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RESPONSE OF CIRCADIAN RHYTHMS OF THE LYMPHOID SYSTEM TO DEEP SCREENING FROM THE EARTH'S GEOMAGNETIC FIELD

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UDC 612.42.014.426.064"52"

KEY WORDS: biorhythms; lymphoid system; hypomagnetic state

Circadian rhythms of the lymphoid system form the spatiotemporal organization for the distribution of functional steps in lymphoid tissue during the 24-h cycle. This applies in particular to the power of migration and recirculation flows of lymphoid cells, maintaining the morphological composition of the lymphoid organs: bone marrow, thymus, spleen, lymph nodes. During the daytime, in the period of rest, proliferative processes predominate in the lymphoid tissue of inbred mice and recirculation processes of cells, which left the circulation during the previous period of activity, come to an end. The period of dark motor activity itself is characterized primarily by more intensive migration of young lymphoid cells and the more rapid migrations of mature lymphoid cell forms [3]. Data have also been obtained on the effect of changes in the intensity of the earth's magnetic field on the level of leukocytosis in peripheral blood [1, 6], and also on the effect of deep screening on young rabbits: the number of large, undifferentiated lymphoid cells in the structure of their spleens was increased [5].

We have studied the state of the set of circadian biorhythms of the lymphoid system of laboratory mice, kept under conditions of deep screening.

EXPERIMENTAL METHOD

Experiments were carried out from May 11 through May 26, 1988, in a reinforced concrete bunker, at a depth of 3-4 m; a cylindrical screening chamber was made in it, from four layers of permalloy steel, reducing by 10^4 times the intensity of the earth's magnetic field. The experimental series of seven inbred male C57BL/6 mice aged 13-14 weeks and weighing 23-26 g was kept under conditions of constant dim lighting and with free access to water and food. A similar control series of animals was kept in the same bunker in the immediate vicinity of the hypomagnetic chamber. On the 1st and 7th days (throughout the 24-h period

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Department of Lymphology, Institute of Physiology, Siberian Branch, Academy of Medical Sciences of the USSR, Novosibirsk. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 2, pp. 191-193, February, 1990. Original article submitted February 16, 1989.

0007-4888/90/0002-0239$12.50

TABLE 1. Differences in Mean Daily Levels of Peripheral Blood Leukocytosis on 1st, 7th, and 14th Days of Hypomagnetic State (by Wilcoxon–Mann–Whitney test)

Days	Control (C)	Hypomagnetic state (H)	p_{C-H}
1st	10,29±1,23 $n=19$	10,87±1,82 $n=1$	>0,05
7th	12,69±1,65 $n=16$	14,72±1,24 $n=16$	>0,05
14th	15,50±3,01 $n=7$	20,86±1,64 $n=7$	>0,05
p_{1-7}	>0,05	>0,05	
p_{1-14}	>0,05	<0,01	
p_{7-14}	>0,05	<0,01	

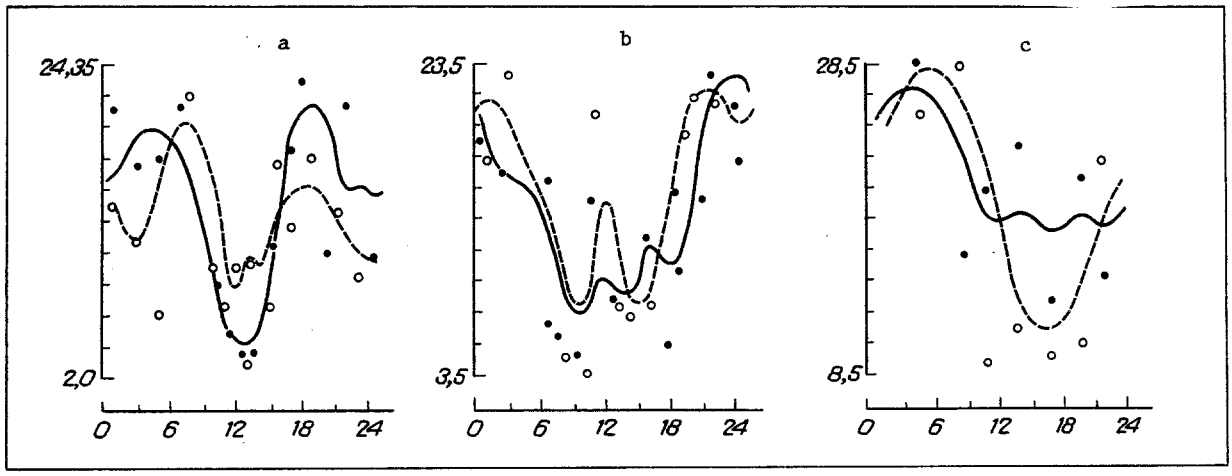

Fig. 1. Dynamics of circadian rhythms of blood leukocytosis ($\times 10^9$/liter – ordinate) on 1st (a), 7th (b), and 14th (c) days of screening. Hypomagnetic: filled circles – initial data, continuous line – twice-smoothed dynamics [4]. Control: empty circles – initial data, broken line – dynamics. Abscissa, clock time.

in each case) blood was taken from the amputated tip of the tail at intervals of 1-1.5 h, with one sample at each point: the absolute leukocytosis was determined by means of a counting chamber. The animals were killed on the 14th day, namely May 25-26, by dislocation of the cervical spine, with one animal at each point, at intervals of 3-4 h. The absolute leukocytosis was counted and the blood formula determined on films stained with azure II-eosin. The concentration of lymphoid cells was determined in a counting chamber in suspensions from bone marrow, thymus, spleen, and inguinal lymph node, after which the absolute number of cells in the organ was calculated. The numerical data were analyzed by RDFA and SMD programs, realized on the "Élektronika-60 M" computer. The RDFA program included periodic regression analysis, Fourier harmonic analysis, construction of smoothed plexograms, and nonparametric methods of estimation of extrema of a dynamic series [4]. The SMD program envisaged a mathematical diagnostic model of statistical type, based on the use of a rank correlation apparatus and the method of cubic splines of dynamic series. By means of the model it was possible to assess migration of cell masses between the lymphoid organs on the basis of data on the absolute number of cells in them during time cuts of a period, revealed by spline analysis.

EXPERIMENTAL RESULTS

Peripheral blood leukocytosis is a sufficiently sensitive marker of the state of the magnetic field surrounding an organism: a hypomagnetic state leads to a certain increase in the number of circulating cells in the blood on the 7th and 14th days of investigation (Table 1). The circadian rhythm of blood leukocytosis on the 1st day of the hypomagnetic state did not differ from

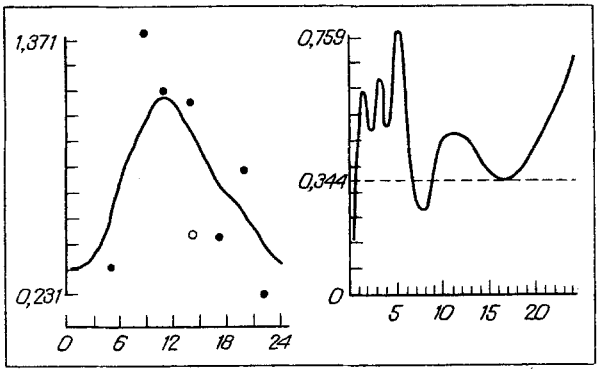

Fig. 2. Circadian rhythm of acidophilic granulocytes ($\times 10^9$/liter — ordinate) on 14th day of screening. Filled circles — initial data, curve — dynamics [4]; empty circles — acidophilic granulocytes in blood of one control animal. Abscissa, clock time.

the control, and only during the first 2 h was a significant fall of the leukocytosis observed (2.42 ± 0.35 compared with 6.58 ± 0.71 · 10^9/liter, $p < 0.01$) in the experimental group; this may be attributed to an orienting reaction of the animals when placed in the metal screen, as we proved later in an experiment with a cardboard chamber, identical in shape with the permalloy screen. On the 7th day the amplitude–phase characteristics also were similar. On the 14th day of the hypomagnetic state the curve of the blood leukocytosis did not differ in principle from the control (Fig. 1). In the course of 14 days the acrophases of the 24-h components moved from noon to the evening, and back again to the morning. By the end of the experiment, acidophilic granulocytes were found in the blood of all animals kept under hypomagnetic conditions, whereas in the control group they were found in only one animal. The rhythm of the number of these cells followed a course similar to that of the leukocytosis (Fig. 2). The strength of the controlled factor (hypomagnetic state) or an increase in the number of acidophilic granulocytes in the blood in a single-factor dispersion complex was A-0703 ($p < 0.05$).

The following cell migrations were diagnosed in the control animals: from bone marrow into thymus — from 9.15 a.m. to 2.10 p.m., from thymus into peripheral blood and lymph nodes from 5 p.m. to 4.25 a.m. Within the interval from 8.15 p.m. to 4.10 a.m. there was a massive outflow of cells from the lymph nodes into the blood, and also passage of thymocytes and cells from lymph nodes into other lymphoid organs. These results were determined by the dynamics of the number of cells in the lymphoid organs (Fig. 3, column on left, broken line).

Twenty-four-hourly peaks came closer to the minima at about midnight, a possible indication of acceleration of migration-recirculation movements of the lymphoid cells during this period (Fig. 3). Rhythms of the number of cells in the spleen and inguinal lymph nodes changed their shape: the impression of inversion of the dynamics was created with the formation of a new 24-hourly maximum in the midnight region. The rhythm of absolute lymphocytosis was reorganized but preserved its basic scheme, evidently because animals in the hypomagnetic chamber and the controls had synchronous programs of motor activity. Analysis of the amplitude maps (Fig. 3, right hand column) revealed reorganization (bone marrow, blood lymphocytosis) or destruction (spleen, lymph nodes) of the spectra of oscillatory processes of the parameters studied. A conclusion of preservation of the spectrum could be drawn only for the cell count of the thymus. In all cases the amplitude of the harmonic component with a period of about 15 h was preserved and intensified. This may perhaps indicate strengthening of synchronization in the processes of migration and proliferation of the lymphoid cells, more especially because the period of the recirculation cycle and the duration of cell division of the lymphoid cell were close to the period of the preserved harmonic. All changes in amplitude–phase schemes were evidently based on reorganization of the migration and recirculation flows of the lymphocytes; differences in sensitivity of the lymphoid organs to the hypomagnetic state can be regarded as one cause of this desynchronization, for the intrinsic temporal order was changed: from 9.35 a.m. to 5 p.m. accelerated recirculation of cells of the peripheral lymphoid pool into the bone marrow was diagnosed, with simultaneous rapid recirculation of cell masses of the lymphoid organs and thymus through the peripheral blood. From 10 p.m. to 11 a.m. a high degree of synchronization of the dynamics of the cell counts of the bone marrow and thymus was observed, evidently due both to synchronization of mitosis and to the intensive recirculation of cells of the peripheral lymphoid pool and of thymocytes which continued during this time.

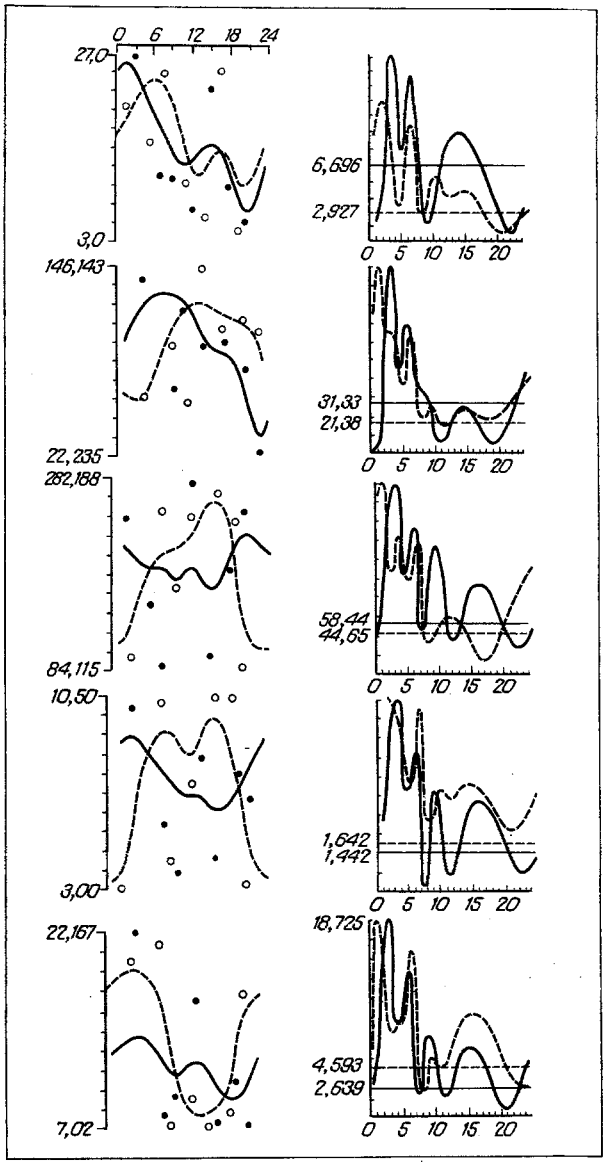

Fig. 3. Circadian rhythms of lymphoid system and their amplitude maps on 14th day of screening. From top to bottom: number of cells in bone marrow, thymus, spleen, inguinal lymph node (in all cases $\times 10^6$) and lymphocytes in blood ($\times 10^9$/liter). column on left represents twice smoothed dynamics [4]: control — broken line, hypomagnetic state — continuous line; initial data — filled (hypomagnetic) and empty (control) circles; abscissa, clock time. First column represents amplitude maps: ordinate, amplitudes of plexograms for sample periods, magnitude of which is indicated on abscissa; hypomagnetic state: continuous horizontal line shows level of confidence interval of mean daily level, continuous curve — value of amplitudes; control: broken line — confidence interval of mean daily level, broken curve — value of amplitudes.

A stay of 2 weeks in the hypomagnetic state did not lead to any threatening deviations in the parameters of the lymphoid system, as shown by preservation of the mean daily levels of all the biorhythms studied. The probable reason for this was the lower informational value of the intensity of the earth's magnetic field for the lymphoid system of adult animals, compared with the state of the "channel of communication" between lymphoid cells, the occurrence of which is postulated at the level of the visible light and ultraviolet bands of the spectrum of electromagnetic oscillations [2].

It can be concluded on the basis of these facts that adaptation of the lymphoid system to the hypomagnetic state is manifested as desynchronization of circadian rhythms on the basis of differences in sensitivity of the organs; reorganization of the rhythms is realized through strengthening of the ultradian components with periods of about 15 h; the data indicate indirectly the acceleration or increased power of recirculation of the lymphoid cells and also of acidophilic granulocytes.

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ROLE OF INTERCELLULAR CONTACT INTERACTIONS AND THE RESPONSE OF MACROPHAGES TO ACTIVATORS CONTAINING AND NOT CONTAINING THE Arg–Gly–Asp SEQUENCE

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UDC 612.112.95.014.46:[615.31:547.96]

KEY WORDS: macrophages; superoxide; intercellular contact interactions.

The superoxide anion occupies a special place in the performance of the effector functions of phagocytes. Meanwhile the mechanisms controlling its production by activated cells under normal and pathological conditions have been inadequately studied. A definite role here may be played by signals transmitted by direct intercellular contact. It has been shown that this is one way by which functional interaction can take place between lymphocytes [6], T-lymphocytes and macrophages [15], and T-lymphocytes and granulocytes [10], but only indirect information was available on the role of direct contacts between phagocytes and the regulation of their activity [5, 11].

The initial aim of this investigation was accordingly to determine whether the level of production of the superoxide radical depends on contacts between macrophages. Since a key role in various kinds of adhesive processes is known to be played by a special group of cell receptors, recognizing the amino-acid sequence Arg–Gly–Asp found in the composition of their characteristic ligands [2, 9, 14], in order to obtain preliminary information on a possible role of receptors of this type in reception of a contact activating signal, we therefore compared the role of intercellular interactions during the response of macrophages to stimuli con-

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(Presented by Academician of the Academy of Medical Sciences of the USSR. R. V. Petrov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 2, 194-196, February, 1990. Original article submitted May 18, 1989.