EXPERIMENTAL BIOLOGY

Chronobiological Parameters of Pain Sensitivity of Rats and Mice

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Circadian and 12-hour rhythms of pain sensitivity to stimuli of different origin were detected in male rats and mice by cosinor analysis in chronobiological experiments. The minimal pain sensitivity to thermal and electric stimulation was observed in rats during the first half of the light phase of 24 h, while in the case of mechanical stimulation it was observed during the dark phase. Biorhythms of sensitivity of mice and rats to thermal pain exposure were similar. Hence, the chronobiological organization of pain sensitivity depends mainly on the type of nociceptive stimulation.

Key Words: *pain sensitivity; nociceptive stimulation; biological rhythms*

Previous experiments [3-5] demonstrated that pain sensitivity varies at different times of the day, but the short duration of these experiments did not permit us to arrive at a conclusion on the rhythmic pattern of the detected daily fluctuations. We detected reliable circadian and ultradian rhythms of pain sensitivity in mice, the parameters of these rhythms depending on the zoosocial status of animals [2]. A group rhythm with a period approximating 24 h and the minimal sensitivity in the middle of the second half of the light phase of 24 h was the most significant and synchronized in aggressive isolated dominants in the circadian interval. In order to find out whether the detected regularities are common to various types of animals and different types of pain exposure and to what extent they are common, we compared biological rhythms of pain sensitivity of mice and rats in the ultradian and circadian intervals during exposure to nociceptive stimuli of different types.

MATERIALS AND METHODS

Experiments were carried out with 56 outbred adult male rats and 12 male mice. Three to four weeks before the experiment, all the animals were kept under standard conditions of artificial illumination with 12-h light and dark periods (the light being switched on at 09:00 h), at a constant air temperature and free access to water and granulated food mix. Pain sensitivity of rats to thermal stimulation was assessed by the latency of jerking back the tail in the tail-flick test, sensitivity to electrical pain exposure by the vocalization threshold during transcutaneous stimulation of the root of the tail, and sensitivity to mechanical compression of the root of the tail, with assessment of the reaction in scores. In mice pain sensitivity to thermal stimulation was assessed by the tail-flick test. Each animal was tested 9 to 12 times in the course of 52-54 hours with 2.5-9-h intervals between the tests. The data were processed by spectral cosinor analysis [1]. The rhythms of changes in pain sensitivity were assessed for the group as a whole and for each animal individually.

RESULTS

Thermal stimulation of rats was associated with an appreciable reduction in the duration of the latency of tail flick in the first half of the dark hours and

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with its increase at the end of the dark period and beginning of the light (Fig. 1). Analysis of the time series of the whole group revealed ultradian and circadian rhythms of pain sensitivity (Table 1). The group circadian rhythm was characterized by a short duration of the period and an acrophase (minimal sensitivity) in the first half of the light hours. Since we did not detect circadian rhythms in all animals, we analyzed the mean values of individual circadian rhythms in order to rule out leveling effect of the parameters in the animals whose rhythms lacked the 24-h periodicity. The mean duration of the period of individual circadian rhythms was much longer and approximated the duration of the 24-h illumination cycle, the acrophase differing negligibly from the group value (Table 2). Rhythms with 12.51-h periodicity in the ultradian range were revealed in the majority of animals. One of the acrophases of the ultradian rhythm occurred during the first half of the light phase.

In the group of mice, pain sensitivity assessed in the tail-flick test did not vary to such an extent over 24 h (Fig. 1). Cosinor analysis revealed a group circadian rhythm of low potency and probability, whose acrophase differed markedly from the acrophase of the rat circadian rhythm and was observed during the second half of the dark phase (Table 1). However, analysis of more significant individual circadian rhythms detected in 67% of mice showed their parameters to differ little from the individual circadian rhythms of rats (Table 2). Semicircadian rhythms with morning and evening

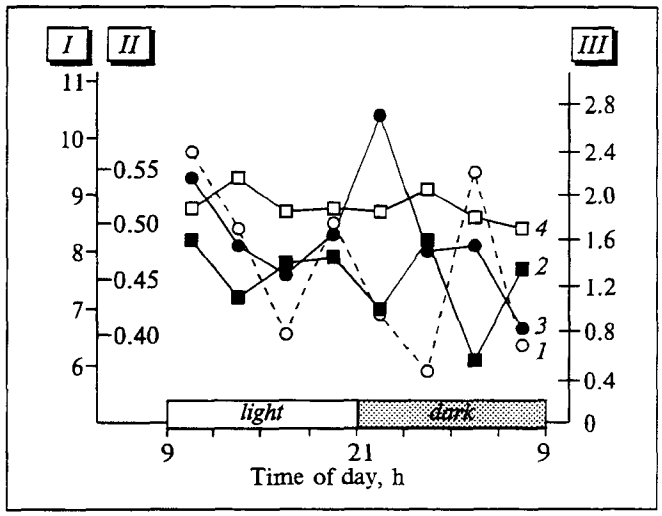

Fig. 1. Changes in sensitivity to nociceptive stimuli of different types in the course of 24 h. Ordinate: *I*) latency of jerking back the tail in the tail-flick test (*1*: rats; *2*: mice); *II*) vocalization threshold in mA during electrical stimulation of rats (*3*); *III*) reaction to compression of the root of the tail in rats, in scores (*4*).

acrophases were observed in the ultradian interval of virtually all mice.

Two rises of the vocalization threshold over 24 h were observed in electric stimulation of the root of the tail of rats: in the first half of the light and dark phases (Fig. 1). Cosinor analysis disclosed a group circadian rhythm with a very short interval and the minimal sensitivity in the middle of the light phase (Table 1). The duration of the period of individual circadian rhythms coincided with the length of the diurnal illumination

Table 1. Parameters of Group Biological Rhythms of Pain Sensitivity

Animals	Parameters of rhythms				
	period, h	amplitude	acrophase, rad	power	probability
			Thermal stimulation		
Rats	8.7620	2.259	1.5349	0.0756	0.997
	19.8929	1.749	3.4755	0.0445	0.995
	14.1187	1.735	5.4005	0.0418	0.994
	Mesor 8.3				
Mice	11.5833	0.337	2.1025	0.0136	0.900
	20.4839	0.258	1.4482	0.0061	0.804
	Mesor 7.48				
			Electrical stimulation		
Rats	19.2992	0.046	3.8530	0.0362	0.991
	12.6317	0.045	4.9272	0.0356	0.991
	Mesor 0.49				
			Mechanical stimulation		
Rats	34.9095	0.170	2.6294	0.0252	0.984
	9.7472	0.171	0.6630	0.0250	0.984
	19.9989	0.094	4.8351	0.0076	0.884
	Mesor 1.89				

Table 2. Parameters of Group and Individual Rhythms of Pain Sensitivity of Mice and Rats

Animals	Group rhythms		Mean values of parameters of individual rhythms	
	period, h	acrophase, h	period, h	acrophase, h
	Thermal stimulation			
Rats	19.89	13.16	22.95	11.38
	14.12	20.34;10.41	12.51	13.40; 02.11
	8.76	5.51; 14.37		
		20.28		
Mice	20.48	5.31	23.09	13.16
	11.58	8.00; 19.35	11.98	9.44; 22.46
	Electrical stimulation			
Rats	19.30	14.41	23.95	14.30
	12.63	18.46; 7.24	11.90	14.40; 2.34
	Mechanical compression			
Rats	20.00	4.25	24.02	00.54
	9.75	7.14; 16.59	13.03	17.49; 6.50
		2.44		
		34.91	10.01	

cycle, while the position of the acrophase of pain sensitivity threshold did not change (Table 2). The power and probability of circadian rhythms in rats were not high, ultradian rhythms with an approximately 12-h period being more potent. On the whole, the parameters of rhythmic organization of pain sensitivity of rats exposed to electrical and thermal stimulation were very similar.

The reaction to mechanical compression of the root of the tail was somewhat less pronounced in the dark phase (Fig. 1). The group circadian rhythm was characterized by very low power and probability and differed considerably from the group circadian rhythms during electrical and thermal stimulation of rats in the time of the minimal pain sensitivity, but not in the duration of the period (Table 1). Individual circadian rhythms were more pronounced, but were likewise characterized by an acrophase inverted vis-a-vis the circadian rhythms of pain sensitivity during electrical and thermal nociceptive stimulation (Table 2), that is, the minimal pain sensitivity to mechanical stimulation was observed during the period of high pain sensitivity to electrical and thermal stimulation. Rhythms with an approximately 12-h period and minimal reactivity during the second half of the dark and light phases predominated in the ultradian rhythms for mechanical stimulation.

Hence, the rhythmic organization of pain sensitivity of mice and rats is characterized by a stable 12- and 24-h periodicity: it drops twice in 24 h and one of these drops, the more pronounced one, forms the circadian rhythm. Sensitivity to thermal and electrical stimulation decreases more appreciably at the beginning of the light phase, and that to mechanical compression during the first half of the dark phase of the 24-hour period.

The data on the diurnal fluctuations of pain sensitivity of mice [3-5] and rats [7,8] are rather contradictory, particularly as regards the shape of the diurnal curve of the pain threshold, length of the period of the rhythm, and time of the maximal values of the function. This may be due to the different conditions under which the animals are kept, specific features of nociception biorhythms in animals of different strains and species, and different types of pain stimulation. We showed that chronobiological parameters of pain sensitivity rhythms are related to the type of pain stimulation. This relationship may be related to previously reported differences in the neurotransmitter systems conducting and regulating different types of nociception [7]. Circadian rhythms of pain sensitivity to electrical stimulation of isolated mice, which we studied exhaustively in previous experiments, differed in the position of the acrophase and in the higher power and probability both from the analogous rhythms in grouped rats and from the circadian rhythms of sensitivity to thermal stimulation of grouped mice. On the other hand, we did not observe any appreciable differences in the chronobiological pattern of pain sensitivity to thermal stimulation of grouped rats and mice. This indicates that the method of pain stimulation, zoosocial effects, and psychoemotional status of animals, which depend on isolated or group maintenance, are more significant for the biorhythm of pain sensitivity than are species-specific features of chronobiological organization.

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Diurnal Time Course of Hexenal Narcosis in Experimental Hepatosis

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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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