EXPERIMENTAL STUDY OF THE EFFECT OF LITHIUM HYDROXYBUTYRATE ON THE CIRCADIAN REST-ACTIVITY RHYTHM AND SLEEP STRUCTURE

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KEY WORDS: lithium hydroxybutyrate; circadian rest-activity rhythm; sleep structure.

Inorganic lithium salts can modify the structure both of the major circadian rhythm of rest and activity, and also the sleep structure [12, 13]. This may perhaps determine their efficacy in the case of treatment of several psychiatric diseases and, in particular, of depressions, whose course is often accompanied by destabilization of these rhythms [2, 14]. Lithium-hydroxybutyrate (LHB) also belongs to a group of psychotropic drugs which normalize mood. A combination of lithium and gamma-hydroxybutyric acid has proved to be so auspicious that it greatly widened the spectrum of activity of the drug and enabled it to be recommended as a therapeutic substance not only in psychiatry, but also in internal medicine, surgery, and emergency care [7]. Information on the effect of LHB on rhythm disturbances is virtually not to be found in the literature, and since the rhythm-modulating properties of lithium mentioned above may be undesirable in the treatment of diseases unaccompanied by psychiatric disturbances, it was decided to study the structure of the circadian rest-activity rhythm and of sleep cycles in intact animals receiving LHB in different phases of the circadian rest-activity rhythm.

EXPERIMENTAL METHOD

Experiments were carried out in the summer on noninbred male rats weighing 180-200 g and kept under conditions of natural illumination (light:darkness = 18:6), with free access to food and water. The circadian rest-activity rhythm was evaluated by the open field test. The total number of ovulatory movements (AC) and the number of holes investigated (investigative component, IC) during 5 min of observation were determined. The rectal temperature was measured after the open field test. Testing was carried out every 4 h continuously during the last 3 days of administration of the compound. LHB was injected in an effective dose of 10 mg/kg subcutaneously once in the course of 10 days [5]. The time of the injection was determined by the initial calculated maximum of the rhythms, i.e., before and after the acrophase of the rest-activity rhythm. The control animals received the equivalent volume of the solvent subcutaneously. Investigations also were carried out on intact animals (without injections). Integral chronograms were analyzed by Halberg's method [9] with certain modifications [4]. Individual rhythms of sinusoidal and nonsinusoidal character were detected by dispersion analysis [1].

The EEG corresponding to the stages of sleep was recorded continuously for 4 h from 11 a.m. to 3 p.m. on an EEG 8S encephalograph (Medicor), through electrodes implanted previously into the sensomotor cortex, dorsal hippocampus, and neck muscles. The stages of sleep were identified and the EEG analyzed visually by the usual methods [6]. The significance of differences was determined by Student's test.

EXPERIMENTAL RESULTS

Stability of the rhythms in intact animals characterized by good agreement one rhythm with another (Table 1). Acrophases of rhythms of AC and body temperature occurred in the depth of the night, between 11 p.m. and 4 a.m. Besides circadian rhythms, 12- and 16-hourly components were identified also for temperature and IC.

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TABLE 1. Effect of Lithium Hydroxybutyrate (LHB, 10 mg/kg) on Rhythms of Activity and Body Temperature of Rats in Summer (M ± m)

Experimental conditions	Characteristics of 24-hourly rhythm	Mesor	Amplitude	Acrophase	Additional periods, h
Intact group	t	37,50±0,28	0,32±0,25	21,3—2,1—4,1	16
	IC	5,71±2,18	0,41	4,1	12
	AC	9,33±4,00	5,60±2,78	2,1—3,3—4,4	—
Control (water, 8:30 a.m.)	t	37,44±0,27	0,32±0,20	19,2—21,8—1,2*	—
	IC	6,90±1,86	1,08	0,5	—
	AC	20,02±8,12	4,38	23,9*	32
Control (water, 7:30 p.m.)	t	37,71±0,45	0,64±0,35	21,9—0,1—1,7*	—
	IC	7,82±3,95	2,08±1,71	1,1—2,1—3,2*	—
	AC	12,97±4,75	9,37±17,21	21,6—1,0—1,7*	—
LHB (8:30 a.m.)	t	37,39±0,22	0,27±0,25	18,6—23,6—2,3	—
	IC	4,07±1,56	1,45±0,98	0,4—2,6—6,0	—
	AC	18,32±9,61	5,12±3,23	21,6—1,6—3,7	—
LHB (7:30 p.m.)	t	37,76±0,38	0,44±0.16	0,2—1,3—2,6	—
	IC	6,01±2,98	0,88	19,4	—
	AC	7,08±1,83	1,36	22,5	—

Legend. Here and in Table 2: *p < 0.05 compared with control.

TABLE 2. Effect of LHB (20 mg/kg) Administered in the Morning (8:30 a.m.) and Evening (7:30 p.m.) on Sleep-Waking Cycle of Intact Rats (M ± m)

Parameter	Control, n = 14	LHB	
		morning	evening
		n=12	n=8
Latent period of falling asleep, min	37,8±5,31	32,8±4,42	38,9±3,84
Latent period of paradoxical phase of sleep, min	63,6±7,56	84,0±10,60	135,5±15,90*
Waking, %	36,2±1,06	43,7±3,08*	37,3±1,59
Slow-wave sleep, %	54,8±1,30	47,8±3,1	56,1±1,86
Paradoxical sleep, %	10,1±0,35	8,6±1,48	6,9±0,56*
Number of episodes of awakening	23,8±3,18	28,2±4,41	25,7±2,12

In the control subcutaneous injection of water in the evening was accompanied by a shift of the acrophages of the circadian harmonics of temperature and AC to earlier times of the night. The 24-hourly harmonic for IC, previously absent, was discovered. The mean diurnal values of the rhythms and their amplitudes did not change significantly except for temperature, the amplitude of which was doubled compared with the intact state. Under the conditions specified above certain ultradiene rhythms were preserved. Thus, injection of water at 24-h intervals led to stabilization of the circadian rhythms, evidently due to a stressor effect, which is known to induce synchronization of circadian rhythms [8].

LHB, administered in the evening, led to approximation of the acrophases of the circadian rhythms to their initial values. However, the temperature rhythm continued to differ significantly from that in the intact animal, because its amplitude was twice as great. The circadian harmonic for IC and the ultradian rhythms for all parameters were lost. Thus the structure of the stable rhythm pattern became less organized under the influence of LHB than in the control and in intact animals.

Injection of the solvent in the morning was accompanied by delay of the acrophase of the temperature rhythm compared with its initial value and the morning control. However, the rhythms studied did not differ statistically significantly. Under these conditions 24-hourly and ultradian harmonics for IC and AC were not discovered, but a 32-hourly rhythm was found for the latter parameter. Thus, by contrast with evening, morning injection of the solvent considerably destabilized the stable circadian rhythm pattern of the rats. Since the time of the evening injection of the solvent coincided with the beginning of the phase of activity of the rats, and the time of the morning injection with its end, it can be postulated that rhythms of motor activity are less resistant to this stimulus if it was applied at a time of a low level of activity of the animals.

Under the influence of morning injections of LHB, short-period components were not detected, the circadian harmonics of AC and IC, lost after injection of the solvent were restored, and their acrophase returned close to their initial values. The mean diurnal values and amplitudes of the lithium rhythms were virtually indistinguishable from the controls and, in particular, from those in intact animals. Consequently, morning injection of the compound had a weaker effect on the rhythmic structure of motor activity of the animals than evening injection. In both cases, however, LHB led to clearer expression of the 24-hourly and loss of the ultradian rhythms.

Because of this last fact it was necessary to study the effect of LHB on the short-period sleep cycles. Sleep, as an independent phenomenon, has its own endogenous oscillators, which are closely connected with the circadian oscillator [10]. Sleep cycles therefore correlate to a certain degree with the major circadian rest-activity rhythm.

In the present experiments the sleep structure of the control animals corresponded to that described in the literature [3]. LHB, injected in the morning, lengthened the duration of wakening, but without changing the other parameters tested (Table 2). More significant changes in the sleep-waking cycle took place after evening injection of LHB: the latent period of onset of the first episode of paradoxical sleep was greatly lengthened but its quantity was reduced. Furthermore, compared with animals receiving LHB in the morning, the duration of slow sleep was increased significantly. The sleep structure of the rats after evening injection of LHB was similar to that after administration of certain antidepressants [11], and also of inorganic lithium salts, which have been shown to lengthen the slow phase of sleep, to reduce the quantity of paradoxical sleep and to increase the number of awakenings in healthy subjects [13]. The sleep of animals receiving LHB in the morning was virtually indistinguishable from the control. LHB evidently affects the sleep cycles of rats differently depending on the time of its injection. The compound acts least of all on the ratio between the phases of sleep when administered in the morning.

Thus LHB has a weaker action on the major circadian rhythm of activity of rats and their sleep structure if administered at the beginning of the phase of the animals' rest.

The circadian rhythms and sleep cycles investigated in intact animals were evidently most sensitive to the chronotropic action of lithium in the waking period, a conclusion which does not contradict Halberg's views expressed on the time of maximal circadian chronosensitivity and chronoresistance to drugs [9].

Our observations indicate that one possible way of diminishing undesirable chronopsychopharmacologic effects of lithium is to take account of the phase when the drug is administered. The results may be of practical interest for physicians using this compound in the treatment of internal diseases.

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^{3}H-tert-BUTYLBICYCLOORTHOBENZOATE – A NEW LIGAND FOR THE GABA$_A$-RECEPTOR GATED ION CHANNEL

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The specific ligand ^{35}S-*tert*-butylbicyclophosphorothionate (^{35}S-TBPS) has been used in recent years to study the chloride ion channel of the GABA$_A$-receptor. For this same purpose, ^{3}H-*tert*-butylbicycloorthobenzoate (^{3}H-TBOB) has been used. Agonists of GABA$_A$-receptors have an allosteric action on specific binding of ^{35}S-TBPS with mammalian brain membranes. Antagonists abolish these effects. As regards ^{3}H-TBOB, no such information is available. The present investigation was undertaken to remedy this omission.

EXPERIMENTAL METHOD

Noninbred male albino rats weighing 180-220 g were used. The synaptosomal-mitochondrial P_2 fraction, isolated from the brain (excluding the brain stem and cerebellum) was homogenized in 50 volumes of 10 mM Tris-HCl, pH 7.4, containing 1 mM EDTA. The suspension was incubated for 10 min at 0°C and centrifuged for 20 min at 20,000g. This procedure was repeated 3 times. The final residue was frozen for 18 h at −18°C. After thawing the membranes were washed twice in 50 volumes of 10 mM Tris-HCl, pH 7.4, followed by centrifugation for 20 min at 20,000*g*. The residue was suspended in 50 mM Tris-HCl, pH 7.4, containing 500 mM NaCl, it being assumed that 1 ml contained 1.8-1.9 mg protein.

Radioligand Analysis. The incubation medium included the membrane preparation (900-950 μg protein), 50 mM Tris-HCl buffer, pH 7.4, containing 500 mM NaCl, 5 nM ^{3}H-TBOB (Amersham International, England, 30.6 Ci/mmole), as well as different concentrations of GABA (Reanal, Hungary) and bicuculline (Sigma, USA). The bicuculline was added to 10 μl of DMSO. The total volume of the incubation medium was 2 ml. Samples were incubated for 30 min with continuous shaking at a temperature of +25°C. The contents of the tubes were transferred to GF/B and GF/C filters (Whatman, England) and washed twice with 5 ml of ice-cold buffer. Binding of ^{35}S-TBPS (2 nM, from NEN, Germany, 87.1 Ci/mmole) was estimated by the method described previously. Some samples were washed on filters without preliminary incubation, so that nonspecific binding of the radioactive label by the material of the filters could be determined. The filters were placed in scintillation flasks containing ZhS-8 scintillation fluid, radioactivity was counted on a 1214 RackBeta II counter. Each value was determined from the results of three separate experiments, conducted in three parallel series. The protein concentration in the samples was determined by Lowry's method. The numerical results were subjected to statistical analysis by Student's t test.

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