EFFECT OF HYPOLIPIDEMIC DRUGS ON BASAL AND STIMULATED ADENYLATE CYCLASE ACTIVITY IN TUMOR CELLS

L. M. Bershtein, I. G. Kovaleva, and O. A. Rozenberg

UDC 616-006-008.931-02:615.272.4

KEY WORDS: adenylate cyclase; hypolipidemic agents; tumor cell.

Much attention has been paid to the study of the role of cyclic nucleotides in neoplastic transformation and progression of tumor growth, primarily in connection with their effect on processes of proliferation and cell differentiation [5]. Irregular changes in basal adenylate cyclase activity in tumor cells are often combined with a decrease in the response of the enzyme to hormonal stimulation [9]. This may lead not only to changes in the hormone receptor apparatus, but also to weakening of hormonal sensitivity (hormone dependence) of the tumor tissue, so that it becomes autonomous, and the effectiveness of treatment of neoplasms is reduced.

Having regard to the lipid-dependence of adenylate cyclase [3, 11] and to data [1] indicating inhibition of activity of the cyclase system in cancer patients during hyperlipidemia and other disturbances of lipid metabolism characteristic of the cancrophilia syndrome [2], in the investigation described below adenylate cyclase activity was studied in Ehrlich's ascites carcinoma (EAC) cells during administration of drugs with a hypolipidemic action.

EXPERIMENTAL METHOD

EAC (about $20 \cdot 10^6$ cells) was transplanted intraperitoneally into 100 male SHR mice weighing 18-20 g, which were divided into four groups. From the first day of the experiment, animals of groups 2, 3, and 4 received one of the following substances respectively with their drinking water: the antidiabetic biguanide phenformin (1 mg daily, from Lääke Oy, Finland), clofibrate (Miscleron, 5 mg daily, from Hinoin, Hungary), and the phospholipid-containing preparation Essentiale (5 mg daily, from Bosnalijek, Yugoslavia). Animals of group 1 (control) received an equal volume of water. The mice were killed on the 7th-8th day after transplantation. Basal and adrenalin ($5 \cdot 10^{-5}$ M) stimulated adenylate cyclase activity in homogenates of EAC cells were determined by the method described previously [4]. Creatine phosphate and creatine phosphokinase were used as the ATP-regenerating system. The cAMP formed was isolated by chromatography on Silufol (Czechoslavakia) plates after incubation of the enzyme preparation with ^{3}H-ATP ("Izotop" Combine, USSR), or was determined by the competitive binding method with protein, using kits from Amersham Corporation (England). No difference in principle was observed between the results. The concentration and relative proportions of the various phospholipid fractions in the washed residue of the tumor cells were studied by thin-layer chromatography on silica-gel [15], the total and free cholesterol were determined by gas chromatography, and protein by Lowry's method.

EXPERIMENTAL RESULTS

Adenylate cyclase in the EAC cell homogenate was stimulated by adrenalin (Table 1), in agreement with data in the literature, and in association with the presence of β-adrenoreceptors in these cells [6]. All the hypolipidemic drugs studied caused an increase in adrenalin-stimulated adenylate cyclase activity of the tumor cells, but definite differences were found in the effects of clofibrate, on the one hand, and of phenformin and Essentiale, on the other hand, in their effects on basal activity of the enzyme.

Laboratory of Endocrinology, N. N. Petrov Research Institute of Oncology, Ministry of Health of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR A. N. Klimov.) Translated from Byulleten' Éksperimental'noi Biologii i Medtsiny, Vol. 100, No. 9, pp. 334-336, September, 1985. Original article submitted September 14, 1985.

TABLE 1. Adenylate Cyclase Activity (in picomoles cAMP/mg protein/min) in Ehrlich's Ascites Carcinoma Cells (M ± m)

Experimental conditions	Basal activity	Stimulated activity
Control	0,99±0,14	4,18±0,66
Phenformin	1,74±0,43	6,50±0,75*
Clofibrate	0,97±0,27	5,53±0,88*
Essentiale	1,20±0,24	5,61±0,65*

Legend. Mean results of five series of experiments. Here and in Table 2: *P < 0.05 compared with control.

TABLE 2. Concentration and Relative Proportions of Lipid Fractions in Ehrlich's Ascites Carcinoma Cells (M ± m)

Experimental conditions	Total CH, μg/mg protein	Free CH, μg/mg protein	CH esters, %	PL, μg/mg protein	Total CH/PL, mole/mole	Free CH/PL, mole/mole
Control	27,23±3,12	22,95±2,50	15,6±2,6	243,2±37,5	0,231±0,009	0,192±0,007
Phenformin	53,68±10,50*	43,78±8,25*	17,4±1,3	443,8±81,5*	0,239±0,007	0,197±0,004
Clofibrate	34,33±6,38	28,50±4,25	15,2±2,5	290,7±48,3	0,242±0,009	0,207±0,012
Essentiale	34,76±3,31	30,38±3,50	11,4±2,3	311,7±33,3	0,222±0,009	0,195±0,004

Legend. Mean results of four series of experiments. CH) Cholesterol, PL) phospholipids.

Clofibrate differed from the other two preparations in its action on the lipid content in EAC cells also. In particular, phenformin was found to increase (about equally) the concentrations of cholesterol and phospholipids in the tumor cells, whereas under the influence of Essentiale there was a tendency for the phospholipid concentration in the cells to rise and the fraction of cholesterol esters to fall. Meanwhile in the group of mice receiving clofibrate, changes in the lipid composition of the tumor cells were not unusual (cholesterol esters) or less marked (phospholipids, Table 2). It was also found that under the influence of phenformin and Essentiale the phosphatidylcholine/sphingomyelin ratio in the EAC cells increased by 46.6 and 34.6%, respectively. When clofibrate was given, it was virtually unchanged. The increase in the cholesterol concentration (mainly on account of the free cholesterol fractions) under the influence of phenformin in EAC cells was evidently peculiar to that particular experimental model. The ability of EAC cells to synthesize cholesterol independently is known to be significantly reduced, and the cholesterol detected in them is mainly brought into the cells from the ascites fluid [7]. In particular, according to our data, when phenformin was given to rats with Pliss lymphosarcoma or with transplantable mammary gland carcinoma (RMZh strain) a fall in the cholesterol concentration was observed (mainly on account of its esters) in the tumor tissue 12-14 h after transplantation, accompanied by a fall in the cholesterol/phospholipid ratio, in harmony with the view that antidiabetic biguanides may inhibit cholesterol biosynthesis [10].

Existing information on the effect of phenformin and clofibrate on adenylate cyclase activity in different tissues is contradictory [8, 12, 13]. In the modern view, an increase in the phospholipid concentration or the phosphatidylcholine fraction in cell membranes may lead to a decrease in their viscosity, on which the nature of contact between the hormone-receptor complex and adenylate cyclase and stimulation of the activity of the latter, in turn, essentially depend [11]. Comparison of this information with our own results leads to the conclusion that potentiation of hormone-stimulated adenylate cyclase activity under the influence of phenformin and Essentiale is probably connected with the above changes in lipid composition of the tumor cells. In this respect it is worth noting that phenformin, like some other antidiabetic biguanides, is able to potentiate the binding of insulin with receptors in the lymphocytes and certain tumor cells [14], and this can also be explained by lipid modification of the cell membranes.

After injection of clofibrate, the increase in adrenalin-stimulated activity of the enzyme was evidently based on a different principle, for example, one involving the character

of interaction of adenylate cyclase subunits with guanyl nucleotide-binding regulatory proteins (G/N-proteins) or a change in concentration of the latter. Despite possible differences in the concrete mechanism of action of the hypolipidemic agents chosen for study on the cyclase system, the use of such agents, which have already been used to abolish endocrine-metabolic disturbances and states of metabolic immunodepression in cancer patients [2], offers definite prospects for oriented modification of the hormone sensitivity of tumor cells.

LITERATURE CITED

1. L. M. Bershtein, Vopr. Onkol., No. 8, 87 (1982).
2. V. M. Dil'man, Endocrinologic Oncology [in Russian], Leningrad (1983).
3. T. S. Saatov, Ukr. Biokhim. Zh., No. 2, 44 (1981).
4. V. A. Tkachuk and G. N. Baldenkov, Biokhimiya, No. 6, 1097 (1978).
5. N. A. Fedorov, Biological and Clinical Importance of Cyclic Nucleotides [in Russian], Moscow (1979).
6. H. P. Bär and J. F. Henderson, Can. J. Biochem., 50, 1003 (1972).
7. D. E. Brenneman, R. McGee, and A. A. Spector, Cancer Res., 34, 2605 (1974).
8. J. D. Brown, J. Beerends, and D. C. Bartels, Endocrinology, 93, 445 (1973).
9. W. E. Criss and F. Murad, in: Clinical Aspects of Cyclic Nucleotides, ed. L. Volicer, New York (1977), pp. 429-448.
10. M. E. Dempsey, Ann. N. Y. Acad. Sci., 148, 631 (1968).
11. M. D. Houslay and L. M. Gordon, in: Current Topics in Membranes and Transport, Vol. 18, New York (1983), pp. 143-178.
12. H. Kather, G. Simon-Crisan, and B. Vogt, Horm. Metab. Res., 9, 300 (1977).
13. D. C. Lehotay, H. S. Paul, J. S. Sindler, et al., Metabolism, 32, 157 (1983).
14. V. Pezzino, V. Trischitta, F. Purrello, et al., Diabetologia, 23, 131 (1982).
15. V. E. Vaskowsky and V. I. Svetashev, J. Chromatogr., 65, 451 (1972).