CLINICAL AND BIOCHEMICAL PARAMETERS OF PARKINSONISM INDUCED BY 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE AND ITS METHYL-PHENYL AND METHOXY-PHENYL DERIVATIVES IN C57B1/6 MICE

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The compound 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induces a combination of clinical, biochemical, and pathological disturbances characteristic of parkinsonism in man and animals [8, 11]. The development of parkinsonism is linked with oxidation of MPTP in vivo to 1-methyl-4-phenylpyridine (MPP^+), and the accumulation of this ion in dopamine neurons of the substantia nigra of the brain, leading to their degeneration [7, 11]. Various derivatives, which can be regarded as analogs of MPTP, are found in products and by-products of the chemical industry, and the development of laboratory methods of assessment of the MPTP-like action of pyridine compounds and determination of the criteria of their potential toxicity is therefore an urgent task at the present time. A method of testing substances for MPTP-like activity on the basis of their ability to induce a long-term decrease in the dopamine (DA) concentration in the brain of C57B1/6 mice [3, 10], was suggested previously. The introduction of substituents into the tetrahydropyridine fragment of the MPTP molecule, lengthening of the carbon chain attached to this atom, and replacement of molecule, lengthening of the carbon chain attached to this atom, and replacement of the phenyl ring by a pyridine ring, lead to loss of parkinsonogenic properties [3, 10].

The aim of this investigation was to study the ability of certain new synthetic MPTP analogs with methyl and methoxy-groups in the phenyl ring to induce parkinsonism in C57B1/6 mice, and also to study the possibility of predicting parkinsonogenic properties of compounds on the basis of their action on spontaneous behavior of mice in the first 3 h after injection.

EXPERIMENTAL METHOD

Female C57B1/6 mice weighing 25-30 g, obtained from the "Stolbovaya" nursery, were used. The compounds (Table 1) I-VI were synthesized in the chemical faculty of Moscow University [1], and substances VII and VIII were synthesized at the Institute of Physiologically Active Substances, Academy of Sciences of the USSR, and characterized by nuclear magnetic resonance and elementary analysis. All the substances were used in the form of hydrochlorides, with the exception of VII (iodide). To determine the parkinsonogenic properties of the compounds they wre injected intraperitoneally in 0.1 ml of physiological saline, twice on alternate days, in doses close to LD_{50}; control animals received an injection of the solvent. The experimental and control animals were divided into two groups, one of which was sacrificed after 5-7 days, after which the concentrations of DA, noradrenalin (NA), and serotonin (5-HT) in the brain were determined by a spectrofluorometric method [2]. The significance of changes in the content of the mediators relative to the control was estimated by Student's test. In another group of animals, the development of symptoms was monitored for 2 months. LD_{50} was determined on male C57B1/6 mice weighing 20-22 g in the course of 24 h after intraperitoneal injections [4]. The animals' motor activity was assessed in the open field test, on the basis of the number of squares crossed during 5 min of observation.

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TABLE 1. Effect of MPTP, MPP^+, and Their Analogs on Brain Levels of Monoamines in Mice 5-7 Days after Injection (M ± m)

No. of compound	Compound	Dose, mg/kg	Per centof control		
			DA	NA	5-HT
I	R_1 – unsubstituted (MPTP)	30×2	44±17*	93±13	100±14
		15×2	72±9*	92±10	93±14
II	R_1 – ortho-CH_3	15×2	37±9*	110±11	96±12
III	R_1 – para-OCH_3	50×2	102±9	120±13	83±14
		15×2	109±20	108±7	107±13
IV	R_1 – para-CH_3	90×2	93±11	100±3	95±5
		15×2	104±7	104±8	109±10
V	R_1 – ortho-OCH_3	40×2	60±12*	108±7	120±18
		15×2	113±21	100±21	96±7
VI	R_1 – para-OCH_3	90×2	94±13	88±4	98±9
VII	R_2 – CH_3 R_3 –unsubstituted (MPP^+I^-)	19×2	98±13	97±11	99±15
VIII	R_2 – unsubstituted R_3 – unsubstituted $R_{3'}$ – ortho-CH_3	100×2	107±10	93±5	108±12

Legend. All data shown as mean value for 2-3 experiments, with 6-10 animals in each experiment; *$p < 0.05$ compared with control.

Only those cases of hyperactivity and of stereotypy, in which activity of the experimental animals was 1.5 times or more greater than that of the controls, are illustrated in Table 2. Hypokinesia was assessed in points: 4) weak response to touch, 3) makes 2-4 steps, 2) activity in the open field test half or less than half of the control level, and 1) activity in the open field test 1.5 times less than in the controls. Rigidity was determined by comparing the muscle tone of the hind limbs during passive flexion and extension of the experimental and control animals. Rigidity and tremor were assessed by the number of animals with the given symptoms, as a percentage of their total number in the experiments.

EXPERIMENTAL RESULTS

The o-methylphenyl (II) and o-methoxyphenyl (V) derivatives of MPTP can depress the brain DA concentration for a long time (Table 1); compound II caused the maximal reduction of DA, and in a dose less than that of the other substances. In response to injection of the other substances in a dose corresponding to that which was active for II (15 × 2 mg/kg) reduction of DA was observed only in the case of MPTP, and it was dose-dependent in character. The animals 5-7 days after the injections had an outwardly normal state, despite reduction of their brain DA concentration to 37-44%. The specific response characteristic of MPTP consisted of hypokinesia (2-3 points), rigidity, and tremor of the anterior part of the trunk and head, was observed on the 20th-27th days after injection of substance II (in three of eight animals) and substance V (in two of nine mice). The mice lost weight, had the characteristic lordotic posture, and an uncared for appearance. Determination of concentrations of biogenic amines in these groups during general depression of the brain DA level showed no significant differences in animals with and without symptoms. The late appearance of clinical features of parkinsonism was evidently connected not only with depression of DA, but also, possibly, with the development of degenerative processes in the nervous system. All MPTP analogs possessing parkinsonogenic properties had a higher acute toxicity (Table 2). Immediately after injections of MPTP, various behavioral symptoms were noted, indicating action on different neurochemical mediator systems in both central and peripheral regions of the nervous system [6]. We distinguished the basic symptoms and examined their development with time (Table 2). For MPTP and other parkinsonogenic substances (II and V), the majority of animals were characterized during the first 30 min by hyperactivity, by a frequenct, low-amplitude tremor, and by stereotypy in the form of sniffing movements. Substance IV showed similar symptoms, but the transition from hyperactivity to hypokinesia took place quickly, in the course of 10-15 min. For further verification substance IV was injected in different doses (15 × 3, 15 × 5, 60 × 2, and 75 × 2 mg/kg), but no decrease in the DA concentration was observed after 5-7 days in any of the animals.

Changes in the symptomatic picture with time reflected mainly metabolism of the phenyltetrahydropyridines and the redistribution of their metabolites in the tissues. The principal metabolic pathway of MPTP in the brain is oxidation by monoamine oxidase (MAO) followed by inhibition of the enzyme by the reaction products [11]. In the presence of methyl substituents in the phenyl fragment of the MPTP molecule, its ability to bind with MAO and for activity of the enzyme to be inhibited by reaction products still continued, but these pro-

TABLE 2. Comparative Action of MPTP and Its Analogs on Spontaneous Behavior of C57Bl/6 Mice during First 3 h (M ± m)

No. of compound	LD_{50}, mg/kg	time after injection, h	Number of animals with symptoms, % of total number in experiment: hyperactivity	low-amplitude tremor	stereotypy (sniffing)	hypokinesia	tremor	rigidity
I	42±11	0—0,5	100	80	100	—	—	—
		1—3	—	—	—	100(4)	80	75
II	28±6,5	0—0,5	85	20	55	—	—	—
		1—3	—	—	—	85(3)	25	35
III	72±10	0—0,5	—	75	—	100(1)	—	—
		1—3	—	—	—	80(1)	15	—
IV	117±21	0—0,5	100	85	100	75(1)	—	—
		1—3	—	—	—	85(3)	15	35
V	50±12	0—0,5	90	35	100	—	—	—
		1—3	—	—	—	75(2)	25	55
VI	102±33	0—0,5	—	95	—	95(1)	—	—
		1—3	—	—	—	85(2)	35	—
VII	29±8,5	0—0,5	—	15	65*	—	—	—
		1—3	—	—	55	90(4)	65	30
VIII	126±26	0—0,5	—	75	—	100(2)	—	—
		1—3	—	—	—	89(1)	—	—

Legend. Results given as mean value from two experiments with 10 mice in each experiment; asterisk indicates stereotypy in the form of grooming movements. Point rating shown in parentheses.

cesses differed from MTP in their kinetic characteristics [1]. For instance, in a series of tolyl homologs of MPTP (II-IV) the p-tolyl analog (IV) was oxidized most slowly by MAO, whereas its biotransformation product 2,3-dihydropyridinium, inhibited the enzyme least of all, evidently reflecting the short-term character of hyperactivity. Hypokinesia, rigidity, and tremor, appearing 1-3 h after injection of MPTP, are linked with accumulation of the oxidation product of MPTP — MPP^+ — in the striatum and substantia nigra [7, 11]; these symptoms were intensified by the action of MPP^+ on the peripheral zones of the nervous system [5]. In response to injections of MPP^+ itself, according to data in the literature, virtually none penetrates into the brain [9]. However, we observed central effects of MPP^+, of which the most marked in the first hour was stereotype (in 65% of animals the number of grooming movements during 5 min was 1.5-4 times greater than in the control). Symptoms after 2-3 h were similar to those of MPTP, but MPP^+ did not induce any long-term fall of the DA level. Since of the fall of DA under the influence of MPTP is does-dependent in character, the MPP^+ penetrating into the brain was evidently insufficient to exhibit the effect. The formation of metabolites of different phenyltetrahydropyridines may take place in different brain cells, for it has been shown that MPP^+ is formed from MPTP during oxidation predominantly by type B MAO in cells of the astroglia [11], and substance IV, unlike other methylphenyl tetrahydropyridines, is oxidized in high concentrations mainly by type A MAO [1], which probably leads to a different distribution of metabolites among the cells than in the case of MPTP. For substances III, IV, VI, and VII, besides the symptoms mentioned in Table 2, the most characteristic features were abduction of the hind limbs, Strabu's sign, and shaking of the head, i.e, symptoms of activation of the serotonin- and noradrenergic systems. The experiments thus showed that ortho-methyl-phenyl and ortho-methoxy-phenyl derivatives of MPTP have the ability to induce a long-term fall of the DA level in the brain and clinical symptoms of parkinsonism in C57Bl/6 mice. All the compounds exhibiting parkinsonogenic properties, immediately after injections were characterized by hyperactivity, stereotype, and low-amplitude tremor, giving way after 1-3 h to a different group of symptoms: hypokinesia, rigidity, and large-amplitude tremor. This symptomatic picture may evidently serve as the basis for a preliminary assessment of the poential parkinsonogenic properties of the phenylpyridines.

LITERATURE CITED

1. S. O. Bachurin, S. O. Sablin, G. V. Grishina, et al., Bioorg. Khim., 15, No. 5, 620 (1989).
2. B. M. Kogan and N. V. Nechaev, Lab. Delo, No. 5, 301 (1979).
3. N. N. Lermontova, L. S. Solyakov, S. O. Bachurin, et al., Byull. Éksp. Biol. Med., No. 6, 699 (1989).

4. V. B. Prozorovskii, M. P. Prozorovskaya, and V. M. Demchenko, Farmakol. Toksikol., No. 4, 479 (1978).
5. R. W. Fuller and S. K. Hemrick-Luecke, Life Sci., 39, 1645 (1986).
6. R. E. Heikkila, A. Hess, and R. C. Duvoisin, Science, 224, 1451 (1984).
7. I. J. Kopin, S. R. Burns, C. C. Chiueh, and S. P. Markey, Alzheimer's and Parkinson's Diseases, New York and London (1985), p. 519.
8. J. W. Langston, P. Ballard, J. W. Tetrud, and I. Irwin, Science, 210, 979 (1983).
9. T. L. Perry, V. W. Yong, K. Jones, et al., Neurosci. Lett., 58, 321 (1985).
10. T. L. Perry, V. W. Yong, R. A. Wall, and K. Jones, Neurosci. Lett., 69, 285 (1986).
11. T. P. Singer, N. Castagnoli, R. R. Ramsay, and A. I. Trevor, J. Neurochem., 49, 1 (1987).

EFFECT OF DALARGIN ON CELL MULTIPLICATION IN THE GASTRIC EPITHELIUM DURING STRESS

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Dalargin, a synthetic Leu-enkephalin analog, is regarded as an effective and promising drug for the treatment of peptic ulcer [8]. The ability of dalargin to normalize the microcirculation [2, 13] and its antistressor [3] and cytoprotective [7, 9] action constitute the grounds for widening the indications for its use.

Dalargin stimulates proliferative processes in epithelial tissues [10, 11]. It has also been found to prevent the formation of a structural trace of disadaptation during stress; administration of dalargin reduced the level of pathological mitoses induced by stress, normalized DNA synthesis, and delayed vertical migration of cells in the corneal epithelium [12].

It was decided to study the effect of dalargin on proliferative processes in the gastric epithelium during stress, for disturbances of structural homeostasis in the gastric mucosa and ulcer formation are an indication of the severity of stress. An essential role in the pathogenesis of structural disorders under the influence of extremal factors is played by activation of peroxidation [1, 6], and it was therefore decided to study the effect of dalargin on malonic dialdehyde (MDH) accumulation in gastric tissue.

In the modern view, injury to the gastric mucosa takes place against the background of exhaustion of endogenous catecholamine reserves. Hence the need to investigate the effect of dalargin on the noradrenalin concentration in gastric tissue.

EXPERIMENTAL METHOD

Experiments were carried out on male rats weighing 150-180 g. The animals were divided into three groups: 1) intact control, 2) rats subjected to immobilization for 4 h, and 3) a group which received dalargin intramuscularly in a dose of 10 μg/kg 40 min before fixation. The animals were fixed in a special frames from 6 a.m. until 10 a.m., by the method described previously [12]. Proliferative processes and biochemical parameters were studied during the first hour after the end of immobilization, and again after 12 and 24 h. The animals were sacrificed 40 min after receiving an injection of ^{3}H-thymidine in a dose of 0.6 μCi/g body weight, with specific radioactivity of 87 Ci/mmole. Autoradiographs were prepared by the method described previously [4]. Proliferative changes in the pyloric division of the stomach were judged from the size of the ^{3}H-thymidine-labeled nuclei (ILN, %) and the

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