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REM Sleep Disorders in Rats with Experimental Depressive Syndrome Caused by Systemic Administration of 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine (MPTP)

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Rats receiving daily injections of the neurotoxin MPTP in a dose of 20 mg/kg for 12 days develop disorders of REM sleep, including increased frequency of REM-sleep episodes, decreased REM latency, and increased REM sleep duration, both absolute and relative. The first two of these REM sleep disorders are characteristic of endogenous depression. The results indicate that systemically administered MPTP causes a state similar to endogenous depression.

Key Words: *REM sleep; MPTP; depressive syndrome; rats*

We showed earlier [4] that multiple systemic administration to rats of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a toxin specific for dopaminergic neurons, leads to the development of depressive syndrome (DS) manifested in reduced motivational activity combining with agedonia and "behavioral despair." Using this model, we also found that the experimental DS is dopamine deficiency-dependent [3] and that its behavioral symptoms can be prevented or mitigated by the tricyclic depressant melipramin [2] and, to a lesser extent, by buspirone, a new antidepressant [1]. Patients with endogenous depression show characteristic disorders of REM sleep (so-called paradoxical sleep phase) [6-8,12,15]. Characteristics of REM sleep and of the sleep-wakefulness cycle and their relevance to clinical forms of depression were therefore assessed in the rat model of depression [13]. It has been shown that reduced functional activity of the dopaminergic system of the brain is also accompanied by alterations in parameters of both slow-wave sleep (NREM sleep) and REM sleep [5]. In the present study we examine the characteristics of REM sleep and the sleep-wakefulness cycle in rats with experimental DS caused by multiple systemic injections of MPTP.

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MATERIALS AND METHODS

The study was conducted on 14 male Wistar rats weighing 270-330 g. They were maintained under standard vivarium conditions with the natural lighting schedule, 2 rats per cage, and had free access to food and water. Under chloral hydrate anesthesia (400 mg/kg intraperitoneally), insulated nichrome electrodes 200 μ in diameter were implanted into the rostral part of the caudate-putamen complex and into

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the dorsal hippocampus according to stereotaxic coordinates [11], and a ball-type silver electrode was installed in the area of the sensorimotor cortex. In addition, nichrome electrodes 200 μ in diameter with a noninsulated tip were implanted into cervical muscles to record their electrical activity (EA). The EA of the brain was recorded monopolarly in unrestrained animals and the EA of cervical muscles bipolarly with an EEG-4314F electroencephalograph (Nihon Kohden). The cage with the rat was placed in a separate room illuminated by dim scattered light, and the behavior of the animal was monitored from the adjacent room by means of a video monitor. The EA was recorded continuously for 4 h. On the following day, daily treatment with MPTP (20 mg/kg) (test group) or physiological saline (control group) was started. MPTP (synthesized at the Institute of Pharmacology, Moscow) was dissolved in physio-

TABLE 1. Parameters of the Wakefulness-Sleep Cycle in Rats Treated with MPTP and Physiological Saline (*M±m*)

Parameter	Observation time				
	before treatment	during treatment		after treatment	
		day 3	day 11	1 week	2 weeks
Number of REM-sleep episodes					
MPTP	5.0±1.3	13.0±3.0	16.0±3.1*	10.0±2.7	6.0±1.0
Saline	6.3±1.0	7.3±0.9	7.4±0.6	5.0±0.7	5.3±0.3
Total REM sleep duration, sec					
MPTP	373.4±147.3	1041.0±150.9*	1382.5±287.4*	803.0±284.6	472.0±272.8
Saline	436.6±74.3	608.0±109.7	689.6±67.5	443.0±111.2	302.0±75.8
Mean duration of REM episodes, sec					
MPTP	67.6±12.4	79.7±9.5	77.2±11.8	83.0±15.2	70.8±32.6
Saline	72.7±12.3	66.1±10.8	92.7±4.9	84.3±14.2	61.1±5.0
REM latency, sec					
MPTP	501.1±58.7	166.6±20.5**	210.0±37.7*	278.0±64.4	332.0±40.4
Saline	344.6±54.4	357.7±39.8	343.8±28.3	341.4±73.1	330.8±72.9
Percentage of REM sleep in total sleep					
MPTP	4.9±1.6	13.1±1.4**	16.4±3.0**	10.9±3.2	6.9±3.5
Saline	5.5±1.1	6.3±1.0	7.3±0.9	6.0±1.4	3.8±0.3
Sleep duration, in % of total observation time					
MPTP	58.3±5.2	62.0±5.0	60.0±5.5	56.5±8.8	47.6±12.9
Saline	62.8±1.8	67.9±4.7	70.1±3.3	60.1±5.4	58.5±2.4
Mean duration of sleep periods, min					
MPTP	11.5±1.7	14.3±2.8	12.6±2.2	13.3±1.4	8.9±2.0
Saline	11.0±0.5	12.7±1.2	11.9±1.2	10.0±0.9	10.7±2.4
Duration of wakefulness, in % of total observation time					
MPTP	41.6±5.2	38.3±4.2	40.0±4.5	43.5±8.8	52.4±12.9
Saline	37.2±1.8	31.6±4.8	29.9±3.3	39.9±5.4	41.5±2.4
Mean duration of wakefulness periods, min					
MPTP	8.0±0.5	8.9±1.2	8.3±1.2	13.2±4.7	12.3±4.1
Saline	7.0±0.5	6.4±1.4	5.4±0.7	7.3±1.3	8.0±0.9
Mean duration of longest (>10 min) wakefulness periods, min					
MPTP	17.5±1.5	22.2±2.2	20.3±1.3	25.5±5.7	24.6±1.0
Saline	22.5±2.8	21.2±2.6	18.9±3.5	19.5±3.2	27.4±3.7

Note. The number of rats in the MPTP- and saline-treated groups was, respectively, 7 and 7 before treatment and on day 3 of treatment, 6 and 7 on day 11 of treatment, 5 and 6 one week posttreatment, and 4 and 3 two weeks posttreatment. *$p<0.05$, **$p<0.01$ by Student's unpaired parametric test.

logical saline immediately before injection. MPTP and saline were injected intraperitoneally in a volume of 1 ml/kg body weight for 12 days. The EA was recorded at the same time of the day (from 10:00) on days 5 and 6 after operation, on days 3 and 11 of treatment, and 1 and 2 weeks after the 12-day treatment period.

The criteria of transition to REM sleep during NREM sleep were a simultaneous occurrence of desynchronization on the electroneocorticogram with a sharp fall in the EA amplitude, development of synchronized EA in the hippocampus in the theta rhythm range, and disappearance of static tone in the cervical muscles. In evaluating parameters of the sleep-wakefulness cycle, the time between the moment the rat was placed in the recording chamber and the moment it fell asleep was excluded from the total observation time. Each minute of recording was defined as "sleep" if more than half of the minute was occupied by sleep and as "wakefulness" if more than half of the minute the animal remained awake. The durations of NREM sleep and wakefulness in percent of the total observation time were determined, as well as was the average duration of these two periods. The number and average duration of REM-sleep episodes, the total duration of REM sleep, and the percentage of REM relative to the total duration of sleep were calculated. The REM latency was defined as the time between the onset of NREM sleep and the onset of REM sleep or as the interval between two REM-sleep episodes during continuous NREM sleep. The average REM latency was calculated for each animal using all the latency values obtained.

The results were statistically analyzed by Student's unpaired parametric *t* test and paired parametric *t* test (Primer software).

RESULTS

Short periods of a markedly changed EA pattern simultaneously in all three leads were observed in both the MPTP-treated and saline-treated groups during NREM sleep, including desynchronization in the neocortex, the occurrence of synchronized EA in the theta rhythm range in the hippocampus, and a fall in the tone of cervical muscles. Such changes were indicative of transition to REM sleep. The characteristics of the sleep-wakefulness cycle and of REM sleep in the two groups are presented in Table 1. In the MPTP-treated group, compared with the control group, the number of REM-sleep episodes increased (day 11 of treatment; $p<0.05$), as did the total duration of REM sleep ($p<0.05$) and the proportion of REM-sleep episodes (days 3 and 11 of treatment; $p<0.01$); REM latency decreased ($p<0.01$ and $p<0.05$ for days 3 and 11, respectively). There were no intergroup differences in the average duration of REM-sleep episodes. The groups did not differ in the total duration of sleep and wakefulness, as expressed in percent of the total observation time or in the average duration of sleep and wakefulness periods. Nor did they differ in the average duration of the longest periods of wakefulness (>10 min). It should be noted that intragroup variations were observed in the test group with regard to the four major REM-sleep parameters for which intergroup differences were recorded (Fig. 1, *a-d*). Thus, there occurred increases in the number of REM-sleep episodes (*a*), decreases in REM latency (*b*), and increases in the total duration of REM sleep (*c*) and in its proportion in total sleep (*d*) in comparison with baseline values of the corresponding parameters. These parameters still differed from baseline levels 1 and 2 weeks after MPTP treatment had been discontinued, although the differences were insignificant. In the control group, no differences from baseline levels were observed with the exception of the total duration of REM sleep which exceeded baseline level on day 11 of treatment (Fig. 1, *c*).

Patients with endogenous depression have four specific REM-sleep abnormalities: shortened REM latency, more frequent REM-sleep episodes, more frequent phasic movements during REM sleep, and abnormal temporal course of the restoration of REM sleep after its deprivation [14], although all four abnormalities do not necessarily occur in the same patient. In the present study, the first two abnormalities were observed in MPTP-treated rats (the last two abnormalities were not studied). In the rat model of depression produced by neonatal administration of clomipramine, abnormalities 1, 2, and 4 were recorded and, in addition, increased lengths of the longest wakefulness periods were observed with nocturnal but not with diurnal monitoring [14]. In our study the absence of intergroup differences in the duration of longest wakefulness periods also may be due to the fact that all recordings were made in daytime. We found previously that behavioral symptoms characteristic of endogenous depression occurred in MPTP-treated rats (motor inhibition, hedonic disturbances, "behavioral despair," and depressed levels of major motivations) [4] and that behavioral abnormalities could be prevented by the tricyclic antidepressant melipramin [2] that effectively eliminates endogenous depression in humans. Therefore, it can be concluded that the occurrence in MPTP-treated rats of REM sleep disturbances specific of endogenous depression indicates that this new animal model of MPTP-induced DS corresponds to the clinical form of this disease. Patients with psychotic

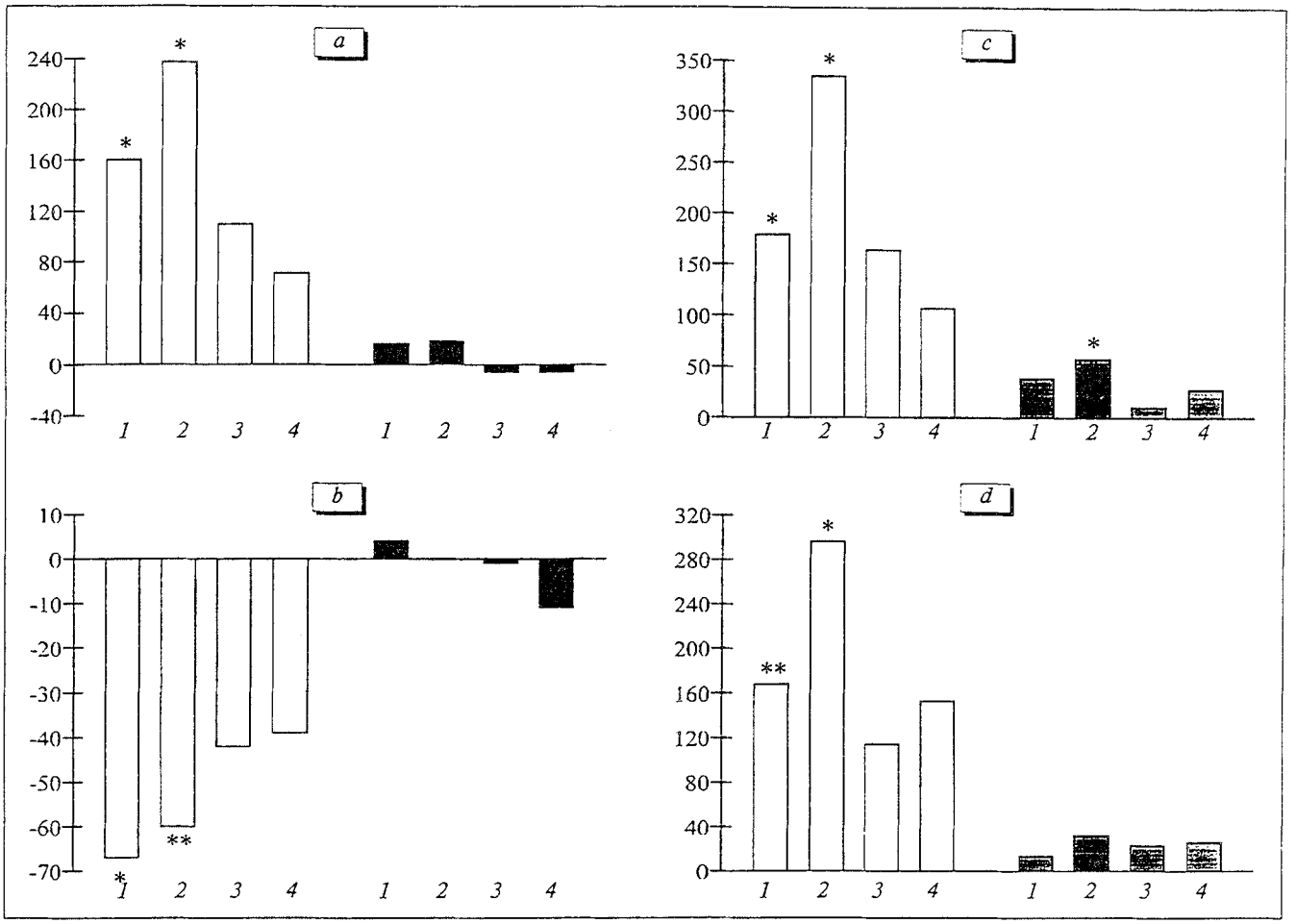

Fig. 1. Variations in the number of REM-sleep episodes (*a*), REM latency (*b*), total duration of REM sleep (*c*), and the proportion of REM sleep in total sleep (*d*) in rats treated with MPTP (white bars) and physiological saline (black bars). *1* and *2*) on days 3 and 11 of treatment, respectively; *3* and *4*) 1 and 2 weeks, respectively, after discontinuation of the treatment. Ordinates: differences between REM-sleep parameters at different times (as expressed in percent of the baseline level) and baseline value of the corresponding parameter in the group taken as 100%. $^{*}p<0.05$, $^{**}p<0.01$ by the paired parametric *t* test in comparison with the baseline.

depression show a decreased percentage of REM-sleep [12]. In the present study, on the contrary, the proportion of REM sleep in total sleep was increased in rats with experimental DS. Dopaminergic function is reduced in patients with melancholic depression accompanied by sleep disorders [5]. Dopaminergic function is also reduced in animals with experimental DS, which is confirmed by the finding that the D_2-dopamine receptor bromocryptine is capable of effectively preventing the development of DS [1].

The paradoxical phase of sleep involves activation of all motivational processes developing through coordinated action of brain structures regulating the formation of multicomponent motivational behavior in awake animals, which is necessary for the maintenance of equilibrium in the body [10]. REM sleep abnormalities are associated with fundamental disturbances in the intensity of affect and with dysfunction of emotional or cognitive processes in depressed patients [9]. Presumably, an increased proportion of REM sleep, which is characteristic of the state of reduced motivational activity in depressive disorders, plays a protective role in the body. Similarly, the increased total duration of REM sleep in the saline-treated control group can be regarded as a manifestation of protective mechanisms in animals daily exposed to a mild depressogenic stress.

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Involvement of Thrombin in Activation of Heparin Secretion by Mast Cells in Immobilization Stress in Rats

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Activation of heparin secretion by connective tissue mast cells under conditions of immobilization stress is determined by activation of the sympathoadrenal system, secretion of adrenocorticotropic hormone, and possible generation of thrombin. Generation of thrombin in the blood under these conditions is confirmed by a significant drop in the proenzyme protein C concentration by 23%, a decrease in the activity of factor V (substrate of protein C) by 36%, and prolongation of activated partial thromboplastin time by 40%. It is shown that 30-min immobilization leads to a 3-fold depletion of the heparin pool in mast cells. Intravenous injection of hirudin, a specific thrombin inhibitor, before immobilization slightly diminishes the stimulating effect of stress on heparin secretion. These data suggest that apart from catecholamines and adrenocorticotropic hormone, thrombin generated in the bloodstream during stress also markedly contributes to activation of heparin secretion by mast cells.

Key Words: *thrombin; protein C; hirudin; mast cells; stress*

Modern concepts of the development of stress reactions do not exclude generation of thrombin in the bloodstream. We have previously demonstrated a sharp increase in the release of heparin, an immediate anticoagulant, from the connective tissue mast cells (MC) [4]. A question arises, is the thrombin generated in the bloodstream involved into stimulation of heparin secretion, i.e., in adaptive reactions aimed at maintenancing homeostasis. The validity of such a question is strengthened by previous findings that intravenous injection of α-thrombin leads to activation of heparin secretion by MC [2].

The aim of the present study is to reveal generation of thrombin in a certain type of stress, to prevent its effect using a specific thrombin inhibitor,

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