PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 145, 975–978 (1974)

Stimulation and Suppression of Erythropoiesis in the Plethoric Mouse by Cyclic Nucleotides[1] (37936)

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It has recently been reported that both adenosine 3′,5′-monophosphate (cAMP) and its dibutyryl derivative (dbcAMP) have the capacity to enhance erythropoiesis (1–3). The role of cAMP as a second messenger in hormone action, proposed by Sutherland (4), has received wide acceptance. The actions and mechanisms of the other naturally occurring nucleotides, such as guanosine 3′,5′-monophosphate (cGMP) still remain much in doubt. The *in vivo* studies were undertaken to determine the effects of cGMP on erythropoiesis.

Materials and Methods. Swiss Webster white, virgin mice (West Jersey Biological Co., Wenonah, NJ), weighing approximately 25 g, were used and supplied with a diet of Purina Rodent Chow and water *ad libitum.* Prior to the experiment, the mice received intraperitoneal (ip) injections of 1–2 mg of iron as iron–dextran solution (Imferon, Lakeside Laboratories, Inc., Milwaukee, WI) to insure adequate iron stores. Mice were made plethoric by exposure to a hypobaric atmosphere (340 mm Hg) for 21 days as previously described (5).

The cyclic adenosine and guanosine nucleotides, and their dibutyryl derivatives, obtained from Sigma Chemical Co., St. Louis, MO, were dissolved in physiological saline, each to a final concentration of 25 mg/ml. Three days posthypoxia, mice were randomized into groups, each containing ten mice. Cyclic GMP, dbcGMP, and dbcAMP were administered individually and in various combinations to designated mice. Each compound was injected at a dosage of 0.5 mg/g body weight. Four groups received, respectively, physiological saline (0.5 ml), cGMP, dbcAMP, and dbcAMP + cGMP at 48 hr prior to ^{59}Fe. Another group of mice was injected with dbcAMP at 48 hr before ^{59}Fe after being treated 12 hr earlier with dbcGMP. To determine possible effects of various time intervals between administration of the guanosine compounds and ^{59}Fe, two other groups received cGMP and dbcGMP at 60 hr before the radioiron.

All mice reecived 0.25 μCi of ^{59}Fe as ferrous citrate (Mallinckrodt, St. Louis, MO) administered iv via the tail vein. Forty-eight hours after ^{59}Fe was injected, mice were exsanguinated by cardiac puncture, at which time 0.5 ml of heparinized blood was collected from each mouse. Red cell radioactivity was counted in a Baird Atomic well-type scintillator, and ^{59}Fe incorporation was calculated as a percentage of injected isotope using a formula previously described (6). Procedures used are depicted in Fig. 1.

Results. Effects of cGMP, dbcGMP, or dbcAMP. Fourty-eight-hours ^{59}Fe incorporation data of mice injected with a single nucleotide, according to the protocol shown in Fig. 1, are listed in Table I. The results indicate that the control group had sharply depressed erythropoietic activity. The administration of dbcAMP caused the most distinct and significant ($P < 0.001$) increase in ^{59}Fe incorporation. By contrast,

[1] This research was supported in part by grants from The John A. Hartford Foundation, Inc., and the Department of Health, Education, and Welfare (AM 11188-06).

[2] Send reprint requests.

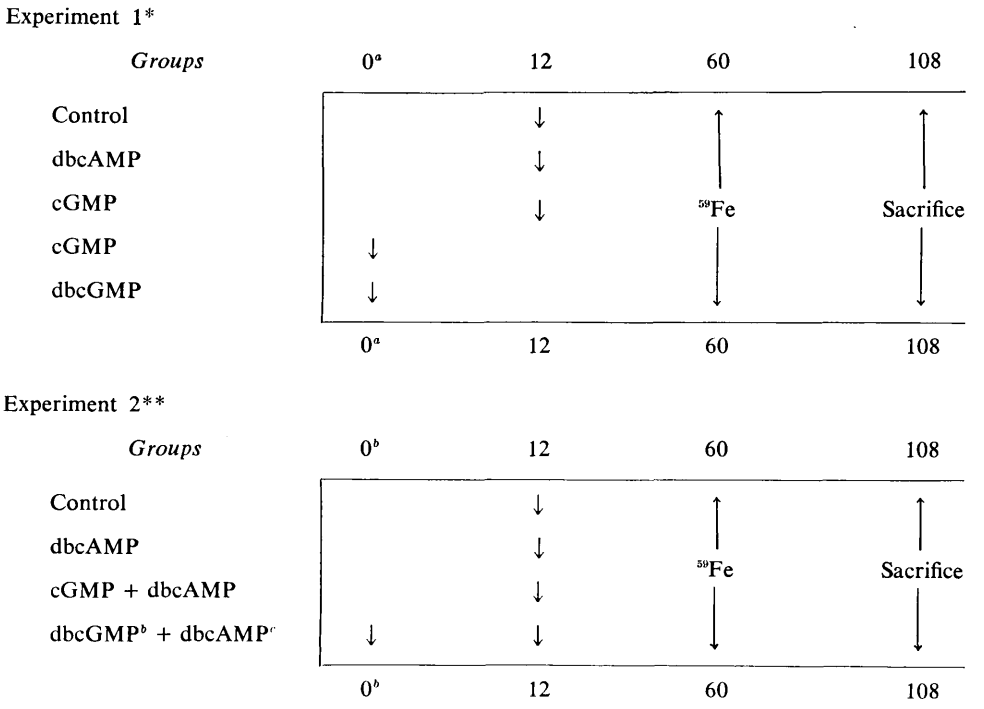

FIG. 1. Protocol used to study the effect of cyclic nucleotides on per cent ^{59}Fe incorporation. (*) Experiment 1 analogous to Table I. (**) Experiment 2 analogous to Table II.

[a] Zero hr represents 3 days posthypoxic exposure.

[b] Simultaneous injection of cGMP + dbcAMP.

[c] dbcAMP injected at 0 hr, dbcAMP injected at 12 hr.

the guanosine compounds, cGMP and dbcGMP, were devoid of the capacity to augment radioiron uptake. This lack of stimulation occurred when either dbcGMP or cGMP was injected at 60 hr previous to radioactive iron and when cGMP was injected 48 hr before ^{59}Fe (Fig. 1 and Table I).

Effects of guanosine and adenosine cyclic nucleotides in combination. The addition of cGMP and dbcGMP to dbcAMP indicates that the guanosine compounds not only fail to stimulate erythropoiesis, but, in fact, block or inhibit the marked erythropoietic stimulatory ability of dbcAMP as shown in Table II. When cGMP was given at the same time as dbcAMP, ^{59}Fe incorporation was depressed 71.4% ($P < 0.001$) below dbcAMP alone. Similarly, marked inhibition of erythropoiesis resulted when dbcGMP was injected 12 hr before the administration of dbcAMP as ^{59}Fe incorporation was

TABLE I. 48 Hr ^{59}Fe Incorporation of Mice Treated with Cyclic Nucleotide at 48 and 60 Hr.

	Hr prior to ^{59}Fe	Percent ^{59}Fe incorporation ±SEM	*P* value
Control	48	1.79 ± 0.44	
dbcAMP	48	20.51 ± 1.48	<0.001
cGMP	48	2.22 ± 0.45	NS
cGMP	60	3.54 ± 1.30	NS
dbcGMP	60	1.36 ± 0.63	NS

TABLE II. Percent ^{59}Fe Incorporation of Mice Treated with Combinations of Nucleotides.

	Percent ^{59}Fe incorporation ±SEM	*P* value
Control	1.85 ± 0.57	
dbcAMP	18.54 ± 1.20	<0.001
cGMP + dbcAMP	5.29 ± 1.30	<0.05
dbcGMP[a] + dbcAMP	6.64 ± 1.30	<0.02

[a] dbcGMP administered 12 hr before dbcAMP.

again significantly ($P < 0.001$) decreased (64.1%) below dbcAMP alone.

Discussion. This present *in vivo* study has demonstrated that guanosine 3′,5′-monophosphate and its dibutyryl derivative do not enhance erythropoiesis in the polycythemic mouse system. It has also been reported (3) that dbcGMP was ineffective in stimulating ^{59}Fe incorporation *in vitro*. Furthermore, either of these two substances cause an inhibition of radioiron uptake when administered in conjunction with dbcAMP, a compound reported (1, 3, 7) to stimulate erythropoiesis. This inhibitory effect was observed when cGMP was administered 12 hr previous to dbcAMP. In the present experiment, the use of cAMP was omitted because of the greater ability of its dibutyryl derivative to increase erythropoiesis (1, 3). Unlike cAMP and dbcAMP, the activity of cyclic guanosine monophosphate and its dibutyryl derivative appear to be about the same. The results of Experiment 1, Table I, indicate that the ^{59}Fe incorporation is not significantly different between cGMP- and dbcGMP-treated mice.

At present, the precise physiological role of cGMP is not known. This nucleotide may be linked to the regulation of the intracellular concentration of cAMP by its ability to alter the rate of hydrolysis of the adenosine nucleotide, through an inhibition or stimulation of the action of a nucleotide phosphodiesterase (PDE). Possibly, cGMP competitively inhibits the hydrolysis of cAMP by PDE (8, 10). The cGMP may act by protecting the cAMP from the PDE or interfering in the metabolic pathway of cAMP via stimulation of the catalytic enzyme adenyl cyclase. This would account for our observations to some extent. If the cGMP either inhibits the hydrolysis of cAMP or stimulates adenyl cyclase, a large increase in endogenous cAMP would result. The decreased ^{59}Fe incorporation could be explained by Graber's proposal (11) of a direct toxic action of high levels of cyclic nucleotides on bone marrow cells. Schooley's *in vivo* observations (2) was that 6 μmoles of dbcAMP had a greater stimulatory effect than 24 μmoles of the same substance.

Another possible mode of action of cGMP is the stimulation of the hydrolysis of cAMP (12). *In vitro* studies (12) demonstrate that small amounts of cGMP (0.08 μmole) which approximates normal levels in tissues (19) can initiate the hydrolysis of cAMP to 5-adenosine monophosphate, the inactive form. Furthermore, cGMP has been shown to penetrate more readily than cAMP (14) in a shorter period of time. Increased intracellular levels of cGMP and its dibutyryl derivative, as compared to dbcAMP, would allow the guanosine compounds sufficient time to decrease the endogeneous levels of cAMP. The resulting low cAMP level may explain the decreased ^{59}Fe incorporation in the groups combining the guanosine nucleotides with dbcAMP. Although cGMP is cleared from plasma more readily than cAMP (15), the high dose injected into the mice would still enable the cyclic guanosine nucleotide to exhibit its effect on the intracellular level of cAMP.

Summary. The cyclic 3,5-guanosine nucleotide and its dibutyryl derivative failed to enhance erythropoiesis in the posthypoxic mouse. This inhibition seemed to be competitive in that cGMP or dbcGMP, when administered in conjunction with dbcAMP, an erythropoietic stimulant, significantly decreased the percent ^{59}Fe incorporation below the level of dbcAMP effect alone.

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Received July 23, 1973. P.S.E.B.M., 1974, Vol. 145.