GENERAL PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY

Monoamine Content in the Rat Brain Structures with MPTP-Induced Depressive Syndrome

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The content of the dopamine precursor dihydroxyphenylalanine in the caudoputamen structures of rats with depressive syndrome induced by the neurotoxin MPTP decreased at the stage of pronounced behavioral depression and increased at the stage of restoration of behavioral activity after discontinuation of the toxin. The monoamine content in the frontal cortex of rats with depressive syndrome did not differ from the control.

Key Words: *monoamines; caudoputamen; frontal cortex; MPTP; depressive syndrome; rat*

There is evidence of the involvement of central dopamine-, serotonin-, and noradrenergic cerebral systems in the development of depressive states [7-10]. However, there is no unequivocal link between depressive state and changes in a specific neurotransmitter system. The clinical evidence is contradictory, which substantiates conception of biochemical heterogeneity of depressive disorders [12]. There are hypotheses on changes in the interaction between the catecholamine- and serotoninergic cerebral systems during depression [10]. We have developed a novel experimental model of dopamine (DA)-deficient-dependent depressive syndrome in rats provoked by systemic administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a neurotoxin specific for dopaminergic neurons [5]. This syndrome can be considered as an endogenous behavioral depression, according to partial neuropharmacological isomorphism with the clinical prototype [1,3] and specific changes in the REM-sleep parameters in rats [1]. Our aim was to study the metabolism of DA, norepinephrine, and serotonin in cerebral structures of rats with MPTP-induced depressive syndrome.

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MATERIALS AND METHODS

The study was carried out on 54 random-bred male Wistar rats weighing 340-420 g. The animals were kept individually under standard vivarium conditions with a natural day-night cycle and food and water ad libitum. The depressive syndrome was induced by daily intraperitoneal injection of MPTP for 14 days (20 mg/kg, dissolved immediately before use in physiological saline, 0.1 ml/kg body weight). Control animals were injected with physiological saline according to the same protocol. The depressive syndrome was assessed according to 1) a decrease in daily liquid intake (moderation of motivated activity), 2) a decrease in the preference for 10% sucrose solution over water (development of hedonic disorders), 3) an increase in immobilization period (development of behavioral despair), and 4) the depression index in the forced swimming test. Evaluation of these parameters and determination of the anxiety and phobia level in the rats were performed as described elsewhere [3,5].

We carried out 3 series of experiments.

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In the first series (referent intact group, n=10), the chemical agents were not administered. In these rats we determined the anxiety-phobia level. In addition, liquid intake and sucrose preference for determined during 4 days. After decapitation, the tissues of caudoputamen (CP) and frontal cortex (50 mg of each sample) were immediately isolated from both hemispheres at 0°C. The specimens were frozen in liquid nitrogen and stored for no more than 3-4 months.

In the second series of experiments two groups of rats were used: the test group treated with MPTP (n=15) and the control group injected with physiological saline (n=14). Behavior was assessed during the entire period of injection of chemicals. Decapitation of the rats of both groups and isolation of the specified cerebral tissue were made at the period corresponding to the state of pronounced behavioral depression in test rats 1 day after the last injection.

The third series of experiments was performed on rats injected with MPTP (n=7) and with physiological saline (n=8). Behavior was monitored during the entire period of MPTP injection and 1 week after MPTP discontinuation (the period of restoration of behavioral activity in control rats). The rats were then decapitated to isolate the specified cerebral tissues.

Monoamine extraction was performed by the method [6]. The content of monoamines in the specimens was determined using high-performance liquid chromatography with electrochemical detection in an HP1050 automatic complex and an HP1049 electrochemical detector (Hewlett Packard). In the first series, a Diasorb 130TC18.5 (100×3 mm) chromatography column (BioRad) was used, and a Spherisorb ODS2 column with a Lichrospher100RP-18 (4×4 mm) primary column were used in the second series. Temperature of the column was stabilized at 35°C. The mobile phase was prepared on citrate-phosphate buffer (20/70 mM, pH 3.0) containing 2 mM sodium EDTA and 2 mM sodium heptanesulfonate as an ion-pair reagent (0.6 mM in the second series). Elution rate was 0.8-1 ml/min. The potential of carbon glass electrode was settled at 550 mV against silver chloride solid reference electrode. DA, norepinephrine, DOPA, DOPAA (3,4-dihydroxyphenylacetic acid), and homovanillic acid standards were from Sigma.

The results were processed using unpaired parametric Student's t test and nonparametric one-factor dispersion analysis (Kruskal-Wallis test) with subsequent multiple comparison of the mean values of the dispersion complex using Student-Newman-Keuls test.

RESULTS

In second and third series, the depressive syndrome manifested itself in decreased daily liquid intake, and body weight, and the preference for sucrose over water (Fig. 1) and increased of immobilization period and depressive index (Table 1). At the stage of pronounced depression in the second series, the body weight of the test rats was 325.3±7.6 g, while the body weight of

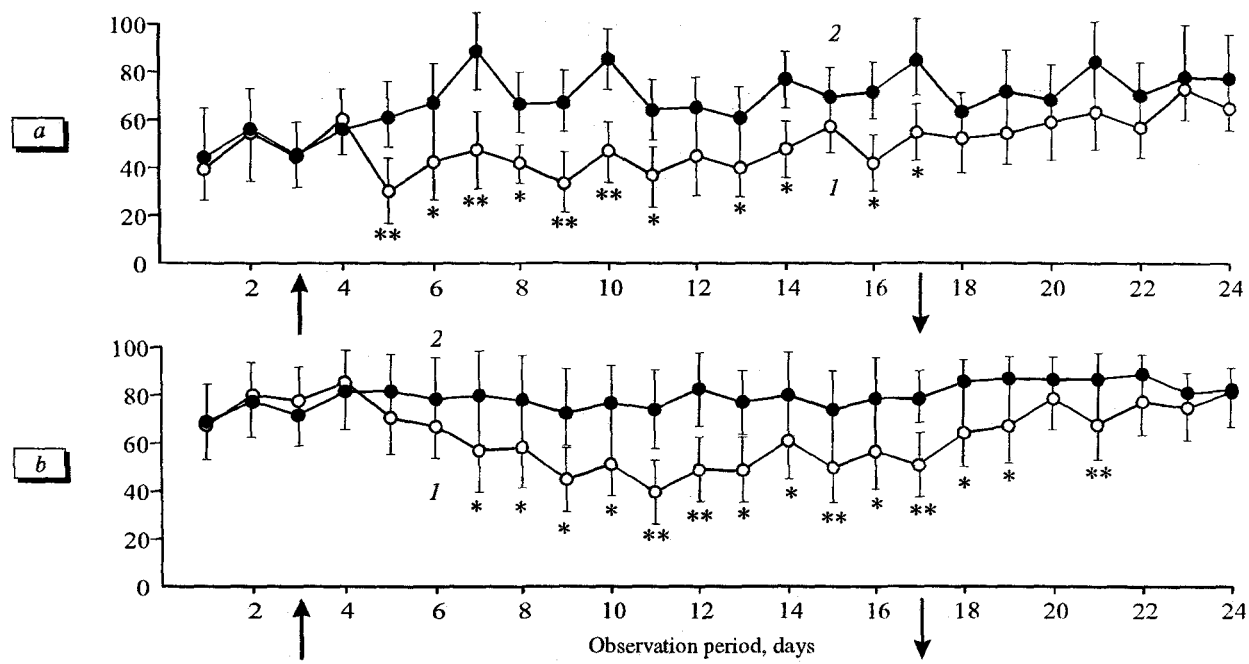

Fig. 1. Dynamics of (*a*) daily liquid intake and (*b*) the preference for 10% sucrose solution over water in (*1*) MPTP-treated rats and in (*2*) the control rats injected with physiological saline. Ordinates: *a*) volume of daily liquid intake, ml; *b*) percentage of sucrose solution intake of the total daily liquid intake. The arrows indicate the first and last days of injections. $^{*}p<0.05$, $^{**}p<0.01$ in comparison with the day-related value of the parameter in rats treated with physiological saline (unpaired parametric Student's t test).

TABLE 1. Characteristics of Disturbances of Swimming Behavior in Rats Treated with MPTP at the Stage of Pronounced Behavioral Depression in Comparison with the Rats Injected with Physiological Saline (*M±m*)

Parameters	Series II		Series III	
	MPTP (*n*=15)	normal saline (*n*=14)	MPTP (*n*=7)	normal saline (*n*=8)
Immobilization period, sec	22.9±3.6*	9.3±4.4	31.7±12.1*	5.6±2.6
Depression index[1]	2.6±0.4**	0.8±0.3	2.7±0.8*	0.7±0.4

Note. [1]Ratio of the number of immobilization periods of less than 6 sec to total number of periods of active swimming in the forced swimming test. *$p<0.05$, **$p<0.01$ in comparison with the corresponding value of the parameter in rats treated with normal saline in the same series (unpaired parametric Student's t test).

the control rats was 367.5±10.3 g ($p<0.05$). Changes in body weight were observed in the test rats in the third series ($F(2,12)=23.16$, $p<0.001$, dispersion analysis for repeated measurements), the body weight at the stage of pronounced behavioral depression being decreased in comparison with the initial value (373.6± 20.0 and 408.6±24.6, respectively, $p<0.05$). One weak after discontinuation of the chemicals, the control rats increased body weight ($F(2,14)=4.26$, $p<0.05$) in comparison with initial value (396.9±13.6) to 402.3±15.6 and 408.1±15.4 g (injection and discontinuation periods, respectively, $p<0.05$). There were no changes in the anxiety-phobia level in the control and test rats in any series of experiments.

At the stage of pronounced depression, the content of DOPA (DA precursor) in CP of the test rats was lower than in the rats treated with physiological saline (Table 2) and in intact rats. The content of DOPA in the test rats at the stage of restoration of behavioral activity was higher than the corresponding values of intact rats and test rats at the stage of pronounced behavioral depression. The DA content did not differ in test and control rats at any stages of the depressive syndrome, although it was higher by 25-30% at both stages in comparison with intact rats. Similar changes were observed with DOPAA but in contrast to DA, the increase in the content of DOPAA was not statistically significant. In the model of experimental depression this was manifested as hedonic disorders in rats subjected to a mild chronic stress, the stress-induced activation of mesolimbic DA system manifested itself in persistent increase in DA production in this system

TABLE 2. Content of Monoamines and Their Metabolites in the CP Structures of the Rats with MPTP-Induced Depressive Syndrome (ng/mg Tissue, *M±m*)

Group	Norepinephrine	DOPA	DA	DOPAA	Homovanillic acid	Serotonin
Series I						
intact	—	0.165±0.015[1] (*n*=10)	7.480±0.531[1] (*n*=10)	0.592±0.044 (*n*=10)	0.410±0.015 (*n*=4)	0.268±0.008[2] (*n*=4)
Series II (the stage of pronounced behavioral depression in the test group)						
normal	—	0.159±0.010 (*n*=11)	10.171±0.346** (*n*=14)	0.759±0.027 (*n*=14)	0.418±0.016 (*n*=13)	0.344±0.020* (*n*=14)
MPTP	—	0.129±0.006*° (*n*=12)	9.300±0.353* (*n*=15)	0.707±0.026 (*n*=15)	0.421±0.019 (*n*=12)	0.336±0.019 (*n*=14)
Series III (the stage of restoration of behavioral activity in the test group)						
normal saline	0.665±0.373 (*n*=6)	0.242±0.139 (*n*=5)	10.117±1.083** (*n*=6)	10.117±1.083** (*n*=6)	0.455±0.105 (*n*=6)	0.165±0.045+* (*n*=6)
MPTP	0.490±0.062 (*n*=4)	0.360±0.131*++ (*n*=4)	10.275±2.425** (*n*=4)	0.763±0.170 (*n*=4)	0.443±0.105 (*n*=3)	0.230±0.112+ (*n*=4)

Note. Here and in Table 3: [1]$p<0.01$, [2]$p<0.001$ (Kruskal-Wallis test, comparison of 5 experimental groups); *$p<0.05$, **$p<0.01$ in comparison with intact rats; +$p<0.05$, ++$p<0.01$ in comparison with the previous stage; °$p<0.05$ in comparison with the control at the same stage of the study (multiple paired comparisons after the Kruskal-Wallis test).

TABLE 3. Content of Monoamines and Their Metabolites in Frontal Cortex of Rats with MPTP-Induced Depressive Syndrome (ng/mg Tissue, *M*±*m*)

Group	Norepinephrine	DOPA	DA
Series I			
intact	0.111±0.007[2] (*n*=9)	0.108±0.009 (*n*=8)	0.176±0.017[1] (*n*=8)
Series II (the stage of pronounced behavioral depression in the test group)			
normal saline	0.160±0.009** (*n*=12)	0.109±0.010 (*n*=7)	0.219±0.024 (*n*=10)
MPTP	0.153±0.005** (*n*=14)	0.119±0.012 (*n*=9)	0.209±0.010 (*n*=13)
Series III (the stage of restoration of behavioral activity in the test group)			
normal saline	0.575±0.047**+++ (*n*=4)	0.240±0.271 (*n*=3)	0.073±0.019**+++ (*n*=4)
MPTP	0.520±0.106**+++ (*n*=4)	0.197±0.202 (*n*=3)	0.090±0.017+++ (*n*=3)

and in the adjacent nuclei [13]. The method of isolation of CP structures used in this work could not separate the tissues of adjacent nuclei from the entire complex. Presumably, the increase in the DA content in the test and control rats, which is unspecific for MPTP-induced depression, indicates pronounced intensification of DA release in CP structures, the adjacent nuclei included, resulting from chronic stress induced by prolonged administration of the chemicals. Serotonin content in the CP of test rats did not differ from the corresponding control values at any stage of syndrome development. The dynamics of changes in serotonin content in test and control groups was similar at different stages: activation during the administration period was followed by normalization one week after discontinuation of the chemicals. Similar to DA, the changes in serotonin content probably attest to disturbance of activity of the serotoninergic system during chronic stress.

At both stages of depressive syndrome, the norepinephrine content of the frontal cortex increased in test and control rats in comparison with intact rats (Table 3). Norepinephrine content was higher at the stage of the behavioral activity restoration than at the stage of pronounced depression. The content of DOPA in this structure did not differ in both groups throughout the entire observation period. The content of DA was decreased in test and control groups one week after discontinuation of the chemicals in comparison with the corresponding values in intact rats and in the respective groups at the stage of pronounced depression. On the depression models in rodents induced by different stressors or their combinations, a decrease in DA in the mesolimbic frontal cortex was revealed during acute stress, while a compensatory increase in central norepinephrine synthesis and moderation of DA utilization rate were observed during chronic stress [14,15]. The direction and nature of changes in catecholamine content under the effect of chronic stress depend on many factors: nature of the stressor, its controllability, specific cerebral structure, the stage of the stress response, animal strain, and others [15]. In this study we revealed a decrease in DA content and a pronounced increase in the frontal cortex norepinephrine content. These changes may reflect the adaptive response of the noradrenergic and DAergic cerebral systems to chronic stress under the specified experimental conditions and indicate the development of plastic rearrangements in the central nervous system.

The most intriguing is the specific decrease of the DOPA content in CP in the test group at the stage of pronounced behavioral depression with subsequent increase in DA precursor level at the stage of behavioral activity restoration. These changes are probably associated with disturbances in the DA synthesis in CP at different stages of MPTP-induced depressive syndrome. By analogy with the study of DA-deficient-depending MPTP-induced experimental parkinsonian syndrome [4], the decrease in DOPA content in CP may reflect the neurotoxin-induced moderation of activity or loss of "pacemaker" enzyme of catecholamine synthesis tyrosine hydroxylase that converts the synthesis DOPA from tyrosine and is a marker of DA-synthesizing neurons. The development of DA-deficient state due to disturbances in the DOPA synthesis in the animals with behavioral depression should be reflected in decreased DA content. However, in chronic stress induced by prolonged administration of the neurotoxin and parallel enhanced release of mesolimbic DA from vesicles, the MPTP-induced decrease of DA-synthesizing function of DAergic neurons is probably masked. At the stage of restoration of behavioral activity the content of DOPA in CP of the test group was higher than in CP of intact rats. This points to restoration of temporarily lost ability of neurons to synthesize DA and/or to adaptive enhancement of the activity of intact DA-synthesizing neurons. Although a similarity between neurochemical mechanisms of development of DA-deficiency-dependent depression

and parkinsonian syndrome has been hypothesized, we failed to reveal severe abnormalities in DA metabolism during experimental depression in contrast to experimental parkinsonism accompanied by a pronounced decrease in DA content and its metabolites in CP [4]. Presumably, the damage to nigrostriatal DAergic neurons is much less severe during experimental depression provoked by half as small doses of MPTP as that used in experimental parkinsonism.

There were no differences in monoamine content in the frontal cerebral cortex in the test and control rats. Previously, we showed that hyperactivation of CP structures is the pathophysiological basis of the MPTP-induced depressive syndrome [2]. The neurochemical data are consistent with the neuropathophysiological indications of specific role of CP structures in the pathogenesis of DA-deficiency-dependent depressive states.

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