EXPERIMENTAL BIOLOGY

Effect of Lithium Hydroxybutyrate on Circadian Rhythms of Brain Serotonin Content and Plasma Corticosteroids in Reserpine-Treated Mice

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Lithium hydroxybutyrate (10 mg/kg daily for 10 days, intramuscularly) administered in the morning prevents reserpine-induced depression of the diurnal steroidogenesis in mice and the development of desynchronosis between circadian rhythms of serotonin in the brain and plasma corticosteroids. Evening injections result in the formation of phase-discordant 24-h periodicities, the mean values of the studied parameters being unaffected.

Key Words: *circadian rhythms; reserpine; serotonin; corticosteroids; lithium hydroxybutyrate*

Phase-dependent resynchronizing effect of lithium hydroxybutyrate (LH) was previously demonstrated by us in the model of reserpine depression [3]. Disturbed rhythmic organization of steroidogenesis in patients with affective psychosis [1] is probably related to dysfunction of the central serotoninergic system [10,12]. Taking into account the mechanism of the normothymic action of LH [4] and the fact that central effects of reserpine are mediated through monoamine system [2,7], the effect of LH on circadian rhythms of serotonin (5-HT) in the brain and plasma corticosteroids (CS) was studied in reserpine-treated mice.

MATERIALS AND METHODS

Experiments were carried out on male mice weighing 25-28 g during the winter solstice. The animals (40-50 mice per group) were kept under natural illumination 1-1.5 months prior to the experiment with free access to food (standard chow) and water. LH (10 mg/kg in a volume of 0.05 ml per 10 g body weight) was injected intramuscularly for 10 days either in the morning (8:30) or in the evening (19:30). Control animals received the same volume of water. On day 8 (15:00) the animals were injected with reserpine (0.75 mg/kg, subcutaneously). On the next day, brain 5-HT and plasma CS were measured every 6 h starting from 9:00. The mice (12 animals per point) were rapidly decapitated (in the night under red illumination), collected heparinized blood was centrifuged, and the plasma was used for glucocorticoid assay. The brain was rapidly removed, and the studied brain structures were isolated and stored in liquid nitrogen. The content of 5-HT (μg/g wet tissue) in brain samples (excluding the cerebellum, medulla oblongata, and olfactory bulb) was measured fluorimetrically using o-phthalaldehyde [5]. The concentration of adrenocortical hormones (glucocorticoids, nmol/liter) was determined using steron-K-^{125}I-M radioimmunoassay kits (Russia) [6].

The primary chronograms were subjected to spectral and cosinor-analysis [4] and statistically significantly rhythms were analyzed ($p<0.05$).

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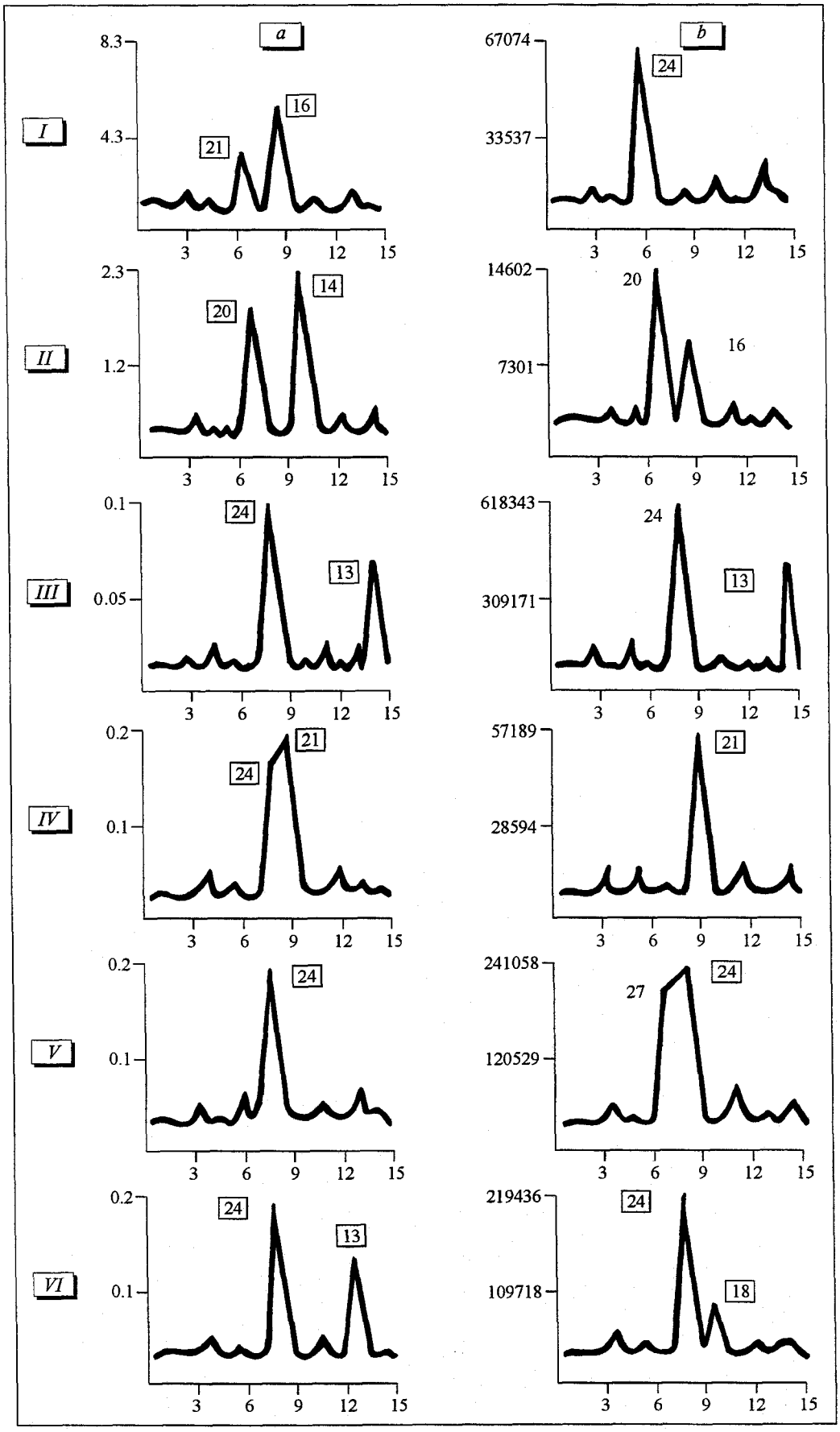

Fig. 1. Effect of lithium hydroxybutyrate (LH) on spectral densities of the rhythms of serotonin content in the brain and plasma corticosteroids in reserpine-treated mice. Abscissa: lags; ordinate: spectral densities. *a*) serotonin; *b*) corticosteroids. *I*: intact mice; *II*: reserpine-treated mice; *III*: reserpine-treated mice injected with the solvent at 8:30; *IV*: reserpine-treated mice injected with the LH at 8:30; *V*: reserpine-treated mice injected with the solvent at 19:30; *VI*: reserpine-treated mice injected with LH at 19:30. Statistically significant rhythms ($p<0.05$) are denoted in rectangles.

RESULTS

Analysis of spectral density of rhythms of brain 5-HT in intact mice during the winter solstice revealed the presence of two equipotent periodicities: 21 and 16 h with acrophases in the morning hours (Fig. 1, Table 1). The latter agrees with published data on a daytime distribution of acrophases of the circadian rhythms of the brain 5-HT content [10]. The presence of a 21-h component in the spectrum points to a switch from the circadian to a free flowing periodicity of this parameter. This probably results from short and weak light phase during the winter time, sharply narrowed capture period [8], and weakened rhythm synchronization by external time-assignor (light). It can be assumed that the circadian rhythm of 5-HT content in the brain can be captured by external time-assignor at an extended capture period.

In contrast to the brain 5-HT content, we observed pronounced circadian oscillations of plasma CS in intact mice characterized by a maximum in the morning hours following the 5-HT peak (Fig. 1, Table 1). When comparing these findings with previous data obtained on rats [3], it should be noted that biochemical rhythms in mice correlate with behavioral rhythms in rats at the same winter period. Our findings and published data suggest that maximum activity of the adrenal cortex preceded the peak behavioral activity regardless of the day or night activity pattern [11].

Reserpine had no effect on the mean diurnal level of 5-HT but reduced 1.5-fold the concentration of CS (Table 1) and increased animal mortality (to 50%). Since the neuroleptic decreased the spectral density of all studied periodicities, no significant peaks (even diurnal peak) were found in the spectra of hormone rhythms. The latter attests to weakened synchronizing effects of the external time-assignor or the rhythm of motor activity on diurnal plasma glucocorticoid level, which is probably related to inhibition of the central catecholaminergic system [2,7]. This presumably results in a more strong interaction between periodicities of the content of 5-HT in the brain and plasma CS level under the influence of the neuroleptic, as evidenced by the presence of 20-h harmonics in these two rhythms. The facts that these two harmonics are discordant in their acrophases and that the CS component is statistically insignificant suggest that this relationship is very weak. It has been previously demonstrated that central 5-HT and catecholamines stimulate secretion of corticotropin releasing factor, adrenocorticotropic hormone, and CS and can modulate circadian rhythms of these hormones [12]. It seems possible that this modulation is reciprocally effected by monoaminergic systems in different daytime and in different seasons.

Thus, single injection of reserpine caused a pronounced external desynchronosis and some internal phase desynchronosis between the rhythms of plasma glucocorticoid level and 5-HT content in the brain.

Reserpine administered against the background of chronic injections of the solvent regardless of the daytime reduced the content of 5-HT in the brain and elevated plasma CS, animal mortality being unchanged in comparison with the previous group. The chronic pain stress caused by subcutaneous injections of the solvent induced hormone release from the adrenal cortex into the blood; these hormones either directly reduced the average daily content of 5-HT in the brain, or increased the sensitivity of 5-HT metabolism to the exhausting effect of reserpine. Analysis of rhythm spectra for both biochemical parameters revealed a considerable (10- to 50-fold) potentiation of circadian harmonics of blood CS level together with an equivalent depression of the spectral density of the 24-h periodicity of 5-HT content in the brain (remained statistically significant). Under these conditions morning injections of water uncoupled acrophases of the diurnal harmonics of these parameters and on the contrary, evening injections timed them best (Table 1). This fact suggests that the synchronizing action of a moderate stressor [9] prevents the reserpine-induced desynchronization only in the case of evening stimulation.

Changes in average daily values of both parameters induced by administration of reserpine against the background of evening or morning injections of LH were similar to those in the control (water). However, the release of glucocorticoids was less pronounced, especially in the morning (Table 1, Fig. 1). Presumably, LH has a direct effect on the average daily level of CS, since lithium has been shown to activate the hypothalamus-pituitary-adrenal system [13,14]. After morning administration of LH, 21-h harmonics with acrophases shifted to earlier morning hours were predominant in the spectra of both parameters; the peak of 5-HT content in the brain preceded the maximum plasma concentration of the hormone (Fig. 1, Table 1), animal survival rate in this phase being considerably increased (to 80%). Evening injections of LH little affected the spectra of both studied rhythms in comparison with the control animals injected with water at the same daytime. Some minor but statistically significant ultradian harmonics appeared; the circadian periodicities were still predominant but compared to control their acrophases drifted further apart. This probably is responsible for desynchronization of behavioral and thermal rhythms in some reserpine-treated rats induced by evening

TABLE 1. Effect of LH on Circadian Rhythms of 5-HT (μg/g) in the Brain and Plasma CS (nmol/liter) in Mice under Conditions of Reserpine-Induced Depression

Group	Rhythm parameter, h		Mesor, rel. units	Amplitude	Acrophase, h×min
Intact mice (n=10)	5-HT,	21	0.77±0.06	0.03-0.29-0.55	0.31—8.35—10.21
		24	0.77±0.06	0.21	11.00
	CS,	24	85.6±4.89	20.3-34.6-67.2	8.17—11.21—18.29
Reserpine (n=8)	5-HT,	20	0.74±0.09	0.08-0.25-0.43	9.59—14.28—18.25
		24	0.74±0.09	0.06	5.08
	CS,	20	55.4±7.9	13.1	13.37
		24	55.4±7.9*	10.2	13.19
Reserpine+solvent at 8:30 (n=12)	5-HT,	24	0.5±0.01**	0.01-0.03-0.04	12.45—15.44—19.0
	CS,	13	124.5±4.3	104-123-142	9.07—9.28—12.11
		24	125.1±20.1**	9.1	9.24
Reserpine+LH at 8:30 (n=11)	5-HT,	21	0.5±0.01**	0.1-0.15-0.16	10.0—10.37—11.50
		24	0.5±0.01**	0.02-0.04-0.08	21.56—7.44—9.18
	CS,	21	90.1±7.1	0.4-45.1-89.9	4.10—7.52—13.48
		24	90.1±7.1**	18.4	7.58
Reserpine+solvent at 19:30 (n=10)	5-HT,	24	0.5±0.01**	0.01-0.05-0.08	12.23—15.10—20.34
	CS,	24	126.8±10.1**	4.6-55.6-107.1	15.43—20.35—1.14
Reserpine+LH at 19:30 (n=11)	5-HT,	24	0.5±0.01**	0.02-0.04-0.08	21.56—7.44—9.18
	CS,	24	112.2±9.9**	28.4-61.8-95.2	15.53—17.37—21.38

Note. p<0.05: *compared with the control group; **compared with reserpine-treated mice.

administration of LH [3]. Survival rate in this group was even decreased in comparison with controls (40%).

Thus, LH administered in the morning hours normalizes the average level of steroidogenesis in reserpine-treated mice and facilitated synchronization between the circadian rhythms of the brain 5-HT content and plasma CS level due to increased mutual sensitivity of the two oscillators. Evening injections of LH promoted the onset of phase-discordant circadian periodicities in reserpine-treated animals, the average daily levels of the studied variables being unchanged.

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