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Diurnal Time Course of Hexenal Narcosis in Experimental Hepatosis

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 119, № 5, pp. 540-543, May, 1995
Original article submitted June 7, 1994

A statistically significant circadian rhythm of hypnogenic effect of hexenal is revealed in intact Wistar rats, with the maximum recorded in the daytime and the minimum at night, and an amplitude of at least 30% of the mesor. Circadian rhythms of the analgetic action of hexenal and of α-tocopherol concentrations in the blood serum are found to be in reciprocal relationship. Experimental hepatosis induced by intragastric administration of CCl_4 is attended by alteration of the time organization of the antitoxic function of the liver and of the concentrations of iron, α-tocopherol, and lipid peroxidation products in the blood collected from the hepatic and portal veins.

Key Words: *biorhythms; lipid peroxidation; antioxidants; liver; hexenal narcosis*

The rate of hexenal degradation is a criterion of the antitoxic function of the liver [4,5]. Activation of lipid peroxidation (LPO) processes during toxic hepatosis is known to depress the hepatocyte monoxygenase system [4,10]. Data on the space-time organization of metabolism in the liver [6], the restructuring of biorhythms of sideremia and LPO activity at the early stages of toxic hepatosis [1] attest to the necessity of studying the diurnal time course of the antitoxic function of the liver, particularly its relationship with the level of sideremia and activity of LPO and the antioxidant system (AOS) in toxic damage to the organ.

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This research was aimed at elucidating the effect of CCl_4 intoxication on the rhythmic organization of xenobiotic detoxication and its relationship with the status of the LPO-AOS system and level of sideremia on the basis of an analysis of the diurnal time course of the narcotic effect of hexenal, as well as of concentrations of free iron, LPO products, and α-tocopherol in blood taken from the hepatic and portal veins.

MATERIALS AND METHODS

Experiments were carried out with male white rats weighing 120 to 150 g. Control and experimental animals were kept under the same conditions of water, food, and light regimens. Toxic hepatosis was reproduced by intragastric administration (through a

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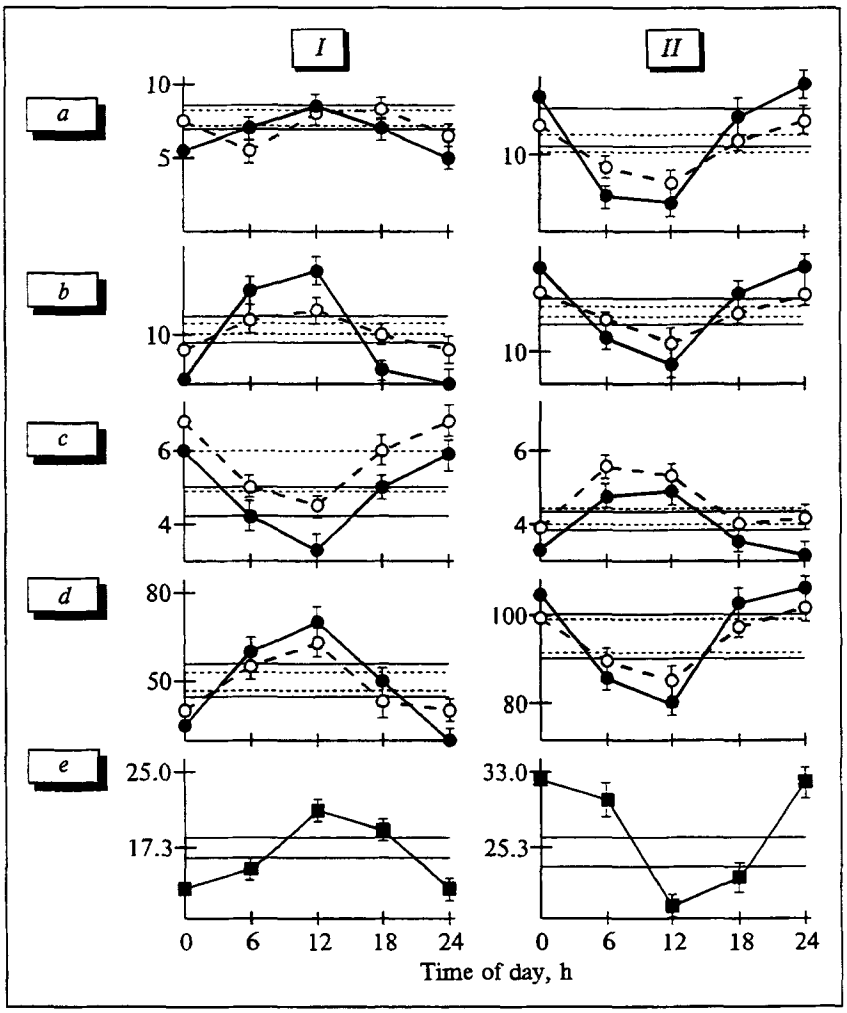

Fig. 1. Circadian time course of concentrations of free iron, erythrocyte MDA, and plasma DC and α-tocopherol in the portal (dashed line) and hepatic (continuous line) veins, and of hexenal narcosis duration in intact rats (*I*) and rats with toxic hepatosis (*II*). Ordinate: *a*) plasma concentration of free iron, μmol/liter; *b*) plasma concentration of DC, nmol/ml; *c*) plasma concentration of α-toco-pherol, μmol/liter; *d*) concentration of MDA in erythrocyte membranes, nmol/ml; *e*) duration of hexenal narcosis, min.

tube) of 10% tetrachloromethane oily solution in a dose of 1 ml per 100 g b.w. once a day for 7 days.

Laparotomy was carried out under intrapleural hexenal narcosis (30 mg/kg b.w.) and blood was collected from the portal and hepatic veins for biochemical analysis 4 times a day at 06:00, 12:00, 18:00, and 24:00 h. The plasma concentration of free iron was measured by the diphenyl phenanthroline method [9]. LPO activity was assessed by the content of diene conjugates (DC) in the plasma [3] and malonic dialdehyde (MDA) in erythrocyte membranes [8]. In parallel with this, the plasma α-tocopherol concentration was measured [12]. The antitoxic function of the liver was evaluated by the duration of hexenal narcosis [4]. The data were analyzed using Student's test and rhythmometric parameters calculated using Cosinor software.

RESULTS

Analysis of the results showed statistically significant circadian rhythms for all the parameters studied (Fig. 1, Table 1). The diurnal time course of the narcotic effect of hexenal in intact rats was synchronous with the indexes of the concentrations of free iron, plasma DC, and erythrocyte MDA in the blood collected from the portal and hepatic veins, but was in counterphase to the plasma level of α-tocopherol (Fig. 1).

Bearing in mind that the highest narcotic effect of hexenal coincides with the lowest level of α-tocopherol and the maximal concentrations of DC in the plasma and of MDA in erythrocytes in the portal vein, and the fact that the diurnal rhythms of MDA concentrations in erythrocyte membranes and hepatic tissue are synchronous [1], it becomes obvious that the circadian rhythmicity of the hypnogenic effect of hexenal depends not only on LPO activity, but on the reserve potential of the antioxidant defense as well.

In animals subjected to CCl_4 poisoning the circadian pattern of sideremia, LPO activity in the plasma and erythrocyte membranes in the blood collected from the hepatic and portal veins was inverted (Fig. 1), with the direct correlation be-

Table 1. Rhythmometric Parameters of Iron Metabolism, LPO and AOS, and of the Duration of Hexenal Narcotic Effect in Intact Rats and Animals with Toxic Hepatosis

Concentration	Plasma from portal vein			
	period	mesor	amplitude	acrophase
	Intact animals			
Free iron in plasma, μmol/liter	23.80±2.1	7.25±0.29	1.65±0.21	16.19 (13.07; 19.31)
Plasma DC, nmol/ml	24.0±1.4	10.36±0.41	2.41±0.19	10.48 (9.00; 12.36)
Erythrocyte MDA, nmol/ml	23.9±0.4	49.5±1.92	12.32±0.69	11.22 (8.38; 13.06)
Plasma α-tocopherol, μmol/liter	23.8±0.4	6.09±0.24	1.46±0.24	22.59 (20.27; 1.32)
Duration of hexenal narcosis, min	23.7±0.5	15.2±0.6	5.86±0.07	11.31 (9.38; 13.31)
	Animals with toxic hepatosis			
Free iron in plasma, μmol/liter	24.0±1.2	9.3±0.18*	2.26±0.36	21.58 (18.06; 21.50)
Plasma DC, nmol/ml	23.9±0.9	13.72±0.38*	8.21±2.24*	2.42* (1.06; 4.18)
Erythrocyte MDA, nmol/ml	23.8±0.9	93.48±4.38*	19.35±1.5*	22.06 (20.38; 23.34)
Plasma α-tocopherol, μmol/liter	24.0±0.8	3.64±0.16*	1.08±0.15	8.01* (6.35; 10.27)
Duration of hexenal narcosis, min	23.7±0.5	24.94±0.27*	9.64±0.23*	2.42 (0.35; 4.19)

Concentration	Plasma from portal vein			
	period	mesor	amplitude	acrophase
	Intact animals			
Free iron in plasma, μmol/liter	23.8±0.7	6.9±0.52	1.65±0.26	12.19 (10.09; 14.30)
Plasma DC, nmol/ml	24.1±0.8	10.36±0.23	5.67±0.41	9.46 (7.22; 12.10)
Erythrocyte MDA, nmol/ml	24.0±0.3	52.42±2.18	14.48±1.24	10.48 (8.36; 11.52)
Plasma α-tocopherol, μmol/liter	24.2±0.45	5.36±0.19	1.78±0.14	22.20 (21.15; 23.35)
	Animals with toxic hepatosis			
Free iron in plasma, μmol/liter	24.1±0.7	9.42±0.26*	7.71±0.44*	22.09* (19.27; 23.49)
Plasma DC, nmol/ml	24.0±1.2	14.15±0.59*	5.06±0.28	22.01* (20.19; 23.41)
Erythrocyte MDA, nmol/ml	24.1±0.6	95.5±5.12*	21.3±2.4*	22.36* (20.36; 0.36)
Plasma α-tocopherol, μmol/liter	23.7±1.1	3.29±0.16*	1.06±0.23	10.00* (7.17; 12.44)

Note. Asterisk shows values reliably differing from those in intact animals ($p<0.05$).

tween the levels of free iron and DC in the plasma from the portal vein remaining unchanged ($r=0.79$; $p<0.05$). It is noteworthy that the mesor of concentrations of LPO products and level of sideremia in the hepatic vein increased ($p<0.05$), while that of α-tocopherol decreased ($p<0.05$).

Similarly to the inversion of the circadian rhythms of activity of LPO products, the concentration of α-tocopherol, and level of sideremia, the rhythm of hexenal narcosis was restructured, with a reliable increase of the mesor in comparison with the control. The highest hypnogenic effect recorded in the night hours coincided with an appreciable drop of the portocaval difference as regards the concentration of α-tocopherol and its maximal rise in comparison with the LPO products in the plasma and erythrocyte membranes and the level of free iron (Table 1).

The initiation of LPO in toxic damage to the liver inflicted by chlorinated carbohydrates is known to be one of the initial mechanisms of impairment of hepatocyte membrane permeability and of pathological changes in these membranes [2]. It is quite possible that these processes potentiate the release of free iron ions from hepatocytes, which, by catalyzing the initial stages of membrane peroxidation [11], promote the accumulation of secondary, highly toxic LPO products, whose excess depresses the processes of detoxication in hepatocytes in the event of inadequate antioxidant defense [7], specifically the activity of the hepatocyte monoxygenase system [5].

Hence, the restructuring of the circadian rhythmicity of the hypnogenic effect of hexenal in rats with experimental toxic hepatosis is the result of impaired antitoxic function of the liver and is

paralleled by intrasystemic desynchronization of the regulation of LPO, AOS, and the level of sideremia in the liver in the course of 24 hours.

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