Effect of the Neurotoxin MPTP on Behavior and Cerebral Content of Neurotransmitters in Mice

T. N. Robakidze, S. E. Metkalova, I. I. Miroshnichenko, N. Yu. Kolgina, and Yu. V. Burov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 128, No. 11, pp. 532-534, November, 1999
Original article submitted January 14, 1999

We compared the effects of the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) on exploratory behavior and conditioning in C57Bl and outbred animals. Two intraperitoneal injections of MPTP suppressed the orienting and exploratory behavior in C57Bl (reductions of both horizontal and vertical motor activity), but not in outbred mice, and impaired conditioning. The data indicate the dopaminergic nature of learning disturbances and suggest that the MPTP model may hold a lot of promise for the search for new drugs for the treatment of cognitive disorders in Parkinsonism.

Key Words: *neurotoxin MPTP; conditioned reflex; dopamine; striatum*

The neurotoxin 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) produces clinical symptoms typical of Parkinsonism in humans and can be applied to model Parkinsonism in animals [3,5,9]. The common subjects of these studies are monkeys, since MPTP-induced Parkinsonism in primates is the best approximation of Parkinson's disease in humans [9]. Attempts to model Parkinsonism in mice and rats showed that rodents are less sensitive to the neurotoxin [8]. The toxic effect of MPTP with selective degeneration of dopaminergic neurons similar to that in primates was observed only in C57Bl mice [3,8]. This mouse strain, therefore, can be used to study different aspects of MPTP-induced Parkinsonism.

Mechanisms of MPTP neurotoxicity are attributed to its oxidation by astroglial monoamine oxidase to 1-methyl-4-phenylpyridine (MPP), which is taken up by the neuronal system of dopamine uptake, interacts with cell structures with the formation of toxic products inducing neuronal death and, as a result, a decrease in the striatal production of dopamine and its metabolites [1,5,6,10]. It was found, that chronic administration of low doses of MPTP to monkeys impaired their cognitive functions. However, the effects of MPTP on similar processes in rodents have been little studied.

The objective of this study was to investigate the effect of MPTP on open field behavior and avoidance conditioning in C57Bl mice and to assess MPTP-induced changes in the striatal content of dopamine, serotonin, and norepinephrine.

MATERIALS AND METHODS

Experiments were carried out on male C57Bl mice weighing 20-30 g. The animals were maintained under standard vivarium conditions (3 mice per cage) at a 12h:12h light:dark cycle with free access to food and water. MPTP-hydrochloride (Aldrich) was injected intraperitoneally for 2 consecutive days (daily dose — 30 mg/kg in 0.2 ml saline). Control animals received an equal volume of saline.

The effect of MPTP on orienting and exploratory activity (EA) was tested in the open field test. The animals were placed in the center of an illuminated box (100×100 cm) with the floor lined by equal segments, and the motor (the number of crossed segments) and exploratory (the number of rearings) activities were monitored for 5 min. These tests were performed 1, 3, 7, and 14 days postinjection.

Research Center for Safety of Bioactive Compounds, Staraya Kupavna, Moscow region

0007-4888/99/0011-1119$22.00

The effects of MPTP on learning and memory were studied using a step-down passive avoidance model [2]. A mouse was placed onto a wooden platform (4×4×4 cm) in the center of a plastic box (21×21×40 cm) with electrified floor illuminated with a 15 W bulb and monitored for 180 sec. Immediately after the mouse stepped down to the floor with all four paws, it received a 5 mA shock. Animals which did not descend from the platform within 15 sec were excluded from further experiments. Retention was tested 24 h after conditioning by measuring the step-down latency. MPTP-treated animals were tested 7 and 14 days postinjection. Behavioral experiments were conducted at the same time interval.

To determine the content of monoamine neurotransmitters, the animals were decapitated, the brain was quickly removed on ice, the striatum was isolated and homogenized in 0.1 N $HClO_4$ with 3,4-ioxibenzylamine (internal standard). The samples were centrifuged at 10,000*g* for 10 min, the supernatant was filtered through 0.2-μ cellulose filters by 2-min centrifugation. Filtrate aliquots were applied to the chromatographic column with electrochemical detection to measure the content of catechol- and indolamines [7]. Noradrenaline, dopamine, DOPAC, 5-hydroxytryptamine, 5-hydroxyindolacetic acid (5-HIAA) were determined with ion-pair reverse phase high-performance liquid chromatography on "Spherisorb ODS" 250×4.6 mm columns. Monoamines were detected with a LC-4A (BAS) electrochemical detector with active graphite and Ag/AgCl reference electrodes with 0.65 V between them. Citrate-phosphate buffer (0.04 M, pH=3.5) containing 0.2 units EDTA was used as a mobile phase, and sodium octylsulfate served as an ion-pair agent. The content of monoamines was measured on days 4, 8, and 15 postinjection.

RESULTS

Horizontal and vertical components of EA in C57Bl mice were significantly inhibited 1 and 3 days postinjection, which indicates that MPTP suppressed the innate behavior (Table 1). Orienting and exploratory behavior returned to normal on day 7 postinjection. No changes in EA activity was observed in MPTP-treated outbred mice.

In outbred mice, two doses (30 mg/kg each) of intraperitoneal MPTP caused no changes in passive avoidance conditioning (Table 2): the step-down latency in the treated animals did not differ from the con-

TABLE 1. Effect of MPTP on Exploratory Motor Activity in C57Bl Mice in Open Field Test (*M*±*m*)

Mice	Time after MPTP administration, days	Number of	
		crossed segments	rearings
Outbred	Control (intact, *n*=15)	105.3±3.8	39.4±4.5
	3 (*n*=12)	100.7±5.4	38.8±3.1
	7 (*n*=12)	101.9±4.3	36.2±5.8
	14 (*n*=12)	104.9±8.6	38.1±7.2
C57Bl	Control (intact, *n*=80)	99.4±6.7	35.8±3.0
	1 (*n*=16)	52.1±6.9*	15.8±2.5*
	3 (*n*=6)	73.8±9.9*	14.2±4.5*
	7 (*n*=14)	84.6±3.4	24.4±2.8
	14 (*n*=22)	95.7±7.0	25.2±2.2

Note. Here and in Tables 2 and 3: *p<0.05 in comparison with the control.

TABLE 2. Effect of MPTP on Acquisition of Step-Down Passive Avoidance Conditioned Response in Outbred and C57Bl Mice (*M*±*m*)

Mice	Step-down latency, sec		
	before MPTP	after MPTP, days	
		8	15
Outbred	70.43±9.81 (*n*=12)	65.32±7.13 (*n*=12)	67.41±4.67 (*n*=12)
C57Bl	55.43±8.14 (*n*=69)	31.20±6.60* (*n*=56)	36.68±12.99 (*n*=22)

TABLE 3. Effect of MPTP on the Content of Monoamines and Their Metabolites in the Striatum of C57Bl Mice (*M±m*)

Time after MPTP, days	Concentration of monoamine, ng/g tissue				
	noradrenaline	dopamine	DOPAC	5-HIAA	serotonin
Control (intact, *n*=69)	0.19±0.02	6.70±0.65	0.42±0.03	0.32±0.05	0.36±0.05
4 (*n*=20)	0.43±0.12*	1.64±0.24*	0.14±0.01*	0.26±0.05	0.27±0.06
8 (*n*=56)	0.34±0.08*	1.32±0.18*	0.14±0.02*	0.28±0.03	0.36±0.04
15 (*n*=22)	0.17±0.01	2.23±0.38*,+	0.19±0.04*,+	0.23±0.02	0.28±0.02

Note. *$p<0.05$ in comparison with respective values on day 8.

trol, while in C57Bl mice the latency of step-down reaction became significantly shorter (by 44 %) on day 8 postinjection and tended to normal after 15 days.

The effects of MPTP on the neurochemical indices of the striatal dopaminergic, serotoninergic, and adrenergic systems are presented in Table 3. A significant decrease in the concentration of dopamine and its metabolite DOPAC in the striatum of C57Bl mice was observed on days 4, 8, and 15 postinjection. After 15 days the content of dopamine 1.7-fold surpassed its value on day 8, which probably indicates the start of recovery. The content of noradrenaline, serotonin and its metabolite 5-HIAA was unchanged. Our results confirm published data [3,8] showing that the dopamine system of C57Bl mice is sensitive to MPTP in contrast to that of outbred animals [8]. It can be suggested, therefore, that MPTP-induced behavioral changes in C57Bl mice result from dopaminergic deficit.

In conclusion, this study showed that MPTP injected in two doses into C57Bl mice suppressed their orienting and exploratory motor activity, decreasing both the horizontal and vertical components.

Impaired learning and memory in the step-down passive avoidance task were observed only in C57Bl mice. The most pronounced impairment of learning observed on day 7 postinjection was accompanied by a dramatic and selective decrease in the striatal content of dopamine and DOPAC.

These data indicate that MPTP-induced impairment of conditioning in C57Bl mice is associated with dopaminergic dysfunction and suggest that this model of pathology is promising for the search of new drug for the treatment of cognitive disorders in Parkinsonism.

REFERENCES

1. Yu. V. Burov, A. A. Trapkova, V. B. Malakhova, *et al.*, *Byull. Eksp. Biol. Med.*, **114**, No. 11, 492-494 (1992).
2. J. Buresh, O. Bureshova, and J. Huston, *Techniques and Basic Experiments for the Study of Brain and Behavior*, Amsterdam-New York (1983).
3. M. Ya. Voloshin, *Neirofiziologiya*, No. 3, 410-414 (1990).
4. R. Barker, *Neuropeptides*, **20**, 73-78 (1991).
5. N. J. G. Barnes, A. J. Bradbery, B. Costall, *et al.*, *J. Neural Transm. Suppl.*, No. 20, 47-63 (1986).
6. R. W. Fuller and S. K. Hemrick-Lueske, *J. Pharm. Pharmacol.*, **41**, No. 7, 493-495 (1989).
7. R. J. Jukhanov, T. M. Tennila, I. I. Miroshnichenko, *et al.*, *Pharmacol. Biochem. Behav.*, **43**, No. 3, 683-687 (1992).
8. E. Sundstrom, J. Stromberg, L. Olson, *et al.*, *Brain Res.*, 26-38 (1987).
9. J. W. Tetrud and J. W. Langston, *Acta Neurol. Scand.*, 35-40 (1989).
10. G. Yoshio, M. Ioshimi, A. Nobutaca, and M. Kazzuaki, *Jpn. J. Pharmacol.*, **55**, No. 1, 93-100 (1991).