Meanwhile the sensitivity of acetylcholine receptors to ACh is much higher in old animals [4]. Consequently, it can be postulated that the increase in the sensitivity of acetylcholine receptors in old age leads to more marked activation of guanylate cyclase by smaller doses of ACh, and to a more considerable rise in the cyclic GMP level, which was responsible for the enhancement of its effects in the old animals.

It must, however, be emphasized that in the intact organism complex interrelations exist between the effects of mediators on the cyclic nucleotide level, as well as mutual influences of these nucleotides on each other and consequent changes in cardiac activity [2, 5, 8]. Under these conditions the increase in the cyclic GMP concentration, according to some workers [10], is not the only cause of changes in heart function under the influence of ACh.

Age differences in the action of ACh on the cyclic GMP concentration are thus an important, but probably not the only, link in the complex mechanism of age changes in the structure of the cholinergic regulation of the heart.

LITERATURE CITED

1. N. S. Verkhratskii, "Mediator metabolism and effector response to cholinergic and adrenergic influences in old age," Author's Abstract of Doctoral Dissertation, Kiev (1971).
2. S. E. Severin and V. A. Tkachuk, in: Metabolism of the Myocardium [in Russian], Moscow (1979), p. 54.
3. V. V. Frol'kis, Regulation, Adaptation, and Aging [in Russian], Leningrad (1970).
4. V. G. Shevchuk, in: Aging of the Cell [in Russian], Kiev (1971), p. 214.
5. J. Beavo, J. Hardman, and E. Sutherland, J. Biol. Chem., 246, 3841 (1971).
6. V. V. Frol'kis (W. W. Frolkis), Mechanismen des Alterns, Berlin (1975).
7. D. Garbes, T. Chrisman, and J. Hardman, in: Eukaryotic Cell Function and Growth. Regulation by Intracellular Cyclic Nucleotides, New York (1976), p. 155.
8. W. George, J. Polson, A. O'Toole, et al., Proc. Natl. Acad. Sci. USA, 66, 398 (1970).
9. J. Hardman, J. Davis, and E. Sutherland, J. Biol. Chem., 244, 6354 (1969).
10. S. Keely and T. Lincoln, Biochim. Biophys. Acta, 543, 251 (1978).
11. J. Kuo, P. Lee, K. Walton, et al., J. Biol. Chem., 247, 16 (1972).
12. M. Schwegler and R. Jacob, in: Recent Advances in Studies on Cardiac Structure and Metabolism, Vol. 7, Munich (1976), p. 391.
13. R. Williams and W. Thompson, Proc. Soc. Exp. Biol. (New York), 143, 382 (1973).

EFFECT OF CYCLIC AMP AND ITS DIBUTYRYL ANALOG ON MACROMOLECULAR BIOSYNTHESIS IN ACTIVELY PROLIFERATING CELLS

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Studies of the role of cyclic adenosine-3,5-monophosphate (cyclic AMP) in cell proliferation and differentiation are of great interest [8, 12-14]. To study the effect of exogenous cyclic AMP on physiological processes in cells both cyclic AMP itself and its dibutyryl analog — N^6O^2-dibutyryl-3,5-cyclic AMP — have been widely used. However, exogenous cyclic AMP does not always produce the effects of the cyclic AMP which is synthesized intracellularly and, furthermore, the effects of exogenous cyclic AMP and of dibutyryl-cyclic AMP are not always equivalent [5, 10].

The object of the present investigation was to compare the action of exogenous cyclic AMPs and of dibutyryl-cyclic AMP on macromolecular biosynthesis *in vitro* in cells in a stage of active proliferation. Ehrlich's ascites carcinoma (EAC) cells and thick embryonic carti-

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TABLE 1. Effect of Cyclic AMP and Dibutyryl-Cyclic AMP on DNA and RNA Synthesis in EAC Cells

Preparation	Concentration, M	Incorporation of ^{14}C-thymidine, cpm/100 mg cells	P compared with control	Incorporation of ^{3}H-uridine, cpm/100 mg cells	P compared with control	% compared with control
Control	—	6 310 (5 466—6 865)	—	6 188 (5 515—6 200)	—	
Cyclic AMP	10^{-3}	13 192 (11 467—14 676)	$<0,05$	12 382 (7 822—13 687)	$<0,05$	209,0
	10^{-4}	7 122 (7 062—7 548)	$<0,05$	7 109 (6 532—8 559)	$<0,05$	112,8
Dibutyryl-cyclic AMP	10^{-3}	3 545 (2 874—4 073)	$<0,05$	3 237 (2 285—4 415)	$<0,05$	56,2
	10^{-4}	4 555 (4 381—5 240)	$<0,05$	4 195 (4 086—5 974)	$<0,05$	72,2

Legend. Here and in Tables 2 and 3, limits of variations given in parentheses.

TABLE 2. Penetration of Cyclic AMP and Dibutyryl-Cyclic AMP into EAC Cells

Group	Preparation	Concentration, M	Quantity of preparation in medium, picomoles	Quantity of nucleotide penetrating into cells (% of quantity added to medium)	P
1	Cyclic AMP	10^{-3}	1 792 000	0,049 (0,048—0,058)	$P_{1-3}<0,05$
2		10^{-4}	192 000	0,047 (0,045—0,047)	$P_{2-4}<0,05$
3	Dibutyryl-cyclic AMP	10^{-3}	1 792 000	0,59 (0,50—0,70)	$P_{3-1}<0,05$
4		10^{-4}	179 000	0,60 (0,54—0,60)	$P_{2-4}<0,05$

TABLE 3. Effect of Cyclic AMP and IBMX on DNA Biosynthesis in EAC Cells

Preparation	Concentration, M	Incorporation of ^{14}C-thymidine, cpm/100 mg cells	P compared with control	% compared with control
Control	—	1 297 (1 189—1 491)	—	—
Cyclic AMP	10^{-3}	2 202 (1 998—2 984)	$<0,05$	169,7
IBMX	10^{-3}	1 087 (840—1 139)	$<0,05$	83,8
Cyclic AMP + IBMX	10^{-3} + 10^{-3}	1 185 (1 060—1 310)	$<0,05$	91,4

lage tissue were used as the test objects.

EXPERIMENTAL METHOD

Biosynthesis of RNA and DNA in EAC cells was studied *in vitro* by the method described previously [3]. The cells were incubated with the cyclic nucleotides for 60 min, after which the appropriate radioactive label was introduced and incubation continued for a further 30 min. To study penetration of cyclic AMP and dibutyryl-cyclic AMP into EAC cells, the cyclic AMP kit from the Radiochemical Centre, Amersham (England) was used; transmembrane transport and metabolism of cyclic AMP were studied with the aid of 8-^{3}H-3,5-cyclic AMP. This substance was determined by column chromatography [7, 11], and its metabolites (adenosine, AMP, ADP, ATP) by chromatography on paper [3, 4]. To study protein biosynthesis in embryonic cartilage tissue, the tibias from 11-day chick embryos were used [2]. The tibias were incubated in Ringer–Krebs bicarbonate buffer for 4 h, after which 2 μCi ^{14}C-proline was added and incubation continued for a further 2 h. At the end of incubation, ^{14}C-hydroxyproline in the samples was determined [2, 9]. The nonparametric U criterion was used for statistical analysis [1].

EXPERIMENTAL RESULTS

Cyclic AMP in concentrations of 10^{-3} and 10^{-4} M stimulated RNA and DNA biosynthesis in EAC cells *in vitro*. Dibutyryl-cyclic AMP in similar concentrations led to inhibition of macromolecular biosynthesis in these cells (Table 1). It was shown previously that sodium butyrate has no effect on RNA and DNA biosynthesis in EAC cells [3]. Dibutyryl-cyclic AMP, being a more strongly lipophilic agent, is known to have greater powers of penetration than cyclic AMP through plasma membranes [6, 10]. The difference in the degree of penetration was 1 order of magnitude (Table 2). These results, and also the fact that dibutyryl-cyclic AMP

TABLE 4. Effect of Cyclic Nucleotides and of IBMX on Collagen Biosynthesis in Cartilage Tissue

Preparation	Concentration, M	Absolute radioactivity of ^{14}C-hydroxyproline, cpm $\cdot 10^{-3}$	% compared with control
Control	—	164,5	—
Dibutyryl-cyclic AMP	10^{-3}	68,6	36,0
Control	—	124,0	—
Dibutyryl-cyclic AMP	10^{-3}	82,6	66,0
Control	—	93,5	—
Dibutyryl-cyclic AMP	10^{-3}	70,6	75,0
Control	—	375,0	—
Cyclic AMP	10^{-3}	425,8	113,0
Control	—	239,0	—
Cyclic AMP	10^{-3}	402,6	168,0
Control	—	371,7	—
Cyclic AMP	10^{-3}	433,1	117,0
Control	—	363,5	—
Cyclic AMP + IBMX	10^{-3} + 10^{-3}	387,6	107,0
Control	—	505,2	—
Cyclic AMP + IBMX	10^{-3} + 10^{-3}	481,2	95,0
Control	—	371,0	—
Cyclic AMP + IBMX	10^{-3} +	213,0	79,0

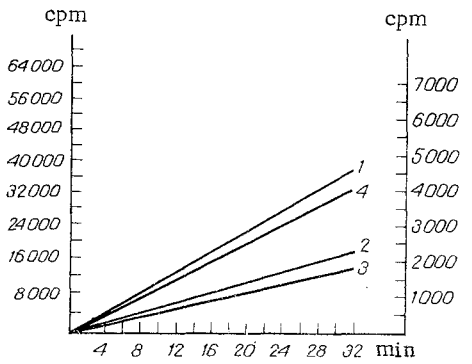

Fig. 1. Total intracellular radioactivity and radioactivity of cyclic AMP after incubation of EAC cells with 8-^{3}H-3,5-cyclic AMP. 1) Total ^{3}H-activity; 2) total ^{3}H-activity (incubation with theophylline, 10^{-2} M); 3) ^{3}H-cyclic AMP; 4) ^{3}H-cyclic AMP (incubation with theophylline, 10^{-2} M). Abscissa, time (in min); ordinate: on left — total radioactivity (in cpm), on right — radioactivity of cyclic AMP (in cpm).

in low concentrations (10^{-6}-10^{-10} M) didnot affect macromolecular biosynthesis in EAC cells, suggest that the stimulating action of cyclic AMP on RNA and DNA biosynthesis is unconnected with the action of the nucleotide itself.

Cyclic AMP is known to undergo rapid hydrolysis under the influence of the specific phosphodiesterase (PDE) to AMP, from which adenosine, which has high powers of penetration through plasma membranes [15], may subsequently be formed. Penetration of adenosine and thè subsequent formation of nucleic acid precursors intracellularly from it may give rise to stimulation of their biosynthesis. The addition of cyclic AMP to the incubation medium together with 3-isobutyl-1-methylxanthine (IBMX), an inhibitor of PDE, to the incubation medium

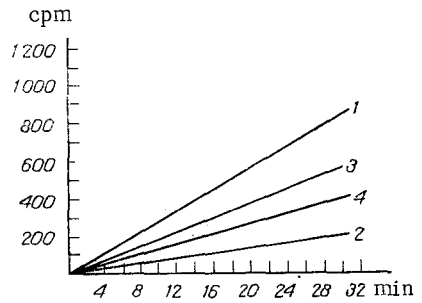

Fig. 2. Intracellular radioactivity of ATP and ADP after incubation of EAC cells with 8-^{3}H-3,5-cyclic AMP. 1) ^{3}H-ATP; 2) ^{3}H-ATP (incubation with theophylline, 10^{-2} M); 3) ^{3}H-ADP; 4) ^{3}H-ADP (incubation with theophylline, 10^{-2} M). Abscissa, time (in min); ordinate, radioactivity of ATP and ADP (in cpm).

prevented stimulation of DNA biosynthesis observed in the presence of cyclic AMP (Table 3).

In the next experiments it was shown that cyclic AMP may be rapidly metabolized extracellularly, and that intracellular resynthesis of ADP and ATP takes place from the products of its metabolism. On incubation with theophylline both the total intracellularly determined radioactivity and the radioactivity of ADP and ATP formed intracellularly from metabolites of exogenous cyclic AMP fell sharply. Meanwhile an increase in the intracellular radioactivity of cyclic AMP was observed in the presence of theophylline (Figs. 1 and 2).

Similar results were obtained on a different model of actively proliferating cells, namely embryonic cartilage tissue. This tissue consists mainly of chondrocytes, synthesizing collagen. Addition of cyclic AMP to the incubation medium led to stimulation of collagen biosynthesis, but under the influence of dibutyryl-cyclic AMP marked inhibition of collagen biosynthesis was observed (Table 4). Addition of cyclic AMP together with IBMX, an inhibitor of PDE, prevented the stimulation of collagen biosynthesis observed in the presence of cyclic AMP.

The results obtained with the PDE inhibitor on both models lead to the conclusion that the stimulating action of cyclic AMP on macromolecular biosynthesis is connected with the metabolism of this nucleotide. Dibutyryl-cyclic AMP, because of its lipophilicity and its resistance to the action of PDE, reproduces the effects of intracellularly synthesized cyclic AMP.

LITERATURE CITED

1. E. V. Gubler and A. A. Genkin, The Use of Nonparametric Statistical Criteria in Medical and Biological Research [in Russian], Leningrad (1973), p. 63.
2. V. I. Mazurov, Biokhimiya, 31, 212 (1966).
3. B. Z. Simkhovich, Vopr. Med. Khim., No. 6, 768 (1978).
4. B. Z. Simkhovich, M. Yu. Lidak, and A. P. Gilev, Khim. Farm. Zh., No. 6, 17 (1978).
5. M. A. Fedorov, Regulation of Proliferation of Hematopoietic Cells [in Russian], Moscow (1977), p. 26.
6. M. M. Bevers, J. Van Rijn, and R. Van Wijk, FEBS Lett., 72, No. 275 (1976).
7. R. J. Haslam and J. A. Lynham, Life Sci., 23, 1143 (1972).
8. A. W. Hsie and T. T. Puck, Proc. Natl. Acad. Sci. USA, 68, 358 (1971).
9. K. Juva and D. J. Prockop, J. Biol. Chem., 241, 4419 (1966).
10. E. Kaukel and H. Hilz, Biochem. Biophys. Res. Commun., 46, 1011 (1972).
11. G. Krishna, B. Weiss, and B. Brodie, J. Pharmacol. Exp. Ther., 163, 379 (1968).
12. K. H. Prasad and A. W. Hsie, Nature New Biol., 233, 141 (1972).
13. R. H. Rixon, J. E. Whitfield, and J. P. MacManus, Exp. Cell Res., 63, 110 (1970).
14. J. R. Sheppard, Proc. Natl. Acad. Sci. USA, 68, 1316 (1971).
15. E. W. Sutherland, J. Am. Med. Assoc., 214, 1218 (1970).