EFFECT OF THYROID HORMONE ON ACTIVITY OF ENZYMES OF CARBOHYDRATE METABOLISM DURING TUMOR GROWTH

L. É. Teras

UDC 616-006-092.9-085.357.441-07:616-008.931:577.152.32

Activity of hexokinase (HK) and its isozymes, glucose-6-phosphatase, and glucose-6-phosphate dehydrogenase and the effect of tri-iodothyronine (T_3) on this activity were investigated in the liver tissue of mice with transplanted hepatoma 22a at different times of growth of the tumor. Changes in the activity of individual enzymes in the liver were observed even while the tumor was small. Persistent changes in the activity of all enzymes studied, and also in the HK isozyme spectrum were observed starting from the 21st day after transplantation of the tumor. In the initial stages of hepatoma development the activity of the various enzymes in the liver is regulated by thyroid hormone. The inducing action of T_3 is gradually lost as the tumor grows in size.

KEY WORDS: tumor; hexokinase; glucose-6-phosphatase; glucose-6-phosphate dehydrogenase; thyroid hormone.

It has been shown recently that activity of the enzymes of carbohydrate metabolism and their sensitivity to the regulatory action of hormones are considerably modified in the various tissues of a tumor-bearing animal [4, 5, 9]. The present writer found [2] that reactivity of hexokinase (HK) and of glucose-6-phosphatase (G6Pase) to the action of thyroid hormone is modified in the liver tissues of animals with tumors.

The object of the present investigation was to study changes in the activity of enzymes of glucose-6-phosphate metabolism and in the spectrum of HK isozymes in the liver and also the action of thyroid hormone on them at different stages of tumor growth.

EXPERIMENTAL METHOD

Experiments were carried out on C3HA mice into which small fragments of Gelstein's hepatoma 22a were implanted subcutaneously. At various times after transplantation of the tumor, HK and glucose-6-phosphate dehydrogenase (G6PD) activity was determined in the soluble fraction of the liver of six to nine mice, by a spectrophotometric method based on reduction of NADP, and G6Pase activity was determined from the increase in inorganic phosphorus. The experimental conditions and composition of the incubation medium were described by the writer previously [2]. HK and G6PD activity was expressed in μmoles reduced NADP/h·mg protein, and G6Pase activity in μg phosphorus liberated during incubation for 20 min, per 10 mg protein. The protein content was determined by Lowry's method [7]. HK isozymes were separated chromatographically on a column of DEAE-cellulose in a KCl concentration gradient (0-0.5 M). Activity of the isozymes in individual fractions was determined spectrophotometrically in medium containing 0.1 M Tris-HCl buffer, pH 7.6, 0.006 M ATP, 0.014 M $MgCl_2$, NADP, G6PD, and 0.5 mM glucose (for determination of the activity of the first three HK isozymes) or 100 mM glucose (for determination of the fourth isozyme).

Tri-iodothyronine (T_3) was injected subcutaneously into the animals in a dose of 90 μg/100 g body weight daily for 3 days.

EXPERIMENTAL RESULTS

Starting from the 21st day after transplantation of the tumor, an increase in HK activity could be observed in the liver tissue (Table 1). On the 21st day, for instance, HK activi-

Institute of Experimental and Clinical Medicine, Ministry of Health of the Estonian SSR, Tallin. (Presented by Academician of the Academy of Medical Sciences of the USSR L. M. Shabad.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 88, No. 7, pp. 71-74, July, 1979. Original article submitted February 21, 1978.

 0007-4888/79/8807-0754$07.50

TABLE 1. Enzyme Activity in the Liver at Different Times after Transplantation of Hepatoma

Day after transplan. of hepato.	HK		G6Pase		G6PD	
	$M \pm m$	P	$M \pm m$	P	$M \pm m$	P
0	0,08±0,01	—	133±10	—	0,28±0,02	—
7-th	0,06±0,01	>0,05	137±14	>0,5	0,42±0,04	<0,05
14-th	0,09±0,01	>0,2	92±5	<0,01	0,33±0,03	>0,1
21-st	0,16±0,02	<0,01	115±17	>0,2	0,58±0,05	<0,01
28-th	0,21±0,05	<0,02	74±9	<0,01	0,50±0,05	<0,01

Legend. Values of P calculated relative to normal liver (day 0).

TABLE 2. Action of Thyroid Hormone on Enzyme Activity in the Liver at Various Times after Transplantation of Hepatoma

Day after transplanta. of hepatoma	HK			G6Pase			G6PD		
	$M \pm m$	%	P	$M \pm m$	%	P	$M \pm m$	%	P
0	0,19±0,02	238	<0,01	212±21	159	<0,01	0,41±0,02	146	<0,01
3-th	0,19±0,06	238	<0,01	228±19	171	<0,01	0,65±0,09	232	<0,01
7-th	0,58±0,09	966	<0,01	433±53	316	<0,01	1,56±0,05	371	<0,01
10-th	0,32±0,07	534	<0,01	168±39	123	>0,5	0,44±0,03	105	>0,5
14-th	0,27±0,07	300	<0,01	148±26	161	>0,1	0,40±0,05	121	>0,1
21-th	0,23±0,03	144	>0,1	133±12	98	>0,5	0,36±0,06	62	<0,02
24-th	0,21±0,05	100	—	79±30	107	>0,5	0,54±0,02	108	>0,2
28-th	0,10±0,02	47	>0,1	48±19	65	>0,2	0,29±0,04	58	<0,02

Legend. Percentage and P calculated for control group (day 0) relative to normal liver without administration of T_3, and in other groups relative to liver tissue of animals with tumors at corresponding times of experiment without injection of T_3.

ty was doubled, and on the 28th day it was increased by 2.5 times compared with the control. An increase in activity of the first three HK isozymes (HK-1, HK-2, and HK-3) was observed at these same times, whereas glucokinase (HK-4) activity was the same as in normal liver or a little lower (Fig. 1).

A sharp decrease in G6Pase activity was observed as early as on the 14th day of the experiment (mean weight of hepatoma 0.89 g). By the 28th day of the experiment only 56% of the original enzyme activity remained. G6PD activity on the 7th day after transplantation of the tumor was increased by 50%. A stable increase in the activity of this enzyme by almost 100% was observed starting from the 21st day of the experiment.

Injection of T_3 caused a significant increase in HK, G6Pase, and G6PD activity in the normal liver tissue (Table 2). Under the influence of thyroid hormone, for instance, HK activity increased by almost 240%, G6Pase activity by 160%, and G6PD activity by 150%, in agreement with values obtained previously and results of other workers [1, 2]. A similar increase in HK activity also was observed on the 3rd day after transplantation of the tumor. HK activity on the 7th, 10th, and 14th day was increased in the liver tissue of animals with tumors after injection of T_3 to a greater degree than in the control animals. The same increase in the first three HK isozymes under the influence of T_3 was observed at these times as in normal liver (Fig. 2a, b). Starting from the 21st day of the experiment, injection of the hormone caused no significant increase in total activity or in activity of the individual HK isozymes (Fig. 2c).

The inducing action of thyroid hormone on G6Pase and G6PD activity was clearly manifested on the 3rd and 7th days after transplantation of the tumor. Starting from the 10th day of the experiment, the hormone had no manifest activating effect.

Changes in the activity of individual enzymes of carbohydrate metabolism in the liver tissue of animals with tumors thus take place even while the tumors are small. This was

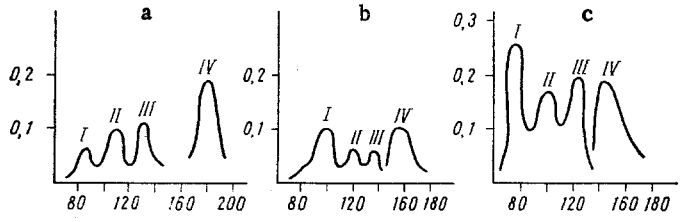

Fig. 1. Chromatograms of hexokinase isozymes in liver tissue. Ordinate, enzyme activity (in μmoles NADPH/ml eluate/h); abscissa, volume of eluate (in ml). I-IV) HK isozymes; a) normal liver; b) liver of animals with tumor on 7th day after transplantation of hepatoma; c) the same, on 28th day.

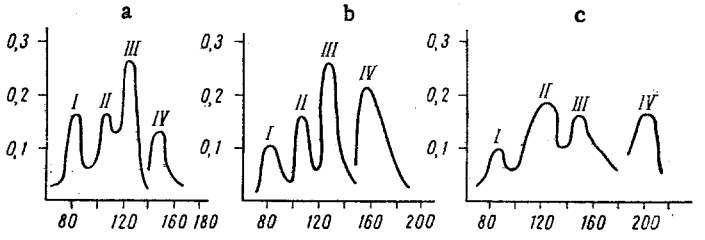

Fig. 2. Chromatograms of hexokinase isozymes of liver tissue after administration of thyroid hormone. a) Normal liver; b) liver of animals with tumors on 14th day after transplantation of hepatoma; c) the same, on 28th day. Remainder of legend as in Fig. 1.

shown by changes in G6Pase and G6PD activity during the first two weeks of development of the hepatoma. Persistent changes in the activity of all enzymes studied and also in the activity of individual HK isozymes came to light when the tumors were larger. In the present experiments this occurred on the 21st day, when the diameter of the tumor reached 1-2 cm and its weight 1.3 g, i.e., 5% of the animals' body weight. Similar dependence of HK activity on the growth of the tumor has also been observed in the liver of rats with Walker's carcinosarcoma [6]. G6PD activity in the liver of rats with sarcoma also was increased during tumor growth [8].

The changes thus detected in enzyme activity in the liver tissue of mice with hepatomas are thus identical in direction with those taking place in the tumor tissue itself [3, 5, 9].

In the initial stages of development of the hepatoma, when the tumor was still small, the activity of the enzymes studied in the liver tissue is regulated by thyroid hormone. The inducing action of T_3 relative to activity of the enzymes of carbohydrate metabolism is gradually lost as the tumor grows in size. Changes in the sensitivity of individual enzymes in the liver tissue of animals with tumors begin to develop at different times after transplantation of the hepatoma. For instance, the inducing action of T_3 relative to activity of HK and its individual isozymes is first manifested before the 21st day, whereas G6Pase and G6PD activity is unchanged during administration of thyroid hormone starting from the 10th day after transplantation of the tumor.

The tendency toward a change in the biochemical characteristics of tissues of individual organs and the decrease in their sensitivity to control factors are features which reflect the profound disturbances caused by the tumor in the host organism. According to Shapot's views [4], the systemic action of a tumor on the host must be regarded as one of the essential properties of a malignant tumor.

The author is grateful to Corresponding Member of the Academy of Medical Sciences of the USSR Professor V. S. Shapot for taking part in the discussion of the results.

LITERATURE CITED

1. R. R. Rachev and N. D. Eshchenko, Thyroid Hormones and Subcellular Structures [in Russian], Moscow (1975).

2. L. É. Teras and M. É. Isok, Vopr. Med. Khim., No. 1, 3 (1974).
3. L. É. Teras and M. É. Lond, Vestn. Akad. Med. Nauk SSSR, No. 10, 84 (1977).
4. V. S. Shapot, Biochemical Aspects of Tumor Growth [in Russian], Moscow (1975).
5. V. S. Shapot, É. G. Gorozhanskaya, and N. V. Lyubimova, Biokhimiya, 41, 1766 (1976).
6. A. Herzfeld and O. Greengard, Cancer Res., 32, 1826 (1972).
7. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
8. T. Nakamura, Y. Matuo, K. Nishikawa, et al., Gann, 61, 177 (1970).
9. G. Weber and M. Lea, Advances Enzyme Regulat., 4, 115 (1966).

PARARENAL ANGIOSARCOMA AS A MANIFESTATION OF SEXUAL DIMORPHISM IN CARCINOGENESIS

V. S. Turusov and N. S. Lanko

UDC 616.615-006.31-092-055

Pararenal angiosarcomas appeared in 42% of male CBA mice receiving 1,2-dimethylhydrazine (DMH) subcutaneously in a dose of 8 mg/kg for 30 weeks. These tumors did not appear in any of the 176 female mice of the same line receiving DMH by the same scheme. Histologically the tumors were variants of angiosarcomas with marked invaive growth into kidney tissue.

KEY WORDS: carcinogenesis; angiosarcoma; sexual dimorphism; 1,2-dimethylhydrazine; tumor of the kidney.

The sex of an animal is known to influence the appearance of tumors developing through the action of various carcinogenic agents. The clearest example of this is the epithelial tumors of the kidneys which develop in male hamsters under the influence of diethylstilbestrol [4], but do not arise under the same conditions in females. In the same way, a higher frequency of epithelial tumors of the kidneys has been observed in male mice of the Swiss line receiving 1,2-dimethylhydrazine (DMH) than in females. In rats receiving DMH, sex-linked differences in the localization of epithelial tumors in different parts of the large intestine, disappearing after castration, have been described [1].

This paper describes an angiosarcoma of the pararenal cellular tissue developing under the influence of DMH in male mice only.

EXPERIMENTAL METHOD

Each of 50 male CBA mice receivied 30 subcutaneous weekly injections of DMH (8 mg/kg, calculated as base, in 0.3 ml distilled water). The injections were started at the age of 10-12 weeks. The mice remained under observation until natural death or sacrifice at 43-45 weeks after the beginning of the experiment. The dying or killed animals were autopsied and material obtained from them was fixed in 10% formalin and embedded in paraffin wax; sections were stained with hematoxylin-eosin.

EXPERIMENTAL RESULTS

In the 46 males which survived the minimal latent period tumors developed in the following situations: angiosarcomas of the pararenal cellular tissue in 20 (43%), epithelial tumors of the kidneys in five, tumors of the anal region in 28, the intestine in 15, and the liver in nine.

The first angiosarcoma in the pararenal cellular tissue was found in an animal which died 28 weeks after the beginning of DMH administration; most of these tumors were found after 35 weeks.

Laboratory of Carcinogens, Department of Carcinogenic Agents, Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR L. M. Shabad.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 88, No. 7, pp. 74-75, July, 1979. Original article submitted March 5, 1979.

