Relationship between the Severity of Hypokinesia Induced by Neurotoxin 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine and Neurochemical Changes in Brain Structures of C57Bl/6 Mice

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The dynamics of hypokinesia in male C57Bl/6 mice induced by single administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine was studied on the model of parkinsonian syndrome. The neurochemical effect of this neurotoxin was evaluated at the peak of locomotor disorders. Severe hypokinesia was accompanied by an increase in serotonin content and decrease in the rate of serotonin biodegradation in the striatum, hippocampus, and frontal cortex. The content of dopamine metabolite 3,4-dihydroxyphenylacetic acid and dopamine turnover decreased in the striatum, but increased in the hippocampus and frontal cortex. Norepinephrine content decreased in the hypothalamus and cortex. Aspartate content decreased in the hypothalamus and hippocampus.

Key Words: *parkinsonian syndrome; 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; hypokinesia*

Dopamine deficiency and hyperactivation of cholinergic and glutamatergic neurons are the major pathogenetic stages of parkinsonian syndrome (PS) [2]. In rodents and primates, neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) causes behavioral disorders that are similar to symptoms of PS. MPTP-induced biochemical changes are manifested in a selective irreversible decrease in the contents of dopamine and its metabolites, dopamine depletion, and inactivation of tyrosine hydroxylase in the striatum, nucleus accumbens, and substantia nigra [6,10,11]. Hypokinesia (HYPK) is one of the main manifestations of MPTP-induced PS in rodents and primates [1,2]. Previous observations revealed that hyperactivity, stereotypic movements, and fine tremor develop immediately after injection of MPTP, HYPK, rigidity, and coarse tremor were observed 1-3 h postinjection [4]. The data indicate that these disorders (*e.g.*, HYPK) are reversible and disappear 5-7 days after neurotoxin treatment [4, 11,12]. Other studies showed that MPTP in high doses (25-30 mg/kg) causes a significant and persistent decrease in dopamine content in the brain of C57Bl/6 mice and primates (up to 4.5 months), which does not depend on the number of injections [4,8].

Here we studied the dynamics of HYPK in male C57Bl/6 mice 90 min, 24 h, and 7 days after intraperitoneal injection of MPTP in a single dose of 30 mg/kg. The neurochemical effect of this neurotoxin was evaluated at the greatest severity of locomotor disorders.

MATERIALS AND METHODS

Experiments were performed on 74 male C57Bl/6 mice weighing 18-20 g and obtained from the Stol-

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bovaya nursery (Russian Academy of Medical Sciences). The animals were maintained under standard conditions and natural light/dark cycle. Control mice (n=34) received intraperitoneal injection of physiological saline. MPTP (n=40) in a dose of 30 mg/kg was injected intraperitoneally to mice of the treatment group. The severity of HYPK was estimated from orientation and exploratory activity (open-field test) and locomotor behavior (Ugo basile actometer) of animals 90 min, 24 h, and 7 days after MPTP injection [1]. Orientation and exploratory activity was evaluated as the sum of horizontal and vertical movements and number of explored holes over 2 min. Locomotor activity of animals in actometer over 10 min was recorded.

The animals were decapitated 90 min after injection of physiological saline or MPTP. The samples were used for neurochemical study. The hippocampus, striatum, and frontal cortex were isolated on ice, weighted, and frozen in liquid nitrogen. The samples were minced in 20-fold volume of 0.1 M $HClO_4$ and 5 nmol/ml dihydroxybenzylamine (internal standard) using a Teflon-glass homogenizer. These samples were centrifuged at 10,000g for 10 min. The contents of dopamine, serotonin, norepinephrine, metabolites of dopamine (3,4-dihydroxyphenylacetic acid, DOPAC) and homovanillic acid, and serotonin metabolite (5-hydroxyindoleacetic acid, 5-HIAA) were measured by high-performance liquid chromatography (HPLC) with electrochemical detection on a LC-304T chromatograph (BAS, West Lafayette) equipped with a Phenomenex analytical column (C_{18}, 4×150 mm, 4 μ) [3]. The contents of aspartate, glutamate, glycine, and taurine were measured by HPLC with fluorescence detection [7].

The results of behavioral experiments were analyzed by nonparametric Mann—Whitney test. The data of neurochemical data were processed using Student's t test.

RESULTS

Locomotor activity of mice in the open filed and actometer was completely suppressed 90 min after MPTP injection (Table 1). Locomotor, orientation and exploratory behavior of animals decreased 24 h after neurotoxin injection (by 50-60% compared to the control). On day 7, no differences were found in the behavior of mice receiving MPTP and physiological saline. During this period, orientation and exploratory activity of MPTP-receiving mice was much higher compared to the control. Our findings illustrate the reversibility of MPTP-induced HYPK, which is consistent with published data [9,12].

Serotonin content significantly increased ($p \leq 0.05$), while the 5-HIAA/serotonin ratio decreased in the striatum, hippocampus, and frontal cortex of animals with the greatest severity of hypokinesia (90 min after MPTP injection). These changes reflect a decrease in the rate of serotonin biodegradation in all structures ($p \leq 0.05$; Fig. 1, *a*). At the initial stage of neurodegenerative processes, MPTP is metabolized into 1-methyl-4-phenylpyridine (MPP^+) under the influence of monoamine oxidase in serotoninergic neurons and astrocytes [2]. The increase in serotonin content and decrease in serotonin turnover are probably associated with the fact that MPTP metabolism involves not only the nigrostriatal system, but also other structures in the central nervous system.

Although dopamine concentration remained unchanged, opposite variations were found in the amount of DOPAC. The content of this dopamine metabolite decreased in the striatum, but increased in the frontal cortex and hippocampus ($p \leq 0.05$; Fig. 1, *b*). Similar changes were revealed in the DOPAC/dopamine ratio, which serves as the integral criterion for the rate of neurotransmitter turnover. Norepinephrine content in the hypothalamus and frontal cortex decreased by 23 ($p \leq 0.05$) and 38%

TABLE 1. Effect of MPTP (30 mg/kg Intraperitoneally) on Locomotor Activity and Orientation and Exploratory Behavior of C57Bl/6 Mice

Substance, time after injection	Locomotor activity, arb. units	Orientation and exploratory behavior, arb. units
Physiological saline (n=10)	45.2±8.8	78.0±16.5
MPTP, 90 min (n=10)	0.3±0.1**	6.5±1.3**
Physiological saline (n=6)	83.2±5.5	50.0±4.4
MPTP, 24 h (n=12)	36.2±6.9**	26.4±3.9**
Physiological saline (n=10)	65.2±3.6	25.3±3.4
MPTP, 7 days (n=10)	71.7±4.0	68.7±7.2**

Note. n, number of animals. *$p \leq 0.01$ and **$p \leq 0.001$ compared to the control.

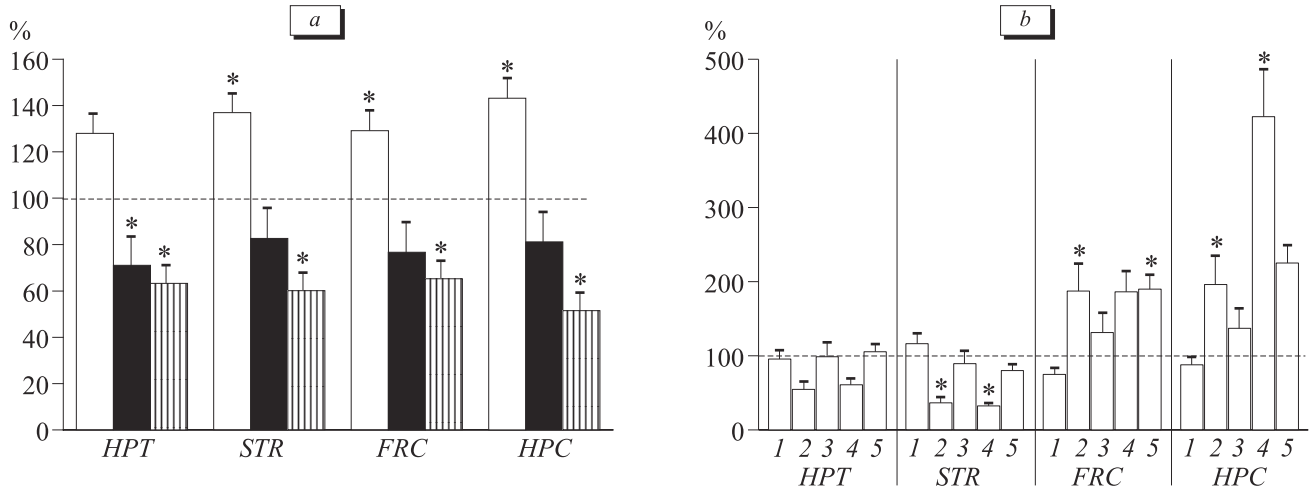

Fig. 1. Effect of single treatment with MPTP on the content and metabolism of serotonin (*a*) and dopamine (*b*) in brain structures of C57Bl/6 mice (90 min after neurotoxin injection). Ordinate: content of catecholamines and their metabolites (%). 100%, content of neurotransmitters in animals receiving physiological saline (0.9% NaCl, dotted line). HPT, hypothalamus; STR, striatum; FRC, frontal cortex; HPC, hippocampus. (*a*) Light bars, serotonin; dark bars, 5-HIAA; shaded bars, 5-HIAA/serotonin ratio. (*b*) Dopamine (*1*); DOPAC (*2*); homovanillic acid (*3*); DOPAC/dopamine ratio (*4*); homovanillic acid/dopamine ratio (*5*). *$p \leq 0.05$ compared to the control.

($p \leq 0.05$), respectively, 90 min after injection of MPTP. We studied the effect of this neurotoxin on the concentration of neurotransmitter amino acids. Aspartate content in the hypothalamus and hippocampus of treated mice decreased by 27 and 19%, respectively, compared to the control ($p \leq 0.05$). Hence, the greatest severity of locomotor disorders is associated with imbalance between monoamines and neurotransmitter amino acids. Reciprocal changes were observed in the content and turnover of dopamine metabolite DOPAC in brain structures. DOPAC content decreased in the striatum and tended to decrease in the hypothalamus. However, DOPAC content increased in the hippocampus and tended to increase in the frontal cortex.

Published data show that treatment with MPTP is followed by complex dynamic changes in the striatal dopaminergic system of C57Bl/6 mice [8]. Dopamine content increases 30-60 min after MPTP administration (by 20%), but decreases after 2.5 h (by 90%). DOPAC content decreases 30 min after MPTP administration and reaches the minimum by the 24th hour. The content of homovanillic acid 2-fold increased 2.5 h after neurotoxin injection, but decreased by the 24th hour. The decrease in functional activity of the striatal dopaminergic system is observed by the 24th hour and persists over 4.5 months after MPTP administration. Dopamine turnover (DOPAC+homovanillic acid) increased by 2 times 2.5 h after administration of this neurotoxin, 3.4-fold exceeds the control level by the 24th hour, and remains high over 4.5 months [8].

Previous experiments showed that death of dopaminergic cells in the substantia nigra is observed only several days after MPTP treatment [14]. The first signs for behavioral recovery in rodents are revealed 24 h after neurotoxin injection, which reflects the compensatory response to MPTP. These processes are not associated with changes in activity of D_1 and D_2 dopamine receptors in the striatum and substantia nigra, which contradicts published data [5]. Recent experiments showed that administration of MPTP is followed by an increase in the ratio of mitoses in the subgranular zone of the hippocampal dentate fascia and appearance of dopaminergic cells *de novo* in the substantia nigra of adult C57Bl/6 mice [13-15]. We showed that serotonin content increases 90 min after MPTP injection, which is consistent with published data. This neurotransmitter serves as a factor that stimulates neurogenesis. Locomotor activity of mice returned to normal 7 days after injection of MPTP. It cannot be excluded that neurodegeneration is accompanied by regenerative processes. Hence, MPTP-induced increase in dopamine turnover in the hippocampus requires further detailed investigations. These neurochemical changes probably serve as a neurochemical correlate for the induction of neurogenesis.

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