ROLE OF THE LIVER IN REGULATION OF THE CIRCADIAN RHYTHM OF THE BLOOD IRON CONCENTRATION IN RATS

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The serum iron (SI) concentration in healthy individuals is subject to regular rhythmic fluctuations during the 24-h period, with a maximum in the afternoon and a minimum at night [2, 14]. The blood iron level has been shown to be an integral parameter, dependent not only on the intensity of absorption of exogenous iron, but also on the rate of deposition, redeposition, and utilization and excretion of this trace element [6, 12]. Data on the circadian rhythm of the SI concentration in rats and its dependence on the intensity of the storage function of the liver during the 24-h period cannot be found in the literature.

The aim of this investigation was to study the temporal organization of iron metabolism in rats on the basis of an analysis of the circadian rhythm of the SI concentration and concentration of the trace element in the liver.

EXPERIMENTAL METHOD

Altogether two series of experiments were carried out on 500 male Wistar rats weighing 150-180 g, from December 15 through 20, 1989 and from December 16 through 21, 1990. The animals were kept under natural lighting conditions (ratio of daylight:darkness = 7:17) and at an ambient temperature of 18-20°C, with free access to food and water. All the tests were carried out continuously for 3 days at intervals of 3 h. The animals were used only once and were then decapitated by means of a guillotine. The SI concentration was determined by the diphenylphenanthroline method, using an SF-26 spectrophotometer (USSR), with laboratory glassware and set of reagents from "Lachema" (Czechoslovakia). The iron concentration in the liver was determined by emission spectral analysis on an STE-1 spectrograph (USSR) with DG-2 arc generator, by igniting the ash of the substrates in the crater of a carbon electrode [5]. Ingestive activity of the reticuloendothelial system (RES) of the liver was estimated as incorporation of colloidal gold isotope (^{198}Au; batch No. 01832002289, registration certificate No. 74.764.4). Radiometry was carried out on an MB 8200 scanner (Hungary) with NK calculator (spectrometer). The intensity of lipid peroxidation (LPO) in the liver and peripheral blood erythrocytes was estimated in the reaction with thiobarbituric acid (TBA), by determining the concentration of TBA-positive material (TBA_{pm}) [8]. The serum alanine aminotransferase (AlAT) activity was determined by the standard dinitrophenylhydrazine method of Reitman and Frankel [13]. The results were subjected to statistical analysis by the Fisher–Student method and by the Cosinor special biorhythmologic program [1].

EXPERIMENTAL RESULTS

Analysis of the circadian rhythm and the rhythmometric parameters shows that the SI concentration in rats has a well-defined circadian dependence (Table 1, Fig. 1). The position of the maximum as a rule corresponds to the evening or beginning of night. The minimum occurs in the morning – at the beginning of the period of daylight. The high amplitude

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TABLE 1. Parameters of Iron Metabolism in Intact Animals (M ± m)

Parameter	Period	Mesor	Amplitude	Acrophase (95% CI)
SI concentration, μmoles/liter	23,5±0,3	24,7±0,6	5,85±0,91	19.12 (17.22—21.08)
Iron concentration in liver, mg/kg	23,8±0,2	9,0±0,1	2,26±0,27	00.24 (23.52—00.57)
TBA_{pm} concentration in erythrocytes, nmoles/ml	23,4±0,6	71,2±6,3	70,53±1,38	20.08 (19.05—21.11)
TBA_{pm} concentration in liver, mmoles/ml	23,2±0,4	28,4±1,7	6,86±0,81	20.22 (18.50—21.54)
AlAT activity, mmoles/liter	23,9±0,1	4,1±0,1	1,07±0,23	22.10 (19.56—23.46)
Incorporation of ^{198}Au isotope by the liver, counts/sec/g	23,2±0,8	314,8±1,9	160,32±18,74	05.58 (05.46—16.10)

Legend. CI) Confidence interval.

of the fluctuations and its relatively limited individual variability are noteworthy: in intact animals the value was not less than 20%, and not more than 27% of the mesor.

The liver, which concentrates more than one-third of the iron reserves [11], probably functions as an oscillator of the circadian rhythm of the blood iron concentration. This view was confirmed by comparing the iron concentrations in the blood and liver (Table 1, Fig. 1). The opposite trends of their levels during the 24-h period and the reciprocal character of their phase relations lead to the conclusion that an essential role in elevation of the blood iron level is played by release of the trace element from the liver, and, correspondingly, its fall is connected with the deposition of iron in the liver.

The importance and complexity of the mechanisms of interaction and participation in "Kupffer cell — hepatocyte" cooperation in iron metabolism are not disputed [4]. The initial role of the RES in the storage of iron and its release into the blood is likewise fairly established [9, 15]. However, the question of the level at which the pacemaker activity responsible for correlation between the temporal organization of iron metabolism and the external medium, has not yet been settled, although the view of the leading role of the neuroendocrine apparatus is not in doubt [10].

If it is considered that the acrophase of the blood iron level in man corresponds to the active phase of the 24-h period (morning and midday) and that it coincides with maximal excretion of products of steroid hormonal and catecholamine metabolism with the urine, it is logical to suggest pacemaker activity of the sympathicoadrenal system [10, 14].

The probability of such a mechanism in the circadian organization of the blood iron level in the rats can be supported by the familiar facts concerning catecholamine activation of LPO in the liver cells [3], which is confirmed by synchronization of acrophases of the SI level and that of one of the LPO products,namely TBA_{pm}. If the circadian rhythm of TBA in erythrocyte membranes and in the liver is compared, the synchronized repetition of its waves can be conclusively demonstrated. Labilization of the membrane structures and the increase in their permeability are evidence of maximal activity of the marker enzyme of hepatocytes (AlAT) in the blood serum. A regular increase in activity of enzymes of the Krebs' cycle in the rats' liver also has been recorded in the period of darkness [7]. If the universal character of LPO activation is extrapolated relative to RES also, and the circadian rhythm of TBA_{pm} and SI compared, it will be found that activation of LPO facilitates iron release from the RES depots. If this view is correct, activity of the deposition processes must also be a component of this circadian complex for the regulation of the blood iron level.

When the ingestive activity of the RES of the liver is estimated on the basis of the intensity of incorporation of the gold isotope ^{198}Au (Table 1, Fig. 1), a statistically significant monophasic circadian rhythm is observed, with a maximum at the end of the period of darkness and a minimum at its beginning.

We have thus discovered for the first time characteristics of the temporal organization of iron metabolism in intact rats and mechanisms influencing the circadian rhythm of this trace element. Intensification of free-radical oxidation of lipids, connected with activation of the sympathicoadrenal system, may perhaps promote weakening of phagocytic activity of the stellate reticuloendotheliocytes and an increase in the permeability of their membranes for iron ions, leading in turn to an increase in the concentration of this trace element in the blood serum due to its redeposition from the liver.

Conversely, when LPO activity declines, the intensity of uptake of iron by the Kupffer cells and deposition of the trace element in the liver is increased, thereby promoting a fall of its serum concentration.

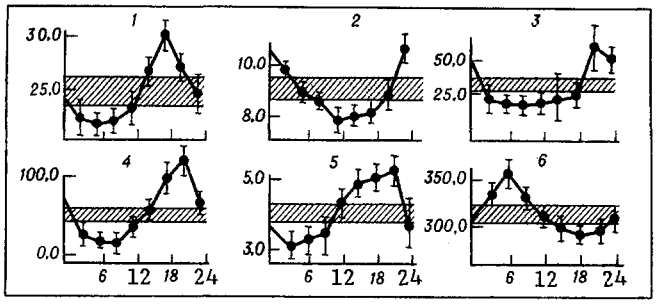

Fig. 1. Circadian rhythm of parameters of iron metabolism in intact rats. Abscissa, clock time (in h); ordinate: 1) SI concentration (in μmoles/liter), 2) iron concentration in liver (in mg/g), 3) TBA concentration in liver (in mmoles/ml), 4) TBA concentration in peripheral blood erythrocytes (in mmoles/ml), 5) AlAT activity (in mmoles/liter), 6) incorporation of isotope ^{198}Au by the liver (counts/sec/g).

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