyclic adenosine 3',5'-monophosphate (cAMP)*

Shigeru Okada, Atsuko Okamoto, Michiyasu Awai, Mitsuko Naito, and Satimaru Seno

**Abstract**

To observe the possible role of cAMP on the DNA synthesis during specialization-division of myelogenous precursor cells, the authors observed the DNA and RNA synthesis of the cells by in vitro autoradiography. And it is concluded that cAMP or its dibutyryl derivative added to the media penetrated into myelogenous precursor cells and metamyelocytes of mice and enhanced the DNA synthetic capacity of them. cAMP hardly enhanced RNA synthesis. Discussion is made on relation between enhancement of DNA synthesis of metamyelocytes and their possible rejuvenation.

*PMID: 4368682 [PubMed - indexed for MEDLINE] 

Acta Med. Okayama **28**, 81—87 (1974)

# ENHANCEMENT OF DNA SYNTHESIS OF MOUSE MYELOGENOUS CELLS BY CYCLIC ADENOSINE 3', 5'-MONOPHOSPHATE (cAMP)

Shigeru OKADA, Atsuko OKAMOTO, Michiyasu AWAI*, Mitsuko NAITO and Satimaru SENO

*Department of Pathology, Okayama University Medical School, Okayama, Japan (Director: Prof. S. Seno)*

*Received for publication, November 17, 1973*

*Abstract*: To observe the possible role of cAMP on the DNA synthesis during specialization-division of myelogenous precursor cells, the authors observed the DNA and RNA synthesis of the cells by *in vitro* autoradiography. And it is concluded that cAMP or its dibutyryl derivative added to the media penetrated into myelogenous precursor cells and metamyelocytes of mice and enhanced the DNA synthetic capacity of them. cAMP hardly enhanced RNA synthesis. Discussion is made on relation between enhancement of DNA synthesis of metamyelocytes and their possible rejuvenation.

It is now recognized that in the multicellular organisms a number of cellular processes are controlled by the intracellular cyclic adenosine 3', 5'-monophosphate (cAMP), the level of which changes in response to environmental stimulation (1). In case of the hormonal stimulation, the hormone combines with the receptor on cell surface, a part of the adenylcyclase system in cell membrane, and releases the catalytic part of the enzyme into the cell which inevitably results in the formation of cAMP from ATP. For example, glucagon elevates the level of cAMP inside the hepatic cells, which causes the enhancement of glycogenolysis by the activation of phosphorylase (1), or pituitary growth hormone again induces the production of cAMP in the thymic lymphocytes as has been revealed by *in vitro* experiments (2).

It is still the matter of debate, however, whether cAMP in nutritional environment penetrates into the cells and if so, whether it acts just as that produced in the cells. But it has been demonstrated that the cAMP in the media mimics the action of glucagon stimulating the glycogenolysis of the liver cells *in vitro* (1), or of pituitary growth hormone promoting the mitotic activities of the thymic lymphocytes (2). These facts seem to suggest that in some cells cAMP given from outside penetrates into the cells and

* Present address: Division of Hematology, Department of Medicine, Washington University, School of Medicine, St. Louis, Missouri, U.S.A.

induces a similar effect to that of hormones on the target cells. Concerning the biological potentialities of growth hormone, its stimulating activity of DNA synthesis and cell proliferation should not be limited on lymphocytes but on general somatic cells. Therefore, cAMP, as the second messenger of growth hormone, may directly affect the somatic cells other than lymphocytes. The growth hormone or cAMP may promote the proliferation of the cells in undifferentiated state or in rejuvenation as seen in lymphocytes, but it is quite uncertain whether it has the same activity on the cells on the way of differentiation and decay. With this problem in mind we have observed the effect of cAMP on the DNA and RNA syntheses of myelogenous cells of mouse bone marrow which are destined to mature and to be discarded, and yet the maturation process proceeds with a few steps of DNA synthesis and cell division. As proposed by some authors, their DNA synthesis and proliferation may be stimulated by a leukocytosis-inducing factor (3), apart from the pituitary growth hormone. Both of the activities of these two humoral factors may be mediated by cAMP involving in DNA synthesis, and if the regulation of DNA synthesis is largely dependent on the phosphorylation of histone by cAMP (4), the activation of DNA polymerase would ultimately result.

In this paper it is shown that cAMP added to the incubation media seems to penetrate into myelogenous precursor cells and to augument DNA synthesis of them without any recognizable enhancement of RNA synthesis, though dibutyryl derivative (db cAMP) stimulates both DNA and RNA syntheses.

## MATERIALS AND METHODS

Fresh bone marrow cells were obtained from the femurs of healthy twenty adult ddN mice, male and female, weighing about 20 gm. The animals were decapitated, femurs were taken and cut at the both ends. The bone marrow tissues were taken out by injecting Hanks' solution containing heparin, 4 u/ml, from one end. They were put together, dissociated by pumping gently with syringe and filtered through nylon mesh, then washed twice with Hanks' solution by repeated centrifugation 1,500 rpm for 5 min. and finally suspended in McCoy 5A medium containing 20% calf serum, $10^6$ cells per ml.

The cell suspension was divided into 28 parts, one ml each. The first 7 parts were added with cAMP dissolved in saline, $10^{-2}$, $10^{-3}$, $10^{-4}$, $10^{-5}$, $10^{-6}$, $10^{-7}$, and $10^{-8}$M, respectively and incubated for 3 h at 37°C. The second 7 parts and the third 7 parts were added with db-cAMP and 5'AMP respectively at the same concentration as in the first 7 part and incubated under the same conditions. The last 7 parts were of saline control. After 3-h incubation, $^3$H-Tdr was added to the incubation medium, 2 $\mu$Ci/ml (specific activity 5 Ci/mmole) in each and reincubated for one hour. The reaction was stopped in ice cold bath, washed twice with Hanks' solution by repeated centrifugation and the cells were smeared by cytocentrifugation (6).

Autoradiogram was made with these smeared cells according to the method reported elsewhere (5). Five hundred myelogenous precursor cells, including myeloblasts, promyelocytes, myelocytes and metamyelocytes, were counted on each sample and labeling index was obtained with these cells.

For the observation of RNA synthesis, additional 20 ddN mice were used. Their bone marrow cells were treated by the way exactly identical with that described above, except that $^{3}$H-Ur, 10 $\mu$Ci/ml (specific activity 2.7 Ci/mmole), and cold thymidine, $10^{-8}$M, were used in place of $^{3}$H-Tdr.

## RESULTS

The viability test of bone marrow cells isolated from the tissue and incubated under the various conditions described proved that the cells retained a good state as revealed by morphologic observations and eosin exclusion tests, which were made at the terminal of 3-h incubation by using a drop of each sample.

The myelogenous cells incubated with $^{3}$H-Tdr, 2 $\mu$Ci/ml for 60 min. smeared and mounted with sensitive emulsion, gave a moderate number of silver grains on the nuclei after one week exposure. Morphologic observation on the cells with May-Grünwald-Giemsa revealed that the younger precursors, myeloblasts and promyelocytes, were well labeled in all series of the experiment. There were no recognizable differences in labeling between the cells of incubated with cAMP and the controls, as far as these younger precursor cells are concerned. But much differences in labeling were found between more matured cells treated with cAMP and controls. Metamyelocytes and some mature cells having segmented nuclei were quite rarely labeled in both saline controls and 5'AMP-treated samples, while a number of metamyelocytes and some mature cells having segmented nuclei were heavily labeled in those treated with cAMP and db-cAMP (Photo 1, a and b).

On these samples the labeling indices were obtained by counting all series of precursor cells, myeloblasts to metamyelocytes. To obtain the labeling index on each cell series, three grains were required as the limiting grain count per labeled cell. In the samples incubated with cAMP, it was found that the labeling index increased at the concentration of $10^{-4}$ to $10^{-7}$M in comparison with the controls incubated with saline. At the concentration of $10^{-7}$ to $10^{-6}$M, the labeling index increased with the increase in cAMP concentration. No effect was found at $10^{-8}$M and the reduced effects at higher concentration than $10^{-3}$M (Fig. 1).

In samples incubated with db cAMP, a similar increase in DNA synthesis was found in myelogenous cells. That is, with the increase in db-cAMP concentration, $10^{-5}$ to $10^{-6}$M, the labeling index of myelogenous cells increased and reached the maximum activation at $10^{-4}$M db-cAMP, 150% of


Photo 1. Picture of mouse bone marrow cells labeled with $^{3}$H-Tdr. Metamyelocytes are labeled rather heavily. The cells were preincubated with cAMP(a) or db-cAMP(b)

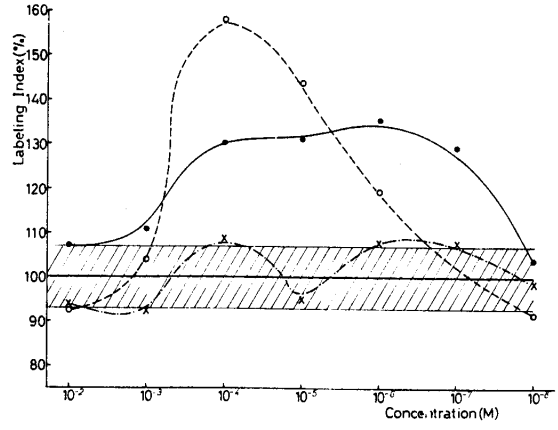


Fig. 1. Labeling indices of myelogenous cells of normal mouse bone marrow cells incubated with $^{3}$H-Tdr for 1 hr at 37°C. Solid line and filled circlles : preincubated with cAMP for 3 hr at 37°C. Broken lines and open circles : preincubated with db-cAMP for three hours Broken lines with dots and cross : preincubated with 5'AMP for 3 hours. Hatched area : range of control

the control. Further increase in db-cAMP concentration, $10^{-3}$ to $10^{-2}$M db-cAMP, the labeling index lowered to the level of controls (Fig. 1).

In the samples incubated with 5'AMP, no activation of DNA synthesis

was found. Labeling indices were found to be within the control level at every concentration tested (Fig. 1).

By incubating with $^{3}$H-Ur and cold thymidine the bone marrow cells showed an active RNA synthesis, the younger precursors were more heavily labeled than more matured cells. The labeling indices of the myelogenous cells preincubated with cAMP and 5'AMP were found to be in the range of control, while indices of those preincubated with db-cAMP increased along with the increase in the concentration of db-cAMP and reached the maximum level at $10^{-5}$M, 145% of the control. A further increase in db-cAMP concentration lowered the index to the control level (Fig. 2)

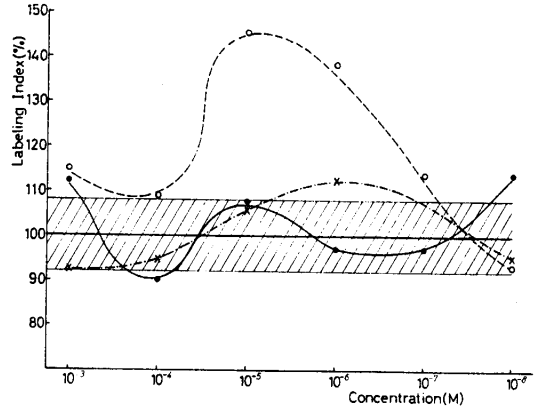


Fig. 2. Labeling indices of myelogenous cells of normal mouse bone marrow cells incubated with $^{3}$H-Ur and cold thymidine for one hour at 37°C. Explanation of lines, see Fig. 1

## DISCUSSION

Now many data are being accumulated on cAMP, which is generally acccepted as the second intracellular messenger of hormones in multicellular organism (1). As just described, it is known that cAMP promotes the syntheses of DNA (7, 8, 20), RNA (10, 17, 15, 16) and protein (12, 19) in some cells, *e.g.* lymphocytes, hepatic cells, *etc.*, but it is still unsettled whether the action of cAMP, which seems to penetrate some cells from outside and has the promoting effect on DNA, RNA and protein syntheses, is specific to some cells or common to general somatic cells. The present experimental results indicate that cAMP seems to penetrate mouse myelogenous cell *in vitro* and to promote the DNA synthesis. This has been clearly observed on metamyelocytes which had a large number of grains after incubation with cAMP and $^{3}$H-Tdr, while those of controls incubated with saline and $^{3}$H-Tdr were poorly labeled. In these cells the activation of DNA synthesis by cAMP was induced without

any enhancement of the net synthesis of RNA. It is generally believed that DNA synthesis requires preliminary synthesis of some RNA and protein (14). The present data showed that cAMP acted to promote the DNA replication but its promoting effect on RNA transcription was very poor in mouse myelogenous cells. This means that cAMP acts mainly to activate DNA replication and related RNA synthesis, but poor in the activity of stimulating RNA synthesis for functioning or structural proteins. The synthesis of RNA for functioning protein may be related to the factors other than those mediated by cAMP.

In this experiment it should be noted that the stimulation of DNA synthesis of cAMP and its derivative involves the ring and segmented nuclei of metamyelocytes which are generally accepted as fully matured cells having no mitotic activity. As DNA synthesis occurs actually in these cells, they may retain mitotic activity which responds to specific stimulation. FANG, HIMEI and SENO have reported the possibility of rejuvenation of mature granulocyte or metamyelocyte of rat grafted to the aplastic bone marrow of rat (11, 16). In their observations RNA synthesis preceded DNA synthesis which seemed to occur only after morphologic changes or swelling of nuclei. We have yet no data to explain this discrepancy but DNA stimulating activity of cAMP and derivative on mature granulocyte may give a clue to observe possible dedifferentiation processes of granulocyte as in lymphocyte rejuvenation by PHA.

## REFERENCES


1. SUTHERLAND, E. W.: Cyclic AMP and hormone action. In *Cyclic AMP*, ed. Robison, G. A., Butcher, R. W., and Sutherland, E. W., Academic Press, New York and London, 1971
2. MACMANUS, J. P. and WHITFIELD, J. F.: Mediation of the mitogenic action of growth hormone by adenosine 3', 5'-monophosphate (cyclic AMP). *Proc. Soc. Exp. Biol. Med.* **132**, 409-412, 1969
3. METCALF, D. and MOORE, M. A. S.: Humoral regulation of haemopoiesis. In *Haemopoietic Cells*, pp. 362, North-Holland Pub. Comp., Amsterdam-London, 1971
4. LANGAN, T. A.: Action of adenosine 3', 5'-monophosphate-dependent histone kinase *in vivo*. *J. Biol. Chem.* **244**, 5763-5765, 1969
5. OKADA, S.: Suppression of erythropoiesis by actinomycin D. II. The change in RNA metabolism by actinomycin D administration. *Acta Med. Okayama* **23**, 265-271, 1969
6. DORÉ, C. F., and BALVOUR, B. M.: A device for preparing cell spreads. *Immunology* **9**, 403-405, 1965
7. MACMANUS, J. P. and WHITFIELD, J. F.: Stimulation of DNA synthesis and mitotic activity of thymic lymphocytes by cyclic adenosine 3', 5'-monophosphate. *Exp. Cell Res.* **58**, 188-191, 1970
8. WHITFIELD, J. F., MACMANUS, J. P. and RIXON, R. H.: The possible mediation by cyclic AMP of parathyroid hormone-induced stimulation of mitotic activity and deoxyribonucleic acid synthesis in rat thymic lymphocytes. *J. Cell Physiol.* **75**, 213-224, 1970
9. DELANGE, R. J., KEMP, R. G., RILEY, W. D., COOPER, R. A. and KREBS, E. G.: Activation of skeletal muscle phosphorylase kinase by adenosine triphosphate and adenosine 3', 5'-monophosphate. *J. Biol. Chem.* **243**, 2200-2208, 1968

10. AVERNER, M. J., BROCK, M. L. and JOST, J-P: Stimulation of ribonucleic acid synthesis in horse lymphocytes by exogenous cyclic adenosine 3', 5'-monophnsphate. *J. Biol. Chem.* **247**, 413-417, 1972
11. FANG, C. H.: A study of granulopoiesis in the aplastic bone marrow of x-ray irradiated rats after parabiosis with healthy littermate by aortic anastomoses. *Acta Med. Okayama* **26**, 1-10, 1972
12. MALLETTE, L. E., EXTON, J. H. and PARK, C. R.: Control of gluconeogenesis from amino acids in the perfused rat liver. *J. Biol. Chem.* **244**, 5713-5723, 1969
13. BURGER, M. and KNYSZYNSKI, A.: The effect of adenosine 3', 5'-monophosphate on $^{3}$H-thymidine incorporation *in vitro* into mouse spleen and thymic cells. *Hoppe-Seyler's Z. physiol. Chem.* **352**, 1019-1024, 1971
14. MONESI, V.: DNA, RNA, and protein synthesis during the mitotic cell cycle. In *Handbook of Molecular Cytology*, ed. A. Lima-De-Faria, pp. 484, North-Holland Pub. Comp., Amsterdam-London, 1969
15. DOKAS, L. A. and KLEINSMITH, L. J.: Adenosine 3', 5'-monophosphate increases capacity for RNA synthesis in rat liver nuclei. *Science* **172**, 1237-1238, 1971
16. SENO, S., FANG. C. H. and HIMEI, S.: Blastformation of granulocyte: An observation of hemopoiesis in aplastic bone marrow of x-ray irradiated rat after parabiosis with non-irradiated littermate by aortic anastomosis. (Abstract from XIV International Congress of Haematology, 1972. 7.)
17. ARNAUD, M., BEZIAT, Y., BORGNA, J. L., GUILLEUX, J: C. and MOUSSERON-CANET, M.: Le récepteur de l'oestradiol, l'AMP cyclique et la RNA polymerase nucléolaire dans l'utérus de génisse. Stimulation de la biosynthèse de RNA *in vitro*. *Biochem. Biophys. Acta* **25**, 241-254, 1971
18. VARMUS, H. E., PERLMAN, R. L. and PASTAN, I.: Regulation of *lac* messenger ribonucleic acid synthesis by cyclic adenosine 3', 5'-monophosphate and glucose. *J. Biol. Chem.* **245**, 2259-2267, 1970
19. GRIFFIN, D. M. and SZEGO, C. M.: Adenosine 3', 5'-monophosphate stimulation of uterine amino acid uptake *in vitro*. *Life Sciences* **7**, 1017-1023, 1968
20. WHITFIELD, J. F., MACMANUS, J. P. and RIXON, R. H.: Cyclic AMP-mediated stimulation of thymocyte proliferation by low concentrations of cortisol. *Proc. Soc. Exp. Biol. Med.* **134**, 1170-1174, 1970