Anti-Cancer Drugs, 2, pp. 305–310

Chronobiological analysis of peripheral lymphocyte dehydrogenase activities in rats with Walker 256 carcinosarcoma

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Alpha-glycerophosphate dehydrogenase (α-GPD) and succinate dehydrogenase (SD) activities were investigated in lymphocytes from peripheral blood of normal rats and rats bearing the Walker 256 carcinosarcoma. Computer analysis using an original algorithm revealed a hierarchy of biorhythmic patterns of dehydrogenase activities. In all rats, mean SD activity was higher than mean α-GPD activity. In rats without tumor, SD and α-GPD activities were both higher than in rats with the Walker tumor. Biorhythm spectra for both dehydrogenases were very similar in rats with or without tumor, but tumor implantation resulted in a change of the phase relationship between α-GPD and SD.

Key words: Alpha-glycerophosphate dehydrogenase, biorhythms, succinate dehydrogenase, Walker 256 carcinosarcoma.

Introduction

Dehydrogenase activity in peripheral lymphocytes is considered to be an accurate index of various effects on the body and may be used to predict development of pathological processes. This is based on statistical analysis of enzyme activity distribution in the lymphocyte population and correlation between selected parameters.[1–3] Temporal fluctuations of mean enzyme activities in cell populations make interpretation difficult[2,4,5] and therefore an investigation of the biorhythms is required.[6–8]

The objective of this paper is to evaluate the possibility of using biorhythmic characteristics of lymphocyte metabolism in peripheral blood for the chronobiological examination of tumor-bearing individuals. Alpha-glycerophosphate dehydrogenase (α-GPD) and succinate dehydrogenase (SD), 2 major components of the dehydrogenase technique, were assayed. These dehydrogenases control glycolytic and Krebs cycle fluxes that feed hydrogen atoms at the same point of the respiratory chain (COQ).[9] Therefore, a comparison of biorhythms of dehydrogenase activity in rats with or without the Walker carcinosarcoma was considered to provide new information on the coupling of glycolysis and respiration through coordination of their rhythmic patterns. The mathematical method proposed by Yu. M. Nikitin and E. N. Chirkova[10,11] was employed to single out biorhythms of different frequencies with arbitrary periods from limited temporal rows.

Materials and methods

Chemicals

p-nitrotetrazolium violet (Reanal, Hungary), D,L-α-glycerophosphate, disodium salt (Ferak, Berlin), sodium succinate (Serva).

Animals

Thirty-two outbred male rats weighing 130–150 g were obtained from the Central Breeding Colony, USSR Academy of Medical Sciences. The animals were randomly assigned to three experimental series each containing one study and one control group. Each group included five animals in experiments 1 and 3, and six animals in experiment 2. The animals were housed under standard laboratory conditions and were acclimatized to the facility at least 10 days before the start of the experiments. They were given fresh food after each blood collection. The tumor was inoculated subcutan-

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eously at 10.00 h as described earlier.[8] Intact rats served as control. Blood samples were taken from the caudal vein at 10.00 h, first every other day and then daily. α-GPD and SD activities were determined based on the count of formazane granules in 50 lymphocytes.[3] Enzyme assays were performed throughout the post-transplantation period till the study animals died between days 11 and 16, and in control animals till days 15 and 20 in experiments 1 and 2, 3, respectively.

Mathematical method

The mathematical method devised by Yu. M. Nikitin and E. N. Chirkova[10,11] was used to elucidate parameters of each biorhythm inherent in the α-GPD and SD dynamics (average level G, period length (days) T, amplitude oscillations around the average level A, and baseline phase characteristic ϕ). The procedure for revealing the unknown set of enzyme biorhythms and determining their parameters requires the biorhythms of different frequencies and noises (non-periodic variables), intrinsic in a given dynamic pattern, to be taken into consideration.

Complexes of significant enzyme biorhythms ($p < 0.05$) were investigated in each individual animal. Parameters of biorhythms belonging to a given frequency class and reflecting the course of the process were summed up to characterize all intact and tumor-bearing individuals. The individual biorhythm phase was adjusted to the beginning of measurements in experiment 1. Control and study groups contained a total of 16 animals each.

Results

Figure 1 demonstrates individual changes of lymphocyte α-GPD and SD activities and the spectra of their biorhythms. For comparison, the dotted line shows activity patterns computed from the summed biorhythms.

To evaluate tumor-induced changes in enzyme activities, the following biorhythmic parameters were determined in normal rats and in animals with the Walker 256 carcinosarcoma: mean α-GPD and

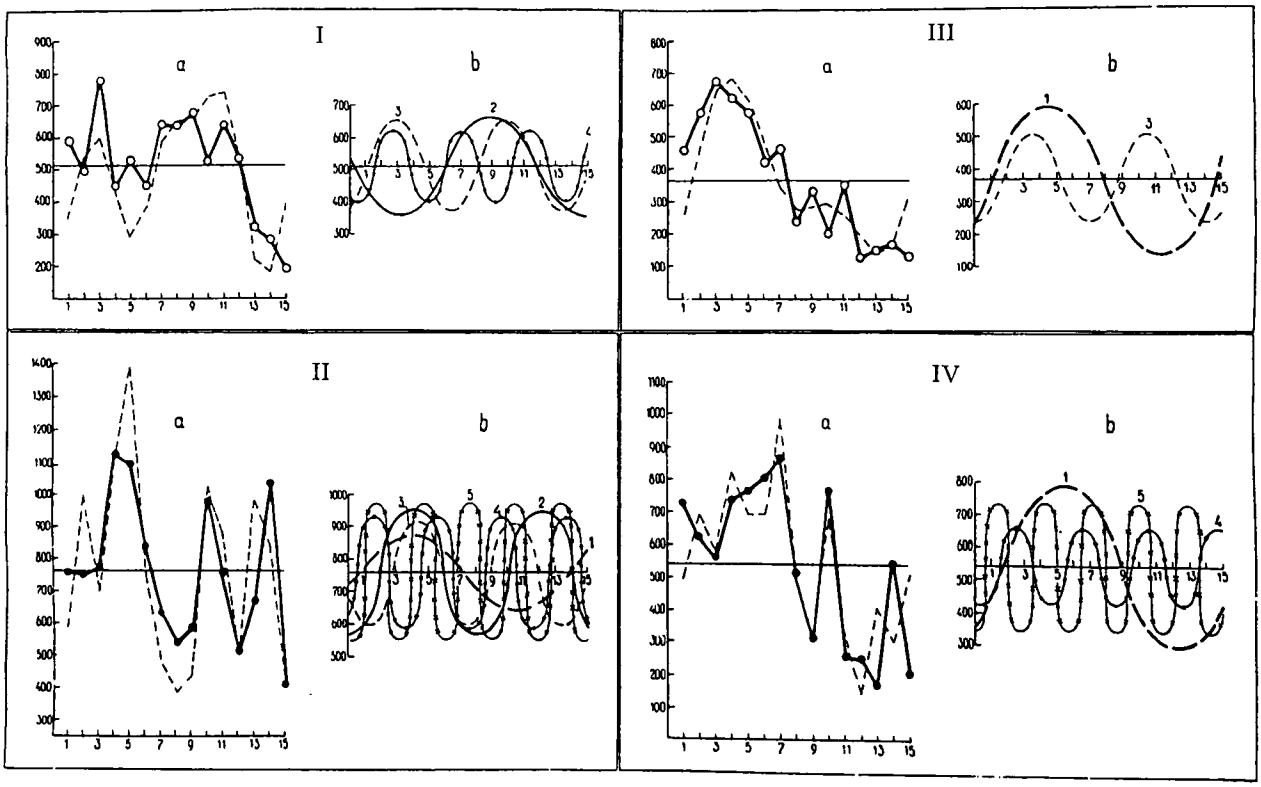

Figure 1. α-GPD and SD activities in peripheral lymphocytes (a) and spectrum of their biorhythms (b) in normal rats (I, II) and rats with Walker 256 carcinosarcoma (III, IV). I, III—α-GPD; II, IV—SD; 1–5: 1/2, 1/3, 1/4, 1/6, and 1/10 month-long biorhythms. Abscissa: time (days); ordinate: absolute activities (number of formazane granules/50 lymphocytes). Dotted line (a): activity changes computed from the summed biorhythms. Straight line parallel to abscissa: mean activity.

SD levels; individual sets of biorhythms and their structure, i.e. diversity and occurrence of biorhythms in a given group of animals; duration, amplitude, and phase of each discrete component of the overall activity rhythm (see Table 1 and Figures 1 and 2).

Measurements of mean enzyme activities in peripheral blood of normal rats and rats with the Walker tumor revealed marked differences between α-GPD and SD level (see Table 1). The activity of SD was invariably higher than that of α-GPD, and both activities were lower in the rats with the Walker carcinosarcoma. However, rhythmic patterns of both activities in the two groups were comparable in that they showed the presence of components with similar duration: 1/2, 1/3, 1/4, 1/6, 1/8 and 1/10 month. The rats differed from another by differing prevalence of these components. The full set of biorhythm types observed in the present experiments was found in a single animal which served as control for the α-GPD assay. There was a marked difference between the α-GPD and SD patterns in one individual (Figure 1). At the same time, different rhythms were more or less equally represented in a given group of rats, i.e. the percentage of animals having a selected type of biorhythm was essentially the same for α-GPD and SD (see Table 1). For all that, the 1/4 month-long rhythm of α-GPD, and the 1/3 and 1/6 month-long rhythms of SD in rats with the Walker carcinosarcoma, were not as common as the rhythms of these durations in normal rats. Occurrence of the 1/8 month-long biorhythm was lower in tumor-bearing rats for both dehydrogenases.

Periodicity of all rhythm types for α-GPD and SD in normal rats was virtually identical and remained unaltered in rats with the Walker carcinosarcoma. Also, biorhythms of similar frequencies in normal animals were alike in terms of amplitude, with the exception of the 1/10 month-long rhythm. In rats with the Walker tumor, the difference in amplitude was found to exist as 1/2, 1/6, and 1/10 month-long biorhythms. Amplitudes of biorhythms in both enzyme activities increased with progressing tumor growth. How-

Table 1. α-GPD and SD activity biorhythms in peripheral lymphocytes of normal rats and rats with Walker 256 carcinosarcoma

Rhythm type, months	Parameters	Normal rats, $n = 16$			Rats with tumor, $n = 16$		
		α-GPD	SD	p	α-GPD	SD	p
	G	614 ± 123	831 ± 99	0.01	544 ± 113	722 ± 97	0.05
1/2	T	13.6 ± 0.7	15.4 ± 1.8		13.1 ± 0.7	13.5 ± 1.6	
	A	86.4 ± 22.0	96.9 ± 27.1		125.0 ± 45.6	211.5 ± 44.8	0.01
	ϕ	203 ± 54	89 ± 52	0.01	148 ± 87	74 ± 51	
	%	50	38		44	38	
1/3	T	9.7 ± 0.7	8.9 ± 0.6		8.9 ± 0.7	9.6 ± 1.5	
	A	125.1 ± 35.7	123.8 ± 32.5		173.6 ± 75.3	145.8 ± 86.6	
	ϕ	349 ± 64	117 ± 53	0.01	86 ± 66	143 ± 66	
	%	56	56		56	38	
1/4	T	6.3 ± 0.5	6.3 ± 0.5		6.7 ± 0.5	6.2 ± 0.7	
	A	89.1 ± 25.5	109.1 ± 40.6		133.9 ± 27.7	145.5 ± 75.4	
	ϕ	191 ± 71	334 ± 87	0.01	201 ± 88	137 ± 113	
	%	63	50		44	38	
1/6	T	4.3 ± 0.2	4.4 ± 0.2		4.4 ± 0.4	4.5 ± 0.4	
	A	85.8 ± 18.3	108.7 ± 23.0		85.8 ± 11.7	137.8 ± 24.3	0.002
	ϕ	138 ± 46	349 ± 60		301 ± 53	96 ± 78	0.002
	%	63	75		56	50	
1/8	T	3.5 ± 0.2	3.6 ± 0.1		3.6 ± 0.5	3.6 ± 0.4	
	A	100.2 ± 20.1	90.7 ± 32.9		76.1 ± 10.2	149.0 ± 76.0	
	ϕ	111 ± 94	355 ± 46	0.05	314 ± 112	88 ± 197	
	%	38	31		19	19	
1/10	T	2.6 ± 0.1	2.7 ± 0.2		2.6 ± 0.2	2.6 ± 0.2	
	A	86.8 ± 16.3	128.7 ± 33.4	0.05	96.0 ± 18.6	136.2 ± 30.7	0.05
	ϕ	63 ± 49	85 ± 44		144 ± 40	307 ± 63	0.001
	%	81	81		81	88	

G—mean dehydrogenase activity (number of formazane granules in 50 lymphocytes); T—period length (days); A—amplitude (number of formazane granules in 50 peripheral lymphocytes); ϕ—phase shift (degrees); %—percentage of animals showing biorhythm.

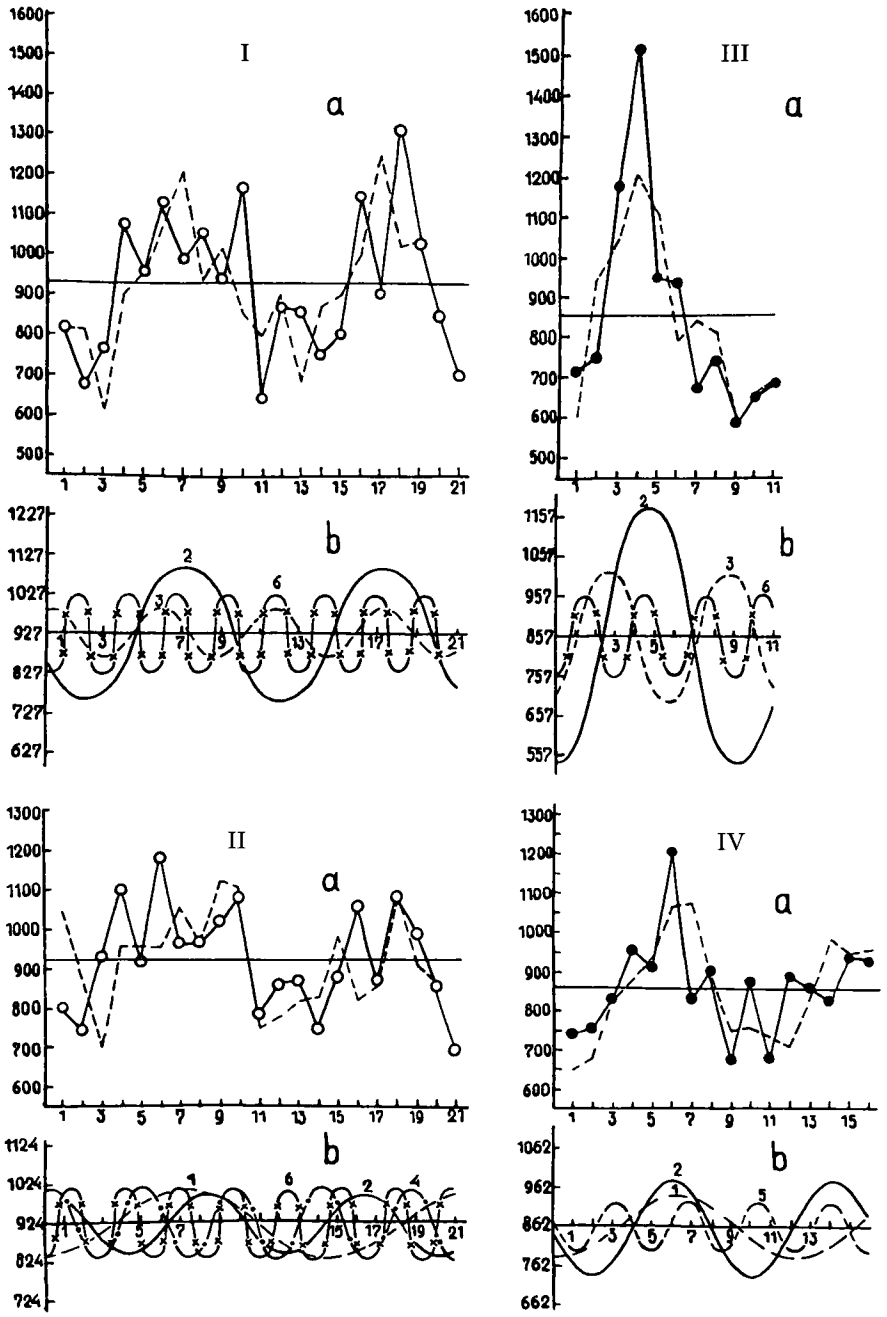

Figure 2. α-GPD activity (a) in peripheral lymphocytes and spectrum of its biorhythms (b) in normal rats (I, II) and rats with Walker 256 carcinosarcoma (III, IV). 1–6: 1/2, 1/3, 1/4, 1/6, 1/8, and 1/10 month-long biorhythms. Other details as in Figure 1.

ever, individual differences were so great that the rise was statistically significant only in the case of one week-long α-GPD rhythm and two week-long SD rhythm, whereas it failed to reach significance in all other cases. For example, the amplitude of the 1/3 month-long rhythm of α-GPD in animals differing in terms of tumor growth rate was subject to changes in the range of 322 to 117 granules/50 lymphocytes (Figure 2). Figure 2 demonstrates individual differences in α-GPD activity and hierarchical organization of its biorhythms in tumor-bearing rats with two extreme longevity estimates, 11 days (III) and 16 days (IV). For comparison, the same parameters are illustrated as

occurring in two normal rats with a similar biorhythmic pattern of α-GPD activity. It can be inferred from the figure that the amplitude of the 1/3 month-long α-GPD biorhythm in rats with retarded tumor growth was close to that in control rats, whereas it was 2–3 times higher in rats with accelerated neoplasm development.

Biorhythms of similar frequencies in both α-GPD and SD activities were significantly different in terms of phase characteristics ($p < 0.01$, see Table 1 and Figures 1 and 2) except for the 1/10 month-long rhythm. The tumor-dependent phase shifts in the biorhythms of either or both enzymes resulted in a change of the phase relationship in all biorhythm variants. One type of change occurred in low-frequency 1/2, 1/3, and 1/4 month-long biorhythms. In rats with the Walker carcinosarcoma, a phase shift in the activity of either dehydrogenase resulted in synphasic rhythms. Another type of change in phase relationship was intrinsic in 1/6 and 1/8 month-long biorhythms. Equal phase shifts in the 1/6 month-long rhythm of both enzyme activities (approximately 150°, $p < 0.002$) resulted in an inverted phase relationship. The phasic changes in the 1/8 rhythms could not be accurately interpreted because occurrence of this type of rhythm, especially in rats with the Walker carcinosarcoma, was very low.

Discussion

Our results indicate that complicated patterns of α-GPD and SD activities can form an integrated process consisting of several enzyme biorhythms interrelated with other biorhythms in the body and the environment through common resonance characteristics. The two dehydrogenases involved in major coupled metabolic pathways are characterized by similar biorhythmic structures, i.e. sets of biorhythms with similar diversity of constituent components (the difference in the occurrence of each component does not exceed 20%). Biorhythmic patterns of α-GPD and SD activities are characterized by the following features: equal duration of all biorhythm types; similar amplitudes in all biorhythms except the 1/10 month-long biorhythm; and the presence of a phase shift in each biorhythm, with the exception of the 1/10 month-long one leading to the inverted phase relationship.

In rats bearing the Walker 256 carcinosarcoma the peripheral lymphocyte metabolism showed reduced activity of α-GPD and SD as compared with rats without tumor. At the same time, the SD/α-GPD ratio remains unaltered (elevated) whereas biorhythmic patterns of both activities undergo marked changes. Specifically, the relative importance of selected biorhythm types in the activity of either dehydrogenase may show significant changes. The period length being unaltered, there may be a rise in the biorhythm amplitude, with its height in some of the SD biorhythm types exceeding that in the corresponding α-GPD biorhythms. A common feature of all biorhythm types was a change in the phase relationship between α-GPD and SD. The phase shift of both or either dehydrogenase in rats bearing the Walker tumor resulted in synphasic patterns of 1/2, 1/3, and 1/4 month-long rhythms, inverted phase relationship in the 1/10 month-long biorhythm, and a change in the sign of the phase shift in 1/6 and 1/8 month-long biorhythms. Results of the present and previous studies [8] indicated that the amplitude of 1/2, 1/3, and 1/4 month-long biorhythms in animals with the Walker 256 carcinosarcoma is higher than that of other rhythm types. These three rhythm types may therefore be responsible, separately or together, for the overall alteration in the patterns of both dehydrogenase activities in tumor-bearing animals. The phase shift in low-frequency biorhythms appears to be of special importance. However, the hierarchical biorhythmic organization as a whole, rather than selected rhythms or their parameters, should be taken into account if the enzyme status of tumor-bearing animals is to be characterized. This is because pathological conditions lead to dissimilar changes in different biorhythm types of dehydrogenase activities and their relationship with other internal rhythms. However, the phase shift and the resultant changes in the relationship between α-GPD and SD activities suggests an alteration in the coupling patterns between glycolysis and the Krebs cycle in peripheral lymphocytes. This may serve as an important biorhythmic index of tumorigenesis since it has been shown[9] that the glycerophosphate shuttle mechanism in tumor cells is virtually ineffective and that the interaction between glycolysis and respiration in tumors appears to be abnormal.

Acknowledgment

We wish to thank Dr G. F. Suslova for helpful advice on enzyme assays.

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(Received 13 November 1990; revised version received 12 March 1991; accepted 19 March 1991)