α-GLYCEROPHOSPHATE DEHYDROGENASE ACTIVITY BIORHYTHMS IN PERIPHERAL LYMPHOCYTES OF RATS WITH WALKER CARCINOSARCOMA

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Dehydrogenase activity of peripheral blood lymphocytes is used experimentally and clinically to evaluate the state of an animal or patient with certain diseases [1, 3]. In the investigation described below, with the aim of optimizing chemotherapy, the dehydrogenase test was used to assess the state of a tumor-bearing animal, using a chronobiological approach. The development of tumor chronotherapy has led to awareness of the necessity to optimize treatment according to several time scales, when the schedule of administration of a drug is determined not only by the circadian marker rhythm, but also by rhythms with lower frequencies [5, 6, 8, 9]. In the present study a mathematical method of detection of latent biorhythms [4, 7] was used to investigated low-frequency rhythms, concealed in the time course of α-glycerophosphate dehydrogenase (α-GPDH) activity of peripheral blood lymphocytes of intact rats and rats with Walker carcinosarcoma.

EXPERIMENTAL METHOD

Noninbred male albino rats weighing 130-150 g were kept under ordinary animal house conditions and were given fresh food after blood had been taken for analysis. There were three experiments, in each of which the animals were divided into two groups with 5 or 6 rats in each group. Animals of one group (intact) served as the control, and rats of the other group underwent subcutaneous transplantation of a Walker carcinosarcoma at 10 a.m. [5]. After 24 h, and thereafter once daily at 10 a.m., blood was taken from the caudal vein of rats of both groups for cytochemical analysis [3]. α-GPDH activity was determined by counting formazan granules in 50 lymphocytes. In the experimental group the duration of the measurements was determined by the survival time of the animals (11-16 days, death of the animals was recorded at 10 a.m.), and in the control group, in two experiments measurements continued for 20-21 days (to increase the size of the sample). The seasonal stability of the biorhythms discovered was evaluated by repeating the experiment on intact animals one year after the beginning of the first experiment. Parameters of significant ($p < 0.05$) rhythms of different frequencies could be discovered simultaneously from a segment of the time course of enzyme activity for each separate animal by a mathematical method [4, 7].

EXPERIMENTAL RESULTS

Examples of the time course of α-GPDH activity of an individual animal and of the hierarchy of the rhythms identified from it are given in Fig. 1. For comparison, the calculated time course obtained after retrograde summation of the isolated harmonics, is shown by a broken line. To evaluate changes caused by development of the tumor process, the following biorhythmologic characteristics of the animals were identified and analyzed: the mean level of activity, the collective structure of the biorhythms (a set of identified rhythms and the degree of representation of each one in the group tested), the parameters (period, amplitude, and phase) of each rhythm of the collective group (Fig. 2, Table 1). Let us examine the changes taking place.

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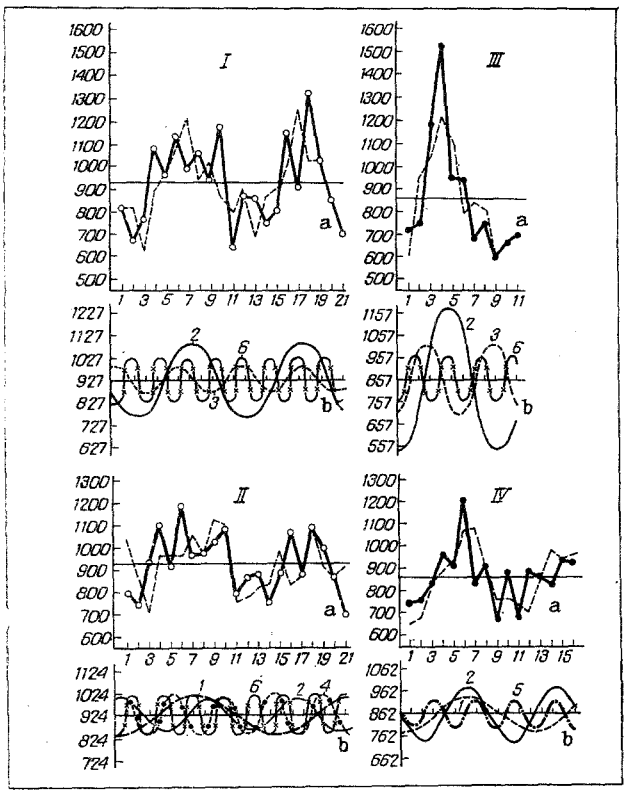

Fig. 1. Natural time course of α-GPDH activity (a, calculated time course shown by broken line) and hierarchy of rhythms isolated from it (b, August 13 to September 2, 1983). Abscissa, time (days); ordinate, absolute value of activity (number of formazan granules in 50 lymphocytes). I, II) Intact rats; III, IV) rats with Walker carcinosarcoma. 1-6) Biorhythms: 1/2, 1/3, 1/4, 1/6, 1/8, and 1/10 months, respectively.

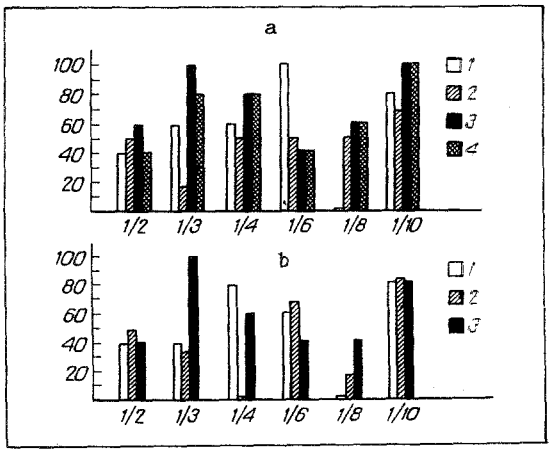

Fig. 2. Seasonal changes in percentage discovery of biorhythms. Abscissa, dimensionality of biorhythm (in fractions of months); ordinate, percentage discovery of biorhythms. a) Intact rats; b) rats with Walker carcinosarcoma. 1a, b) April 17 to May 1, 1983; 2a, b) May 19 to June 6, 1983; 3a, b) August 13 to September 2, 1983; 4a) April 25 to August 17, 1984.

TABLE 1. Parameters of Biorhythms of Change in Peripheral Blood α-GPDH Activity of Intact Rats and of Rats with Walker Carcinosarcoma (M ± 2m)

Rhythm, months	Parameter	Time when experiment performed						Combined result (n = 16)	
		May 1		May 19-June 7		August 13-September 2			
		(n = 5) control	experiment	(n = 6) control	experiment	(n = 5) control	experiment	control	experiment
		506±11	446±62	453±20	413±22*	916±24	831±32**	614±123	554±113
1/2	T	12,50	13,75	14,00±0,00	13,33±1,38	14,00±1,37	12,00	13,63±0,66	13,07±0,76
	A	72,93	185,30	68,47±10,49	102,84±80,73	113,21±126,13	97,92	86,36±21,98	125,00±45,47
	φ	171	103	170±130	90±92	117	122	203±54	148±87
	P	0,029	0,015	0,019	0,035	0,024	0,053	0,020	0,036
1/3	T	10,50±2,07	8,86±1,60	8,25	8,00	9,47±0,99	9,27±1,00	9,68±0,73	8,90±0,69
	A	115,00±80,50	152,25	67,93	57,02	142,62±53,20	228,71±104,81	125,12±35,69	173,58±75,26
	φ	272±62	55	90	55	257±53	169±45***	349±64	86±68**
	P	0,019	0,014	0,015	0,017	0,022	0,033	0,020	0,025
1/4	T	6,67±1,38	6,81±0,90	6,16±1,62		6,14±1,23	6,50±1,02	6,30±0,51	6,68±0,54
	A	87,61±101,55	134,80±36,47	67,33±20,62		106,52±44,47	132,70±100,70	89,09±25,50	133,88±27,75**
	φ	116±35	135±39	56	Not found	292±118	209	191±70	201±88
	P	0,040	0,023	0,027		0,021	0,014	0,029	0,019
1/6	T	4,33±0,31	4,40±1,33	4,18±0,38	4,50±0,99	4,25	4,42	4,27±0,17	4,45±0,36
	A	79,86±20,55	4,45±5,90	70,44±41,53	75,42±32,93	123,74	93,75	85,81±18,05	85,84±11,67
	φ	169±64	241±55*	352±66	80±86*	337	250	138±46	301±53**
	P	0,030	0,032	0,013	0,028	0,011	0,017	0,021	0,026
1/8	T	Not found	Not found	3,52±0,70	3,33	3,45±0,45	3,71	3,49±0,25	3,58±0,52
	A			90,64±43,85	41,35	109,68±31,91	93,52	100,16±20,10	76,13±10,80
	φ			115±16	102	83	199	111±94	314±112***
	P			0,012	0,032	0,018	0,041	0,014	0,038
1/10	T	2,59±0,20	2,59±0,21	2,58±0,18	2,56±0,32	2,50±0,32	2,65±0,53	2,56±0,11	2,60±0,18
	A	102,18±56,67	73,38±48,94	57,54±9,57	100,44±56,74	95,85±13,43	113,19±48,14	86,78±16,28	96,03±18,59
	φ	67±134	89±63	94	140±95	91±96	36±36	63±49	144±40***
	P	0,039	0,025	0,009	0,029	0,026	0,031	0,025	0,028

Legend. Here and in Table 2: T) period of rhythms, A) amplitude, φ) phase. Asterisks indicate significance of differences between values in experiment and control: *$p < 0.05$, **$p < 0.02$, ***$p < 0.01$, 4**) $p < 0.001$.

Mean Level of α-GPDH Activity. It will be clear from Table 1 that the mean level of activity (G, the number of formazan granules in 50 lymphocytes) in different groups of experiments shows significant changes connected both with the development of the tumor and with seasonal factors. First, a twofold seasonal (April-August) increase in G was observed in the experimental and control groups (experiment No. 3) Second, the value of G was below the control level in rats with a tumor in experiments conducted during different seasons (experiments Nos. 2 and 3).

Structure of the Group of Identified Rhythms. The same set of rhythms was discovered in the experimental and control groups, and comprised six components: rhythms of 1/2, 1/3, 1/4, 1/6, 1/8, and 1/10 months. A complete spectrum of the six components was found in only one animal (of 16) under normal conditions. The degree of representation of the identified rhythms in the experimental and control groups is shown in Fig. 2. The representativeness of a rhythm is the ratio of the number of animals in the time course of whose α-GPDH activity this particular rhythm was identified to the total number of animals in the group, expressed as a percentage. Under normal conditions this characteristic is not stable but exhibits seasonal and annual fluctuations (Fig. 2a): seasonal fluctuations are perceptible in rhythms with dimensionality of 1/3, 1/6, and 1/8 months, annual in rhythms of 1/6 and 1/8 months. In the group consisting of all animals with a tumor, the percentage of discovery of rhythms was close to that in the combined control, with the exception of rhythms of 1/4 and 1/8 months, with a lower degree of representation in tumor-bearing rats.

If the percentage of discovery of rhythms (and later, of other parameters also) is compared with the duration of survival of the tumor-bearing animals, general rules are observed which have prognostic value in the future. For example, the representation of a weekly rhythm (1/4 months) in the combined results falls in the following order: intact animals (C, control) > animals with tumors (O) with survival of 15-16 days (O_{15-16}) > tumor-bearing animals with a length of survival of 11-13 days (O_{11-13}). Again, for example, rhythms of 1/3 and 1/6 months in the O_{15-16} group are observed in fewer animals than in the O_{11-13} group. An increase in the representation of a 2-week rhythm in animals with tumor (O_{15-16}) compared with the control will be noted.

Parameters of Rhythms. The development of the tumor affects all three parameters of the rhythms to some extent.

The period (T) of rhythms of all dimensionalities discovered under normal conditions showed no seasonal or annual fluctuations but remained unchanged in the animals with tumors

TABLE 2. Parameters of Biorhythms of Change in α-GDPH Activity in Animals with Tumors and Differing in Length of Survival (M ± 2m)

Rhythm, months	Control	Parameters of rhythm		
		T	A	φ
1/2	Control	13,63±0,66	86,36±22,89	203±54
	O_{15-16}	13,07±0,76	125,00±45,47	148±87
	O_{11-13}			
1/3	Control	9,68±0,73	125,12±35,69	349±64
	O_{15-16}	8,73±1,13	138,85±82,81	80±112
	O_{11-13}	9,11±1,59	216,98±180,52	92±126
1/4	Control	6,30±0,51	89,09±25,50	191±70
	O_{15-16}	6,75±0,68	132,71±36,58*	187±97
	O_{11-13}	6,50	136,81	78
1/6	Control	4,27±0,17	85,81±18,05	138±46
	O_{15-16}	4,77±0,43*	89,55±16,51	290±51**
	O_{11-13}	4,05±0,20	81,20±30,57	316±160*
1/8	Control	3,49±0,25	100,16±20,10	111±94
	O_{15-16}	3,49	153,48	291
	O_{11-13}	3,75	192,27	270
1/10	Control	2,56±0,11	86,78±16,28	63±49
	O_{15-16}	2,50±0,19	103,83±32,69	136±47*
	O_{11-13}	2,75±0,36	83,56±43,26	156±97

Legend. Asterisks indicate significance of differences between parameters in experiment and control: *$p < 0.05$, **$p < 0.001$.

(Table 1). However, comparison of two groups of tumor-bearing animals (O_{15-16} and O_{11-13}) revealed significant differences ($p < 0.01$) in the length of T of the 1/6 month rhythm (Table 2).

However, the essential characteristic of tumor development is the phase shift. The phase (φ) is the shift of the cosinusoid, measured in degrees, at the time of beginning of the measurement. To determine the group characteristic from the combined results, values of φ of individual rhythms were extrapolated to April 17, 1983, the beginning of the measurements in the first experiment. When group values of φ of the rhythms of the tumor-bearing and intact animals were compared during the same experiment, significant differences were found for rhythms of 1/3 months (experiment No. 3; $p < 0.01$) and 1/6 months (experiments Nos. 1 and 2; $p < 0.05$; Table 1). Differences for φ of 4 of the 6 rhythms (1/3, 1/6, 1/8, and 1/10 months) were demonstrated from the combined results with a high degree of significance. For the 1/3 months rhythm the shift of φ in the tumor-bearing animals was ~100° to the left, and for rhythms of 1/6, 1/8, and 1/10 months, ~160, 200, and 80° to the right, respectively. No phase differences were found for O_{15-16} and O_{11-13}. Under normal conditions no seasonal shifts of φ were observed, except φ of the weekly rhythm: φ in experiments Nos. 2 and 3 was 276 ± 117 and 274 ± 139, respectively, compared with 116 ± 35 for experiment No. 1 (extrapolation of all phases to April 17).

The development of the tumor led to marked changes in amplitude (A, number of formazan granules in 50 lymphocytes) of the 1/2, 1/3, and 1/4 month rhythms (Fig. 1, Table 1). However, individual fluctuations of A in the group of tumor-bearing animals were so great that the significance of differences between groups of values of this parameter for tumor-bearing and intact animals could not be discovered from one experiment. Significant differences from the pooled results were found only for the weakly rhythm.

Since A of 1/2, 1/3, and 1/4-monthly rhythms in animals with tumors was much higher than A of the other rhythms of the series studied, rhythms of these dimensionalities, taken together and separately, determined the pattern of the α-GPDH dynamics. A seasonal feature can be noted: in spring the dominant member of the hierarchy of rhythms in the tumor-bearing animals was 1/4-monthly, whereas in summer a special role in the hierarchical shifts was played by the 1/3-monthly rhythm (Figs. 1 and 2). It will be clear from Fig. 1 that the individual trend and hierarchy of rhythms in the tumor-bearing animals, constituting the two extreme versions with respect to life span (11 days – III and 16 days – IV), differed significantly. A of the 1/3-monthly rhythm of these animals was 322.41 and 117.11, respec-

tively. On the left side of Fig. 1 for comparison, data are given (I, II) for two intact rats with a similar combination of rhythms in the group and with A of the 1/3-monthly rhythm equal to 173.32 and 84.77 formazan granules respectively in 50 lymphocytes, i.e., during a slow course of the process A of the tumor-bearing animals was close to normal, but during a more rapid course, it was 2-3 times higher than normally. Analysis of the pooled results confirms this conclusion. In the series intact animals → O_{15-16} → O_{11-13}, group values of A for the 1/3-monthly rhythm can be arranged in the order: 125.12 → 138.85 → 216.98 granules in 50 lymphocytes. However, despite the great magnitude of the effect, because of strong individual differences this rule could not be proved statistically and it can be regarded purely as a tendency.

Thus, the development of a tumor leads to lowering of Φ of α-GDPH and to a marked change in the abundance and parameters of the rhythms, demonstrated by the time course of activity of this enzyme. The present investigation showed that, when characterizing the state of an organism, the whole hierarchy of rhythms must be taken into account and not just one particular rhythm or one parameter of a rhythm, for pathology induces unequal changes in different dimensions of biorhythms, each of which is linked by resonance with definite rhythms within the body and in the external environment. However, phase changes which can reflect changes in the level of coordination of phases of components of the glycerophosphate shuttle mechanism [2], involved in the coordination of glycolysis and the citric acid cycle, must be pointed out in particular. In this connection, a priority task in the future will be to study the connection between biorhythms of α-GDPH and biorhythms of other dehydrogenases of the lymphocytic enzyme profile and, in particular, succinate dehydrogenase.

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