PYRUVATE KINASE ACTIVITY IN THE LIVER OF RATS WITH INDUCED TUMORS

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The activity of pyruvate kinase (PK) and its isozymes was studied in the liver of rats with macroscopically visible tumors induced by p-dimethylaminoazobenzene. At the same time the action of a prolonged dietary intake of NaF by the rats on PK was studied. An increase in activity of the M-isozyme of PK during development of a malignant liver tumor was demonstrated. An excess of NaF in the diet of rats prevents the increase in the activity of this isozyme.

KEY WORDS: pyruvate kinase and its isozymes; hepatoma; fluorine.

Pyruvate kinase — PK (ATP:pyruvate phosphotransferase, EC 2.7.140) — a key enzyme of glycolysis, exists as several isozymes [7], the composition of which in established hepatomas differs from that in normal liver [10].

The object of this investigation was to study the activity of PK and its isozymes in the liver of rats with macroscopically visible tumors induced by p-dimethylaminoazobenzene (DAB). It has been stated [2] that NaF can inhibit the development of experimental tumors. At the same time, therefore, the action of NaF which, *in vitro*, inhibits glycolysis and several enzyme systems of the liver [6, 11], on PK also was studied.

EXPERIMENTAL METHOD

Experiments were carried out on rats divided into six groups. The animals of groups 1-3 received no carcinogen (control), but DAB was added to the diet of the rats of groups 4-6 in a dose of 0.06%. The diet of the rats of groups 1 and 4 contained no NaF, but the rats of groups 2, 3, and 6 received NaF for 5 months in doses of 0.1 and 1.5 mg F /kg, respectively. The rats were given distilled water to drink and also with the food. Pieces of liver wherever possible not containing visible tumors were taken for analysis. The homogenate (1:4) was centrifuged at 40,000 g. PK isozymes were isolated by fractionating the supernatant with ammonium sulfate [3]. PK activity was determined spectrophotometrically with lactate dehydrogenase by the method of Bücher and Pfleiderer [4].

EXPERIMENTAL RESULTS AND DISCUSSION

The results in Table 1 show that PK activity in the liver hyaloplasm of the rats of groups 1-3 was unchanged. The L- and M-isozymes tested correspond to liver and muscle forms of PK [9]. The L-isozyme, which is much more thermolabile, lost 44-60% of its activity during incubation for 3 min at 50°C. Under the same conditions the M-isozyme was inactivated on average by 9%. This sensitivity to temperature still remained when the isozymes were tested in the liver of the control and experimental rats. The activity of the L-isozyme was much higher than that of the M-isozyme, in agreement with data in the literature [5]. In the course of the experiments the ratio between their activities altered: In the liver of the control rats of groups 1-3 it was 15.4, 15.5, and 13.6, respectively, In the liver

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TABLE 1. Activity of PK and Its Isozymes (in μmoles phosphoenolpyruvate/mg protein/min, at 25°C) in Liver of Control Rats and Rats with Induced Tumors (M ± m)

Group of rats	PK in liver hyaloplasm	L-Isozyme	M-Isozyme
1	2,2±0,2	32,3±4,1	2,1±0,5
2 (0,1 mg F^-/kg)	1,9±0,2	43,5±9,8	2,8±0,4
3 (1,5 mg F^-/kg)	2,17±0,3	38±11,1	2,8±0,4
4 (DAB)	2,07±0,2	33,2±29,7	4,3±0,3
5 (DAB +0,1 mg F^-/kg)	1,88±0,2	10,5±4,6	4,1±0,8
6 (DAB +1,5 mg F^-/kg)	2,45±0,1	30,7±7,8	3,3±0,34

of the rats with tumors belonging to groups 4-6 the ratio was reduced to 7.7, 2.5, and 9.5, respectively, on account of an increase in the activity of the M-isozyme of PK.

It will be clear from Table 1 that the activity of the M-isozyme was increased in the liver of rats of all the experimental groups. Activity of the L-isozyme was reduced only in the liver of rats with tumors and receiving 0.1 mg F^-/kg, and was unchanged in the other cases. A significant difference was found in M-isozyme activity in the experiments between groups of animals not receiving NaF and receiving it in a dose of 1.5 mg F-/kg ($P < 0.01$). An excess of NaF led to a relative decrease in the activity of the M-isozyme in the liver of rats of this group.

PK activity in the hyaloplasm was thus not significantly altered in the liver of rats with tumors, but the isozyme composition of the enzyme was changed on account of an increase in the activity of the M-isozyme. An excess of NaF in the diet caused a relative decrease in the activity of this isozyme in the liver of the rats with tumors.

An increase in the content of "embryonic" M-isozyme of PK has been demonstrated in Morris hepatomas and it correlated with the degree of their differentiation and malignancy. The content of the M_2-isozyme of PK was increased in the tissues of hyperplastic nodules in the rat liver during chemical carcinogenesis caused by 3-methyl-DAB [10], and also in the liver of mice with tumors induced by N,N'-2,7-fluorenylbisacetamide [8].

The present experiments showed an increase in the activity of the PK M-isozyme in the liver of rats with tumors induced by DAB. According to Tanaka and co-workers [9], the process of glycolysis, controlled by hexokinase and the M-isozyme of PK, assumes great importance in the embryonic liver and in hepatomas. In a previous investigation the present writers obtained evidence of the ability of NaF *in vivo* to prevent the increase in glycolysis in the liver in the last stages of carcinogenesis [1]. This effect may be connected with the observed ability of NaF to inhibit the activity of the M-isozyme of PK *in vivo*.

LITERATURE CITED

1. G. P. Gorban', O. D. Karpilovs'ka, L. I. Laevs'ka, et al., Ukr. Biokhim. Zh., No. 4, 473 (1973).
2. O. G. Prokof'eva, Sov. Vrach. Sbornik, No. 10, 1 (1947).
3. S. Bailey, F. Stirpe, and C. Taylor, Biochem. J., 108, 427 (1968).
4. T. Bücher and G. Pfleiderer, in: Methods in Enzymology (ed. by S. P. Colowick and N. O. Kaplan), Vol. 1, Academic Press, New York (1955), p. 435.
5. H. Carminatti, L. De Asua, E. Recondo, et al., J. Biol. Chem., 243, 3051 (1968).
6. A. Doberenz, A. Kurnick, E. Kurtz, et al., Proc. Soc. Exp. Biol. (New York), 117, 689 (1964).
7. B. Hess and C. Kutzbach, Hoppe-Seyler's Z. Physiol. Chem., 352, 453 (1971).
8. S. Janagi, T. Kamija, Y. Ikehara, et al., Gann, 62, 283 (1971).
9. T. Tanaka, Y. Harano, F. Sue, and H. Morimura, J. Biochem. (Tokyo), 62, 71 (1967).
10. R. Walker and V. Potter, Adv. Enzyme Reg., 10, 339 (1971).
11. O. Warburg and W. Christian, Biochem. Z., 310, 384 (1942).