GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

Experimental Modeling of Preclinical and Clinical Stages of Parkinson's Disease

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Degeneration of dopaminergic (DAergic) neurons of the nigrostriatal system is the key stage in the pathogenesis of Parkinson's disease. The first symptoms of this disease are observed after degeneration of 70-80% neurons, which occurs over 20-30 years. The clinical stage of Parkinson's disease begins after this period. Late diagnostics of Parkinson's disease contributes to low efficiency of therapi for this disorder. Detailed study of the pathogenesis and development of preclinical diagnostic methods for Parkinson's disease are the urgent problems. This work was designed to develop a new experimental model of the preclinical and clinical stages of the disease. Experimental modeling was performed on C57Bl/6 mice using 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). This agent is converted into the MPP^+-neurotoxin in brain DAergic neurons. We showed that MPTP in a dose of 4 mg/kg has no effect on the nigrostriatal DAergic system. MPTP in a dose of 8-16 mg/kg produced the toxic effect only on DAergic axons, which simulates the preclinical stage of Parkinson's disease. MPTP in a dose of 20-40 mg/kg had the toxic effect on neuronal axons and bodies, which simulates the clinical stage of Parkinson's disease. The data suggest that progressive degeneration of DAergic neurons is accompanied by activation of compensatory mechanisms for functional deficiency of these cells.

Key Words: *brain; dopamine; substantia nigra; tyrosine hydroxylase; neurotoxin*

Parkinson's disease is one of the most serious and common chronic neurodegenerative disorders. This disease is typical of elderly people and manifested in locomotor disturbances [1]. Degeneration of dopaminergic (DAergic) neurons of the nigrostriatal system is the key stage in the pathogenesis of Parkinson's disease [2]. Over the first 20-30 years, the disease has no clinical manifestations (preclinical stage). The first symptoms of this disease are observed after degeneration of 70-80% DAergic neurons (clinical stage) [1]. Over the past 200 years after identification of Parkinson's disease, none of the patients recovered from this disorder. It is mainly associated with late diagnostics of Parkinson's disease.

Detailed study of the cellular and molecular pathogenetic mechanisms of Parkinson's disease is an urgent problem. Preclinical diagnostics should be based

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on the search for peripheral endogenous markers of Parkinson's disease. It is necessary to develop new approaches to the preventive treatment of Parkinson's disease reducing the rate of neuronal degeneration and activating the brain compensatory mechanisms. Clinical investigations and trials should be preceded by experimental modeling of the preclinical (asymptomatic) and clinical (symptomatic) stages of Parkinson's disease. This work was designed to solve this problem.

MATERIALS AND METHODS

Experimental modeling was performed on C57Bl/6 mice (22-26 g) using 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). MPTP is converted into 1-methyl-4-phenylpyridine (MPP^+, end product) in the brain. This compound is captured by DAergic neurons with the DA transporter and uncouples oxidative phosphorylation in mitochondria, which results in neuronal degeneration [9]. The effects of MPTP were evaluated from changes in locomotor behavior, DA metabolism in the substantia nigra (SN, containing neuronal bodies) and striatum (containing axon projections), and number of DAergic neurons in SN.

MPTP was injected subcutaneously in single doses of 4, 8, 16, 20, and 40 mg/kg. Control mice received physiological saline (0.9% NaCl). Locomotor activity of animals in the open-field test was studied using an Opto-Varimex-3 system (Columbus Instruments). The total length of the track, time of immobility, and number of vertical rearing postures were measured over 3 min [5]. The step length was estimated when the animal moved along a straight line [8].

The animals were decapitated 2 weeks after injection of MPTP or 0.9% NaCl. The brain was removed and cut (midsagittal plane). SN and striatum (bodies and axons of DAergic neurons) were dissected from the right part of the brain. The samples were weighted, frozen in liquid nitrogen, and stored at -70°C until further HPLC and electrochemical detection for monoamines and metabolites. The concentration or content of these substances was calculated (Multichrom software). The results were analyzed by Student's *t* test. The data are presented as a change in the content or concentration of study substance in animals of the treatment and control groups (100%, Fig. 1). The left part of the brain was fixed by immersion into 4% paraformaldehyde in 0.2 M phosphate buffer (pH 7.2-7.4) at 4°C for 12 h, washed with 0.9% NaCl in 0.02 M phosphate buffer (pH 7.2-7.4, phosphate buffered saline), incubated in 20% sucrose for 48 h, and frozen in hexane at -40°C. The frozen samples were stored at -70°C. Peroxidase immunolabeling of tyrosine hydroxylase (TH; first rate-limiting enzyme of DA synthesis) was performed routinely on serial sections of SN [3]. The samples were examined under an Olympus BX51 light microscope. For quantitative study of TH-immunopositive cells, AnalySIS 5.0 software (Olympus) was used.

The results were analyzed by *F* test (homogeneity of the sample) and Student's *t* test (significance of differences). Both tests were performed using Sigma Plot 9.1 and GraphPad Prism 5.0 software (GraphPad Software).

RESULTS

Administration of MPTP in a dose of 40 mg/kg caused death of 60% animals. Survivors were characterized by impairment of locomotor behavior (total length of track and step length, Table 1) and 80% decrease in DA concentration in the striatum (Fig. 1, *a*). Our results provide support to the concept that the 70-80% decrease in the concentration of DA in DAergic axons is accompanied by locomotor disturbances [1]. The number of DAergic neuron bodies in SN was reduced by 72% (Fig. 1, *c*). However, the concentration of DA decreased only by 45% (Fig. 1, *b*). The data reflect compensatory activation of DA synthesis in non-degenerated DAergic neurons, which indicates that the brain plasticity plays an important role in the pathogenesis of Parkinson's disease. The concentration of DA degradation products (dihydroxyphenylacetic and homovanillic acids) in the striatum and SN of MPTP-treated mice was lower than in control animals. However, the concentration of these substances decreased less significantly than that of DA. It is probably associated with a compensatory decrease in activity of catecholamine degradation enzymes (*e.g.*, monoamine oxidase B).

Surprisingly, the concentration of serotonin in the striatum was elevated by 60%, which attested to intensified synthesis and accumulation of serotonin in serotoninergic axons of the striatum. It is more obviously that serotoninergic and DAergic axons compete with each other for innervation of the target neurons in the striatum. Hence, the decrease in innervation of target neurons with DAergic fibers due to their degeneration may be accompanied by an increase in innervation of these targets with serotoninergic axons (growth of the newly formed serotoninergic axons or branching of the existing processes) [7]. The same competitive interactions between serotonin-immunopositive and TH-immunopositive axons were previously found in the suprachiasmatic nucleus of rats [10]. The animals survived after administration of MPTP in a dose of 20 mg/kg. Locomotor disorders in these mice were observed after 2 weeks. These disorders were revealed only in the most sensitive test for step length (Table 1). The lowest degree of locomotor disturbances is re-

lated to the fact that DA concentration in the striatum decreases by 69% (threshold level; Fig. 1, *a*). These changes in the concentration of DA correspond to the appearance of the first symptoms in humans [4]. The concentration of DA metabolites in the striatum decreased by 53 and 38% under the influence of MPTP in a dose of 20 mg/kg. The ratio of DAergic neurons and DA concentration in SN were reduced by 41% (Fig. 1, *b*, *c*). No changes in serotonin concentration were revealed in the striatum and SN. These data indicate that the early and late phases of the clinical stage of Parkinson's disease can be induced by administration of MPTP in doses of 20 and 40 mg/kg, respectively. It should be emphasized that the late phase of this disease is followed by the death of some patients.

All animals survived after administration of MPTP in a dose of 16 mg/kg. The absence of locomotor disorders under these conditions (Table 1) can be explained by minor decrease in DA concentration in the striatum (by 35%; Fig. 1, *a*). The degree of variations in DA concentration is 2-fold lower than the threshold level for the first symptoms of locomotor disorders.

TABLE 1. Step Length (cm) as a Measure of Locomotor Activity in Mice Two Weeks after Administration of 0.9% NaCl or MPTP in Various Doses (*M*±*m*)

Number of observation	MPTP dose, mg/kg	Control	Treatment
1	40	6.6±0.2	4.90±0.24*
2	20	6.2±0.1	4.39±0.20*
3	16	4.7±0.2	5.20±0.25
4	8	4.6±0.1	4.85±0.15
5	4	5.3±0.1	5.8±0.2

Note. The time of testing is 180 sec. *$p<0.05$ compared to the control.

The concentration of DA metabolites decreased in the striatum. However, the concentration of DA, DA metabolites, and serotonin in SN remained unchanged (Fig. 1, *b*). No changes were found in the number of TH-immunopositive neurons in SN (Fig. 1, *c*). Comparison of changes in the striatum and SN suggests

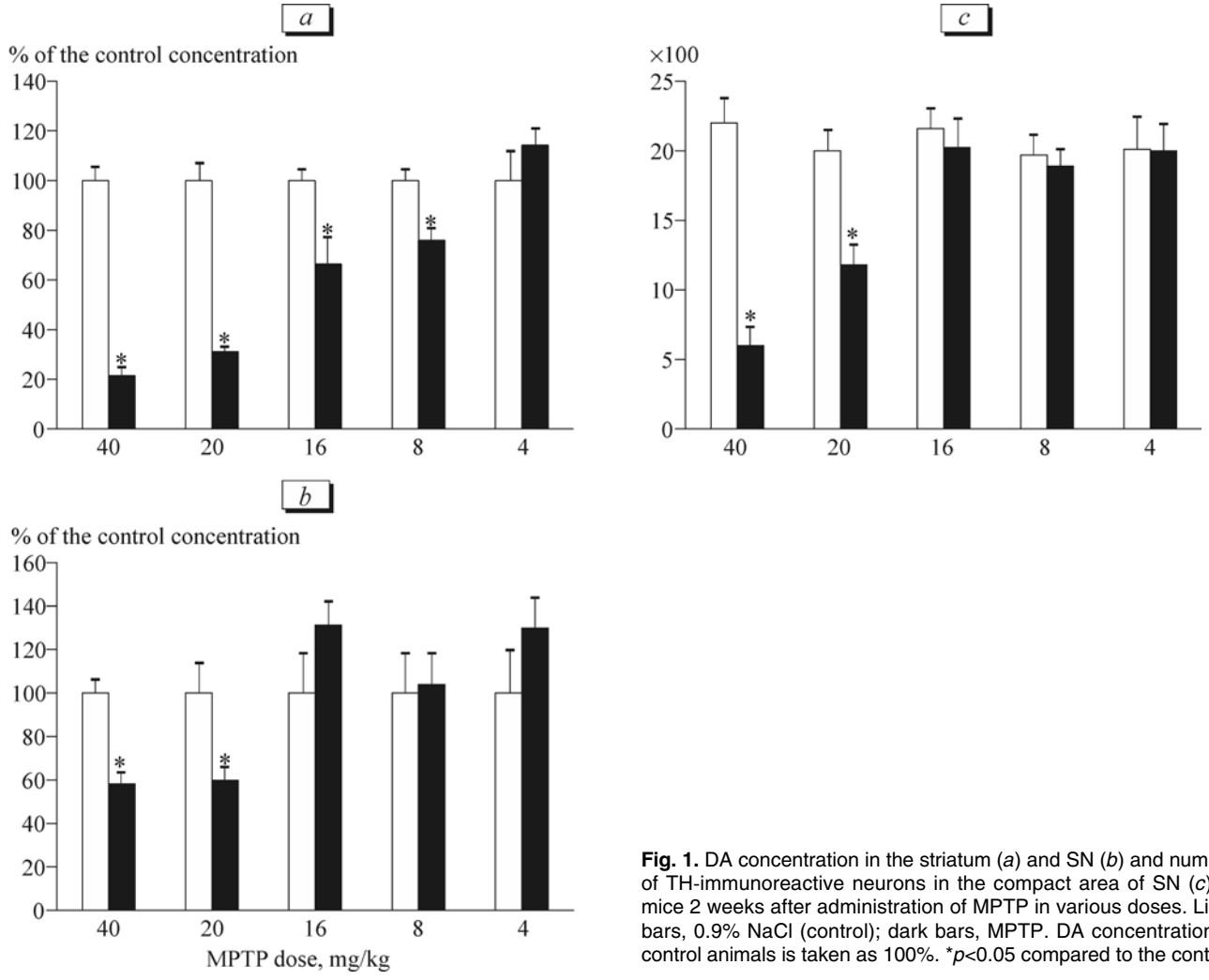

Fig. 1. DA concentration in the striatum (*a*) and SN (*b*) and number of TH-immunoreactive neurons in the compact area of SN (*c*) of mice 2 weeks after administration of MPTP in various doses. Light bars, 0.9% NaCl (control); dark bars, MPTP. DA concentration in control animals is taken as 100%. *$p<0.05$ compared to the control.

that 35% decrease in DA concentration in the striatum is associated with degeneration of DAergic axons. The neuronal bodies remain intact under these conditions. The selective toxic effect of MPTP on DAergic axons is probably related to a higher concentration of membrane DA transporter molecules in the axon terminals (as compared to that in neuronal bodies) [6].

In contrast to experiments with administration of MPTP in a dose of 16 mg/kg, changes in the nigrostriatal system after injection of 8 mg/kg MPTP were observed in only 10 of 30 animals. We revealed an insignificant decrease in the concentrations of DA, dihydroxyphenylacetic acid, and homovanillic acid in the striatum (by 24, 25, and 18%, respectively). The content of DA and DA metabolites did not change in SN (Fig. 1, *b*, *c*). It can be hypothesized that treatment with MPTP in doses of 8 and 16 mg/kg induces similar pathological processes, but the degree of abnormalities and number of affected animals are lower after administration of MPTP in a dose of 8 mg/kg. Locomotor disturbances are not found under these conditions. The observed changes correspond to the preclinical stage of Parkinson's disease.

Treatment with MPTP in a dose of 4 mg/kg did not induce locomotor disturbances (Table 1) and changes in the DAergic nigrostriatal system (Fig. 1). Therefore, MPTP in a dose of 4 mg/kg does not cause damage even to the most sensitive component of DAergic neurons.

We conclude that MPTP in a dose of 4 mg/kg does not produce the toxic effect on the nigrostriatal DAergic system. MPTP in a dose of 8-16 mg/kg produced toxic effect only on DAergic axons in the striatum, which simulates the preclinical stage of Parkinson's disease. MPTP in a dose of 20-40 mg/kg has the toxic effect on axons and bodies of DAergic neurons, which simulates the clinical stage of Parkinson's disease.

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