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ABILITY OF 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE AND SOME OTHER PYRIDINE DERIVATIVES TO CAUSE PARKINSONISM

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The compound 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) gives rise to clinical features of parkinsonism (akinesia, rigidity, tremor) and also to lowering of the dopamine (DA) level in the brain and to degeneration of neurons of the substantia nigra (SN), characteristic of this disease, in man and monkeys [6, 10]. The mechanism of action of MPTP is linked with a number of consecutive processes: oxidation to 1-methyl-4-phenylpyridinium (MPP^+) under the influence of monoamine oxidase, uptake of MPP^+ by the retrograde transport system of DA into neurons, binding with neuromelanin, and inhibition of various intracellular processes, including oxidative phosphorylation in mitrochondria [12].

The widespread distribution of various pyridine derivatives, which can be regarded as structural analogs of MPTP and MPP^+, as waste and end products of the chemical industry raises the question of a possible link between the prevalence of idiopathic parkinsonism and the use of compounds of this sort in practice. We know [5] that the intensity of application of the herbicide paraquat (1,1-dimethyl-4,4'-dipyridinium dichloride) correlates with the prevalence of parkinsonism in some provinces of Canada.

The development of laboratory methods of assessment of the MPTP-like action of various chemical compounds and testing of some known and newly synthesized pyridine and dipyridyl derivatives, which are potential pesticides, for these properties are thus matters of urgency.

EXPERIMENTAL METHOD

Male C57BL/6 mice weighing 22-25 g were used at different times of the year. The substances for testing, in doses close in acute toxicity to LD_{50}, were injected intraperitoneally in 0.1 ml of physiological saline daily. Control animals received the same volume of physiological saline. Some of the animals were left for 2 months to allow the development of a behavioral syndrome to be observed. Animals of the other group were killed a few days after the injection by cervical dislocation, the brain was removed, and concentration of DA, noradrenalin (NA), and serotonin (5-HT) were determined spectrofluorometrically [1]. The significance of the measurements of the DA, NA, and 5-HT concentrations was determined by Student's test. Pyridine derivatives were synthetized in the writers' institute and their

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TABLE 1. Effect of Schedule of MPTP Administration on Brain Dopamine and Noradrenalin Levels in C57BL/6 Mice (M ± m)

Dose, mg/kg	Number of days after injections	Percent of control	
		DA	NA
30	4	51±9*	92±20
30×2 at interval of 3 h	5	44±19*	87±3
25×3 at intervals of 6 h	5	60±8*	90±19
30×3 daily	7	49±13*	78±12
30×5 daily	7	51±3*	74±23
30×2 daily	10	45±20*	94±13
30×3 daily	76	56±21*	90±14

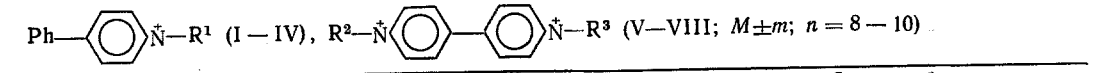
Legend. *p < 0.05 compared with control.

structure and correspondence to the formula were demonstrated chromatographically and by NMR spectra. Substance I (1,1-dimethyl-4,4'-dipyridinium dichloride) was obtained from "Sigma" (USA).

EXPERIMENTAL RESULTS

It has frequently been demonstrated that the basic features of parkinsonism, named the behavioral syndrome, the marked and prolonged (for several months) lowering of the brain DA level, and degeneration of SN neurons, under the influence of MPTP, are most marked in monkeys and in C57BL/6 mice [6, 7, 9, 13]. In order to evaluate the MPTP-like action of the pyridine derivatives, we therefore used C57BL/6 mice, and the basic indicator of the potential activity of the compound was taken to be lowering of the brain DA level of the animals (Tables 1 and 2). Since MPTP possesses considerable acute toxicity (30-50 mg/kg), possibly unconnected with its action on catecholaminergic neurons, in order to obtain experimental parkinsonism the cumulative effect of several doses is used [7, 9, 11]. We tested schedules of MPTP administration described in the literature (Table 1) and showed that, virtually independently of the number of near-toxic doses (with respect to acute toxicity), MPTP induced a considerable and prolonged (up to 76 days) fall of the brain DA con-

TABLE 2. Effect of Preparations of MPTP and Some Pyridine Derivatives on Brain Monoamine Levels in C57BL/6 Mice



Compound	Substance	Dose, mg/kg	Days of injection	Percent of control		
				DA	NA	5-HT
	MPTP	30×2	10	45±20**	94±12	91±8
		30×2	5	44±16**	93±17	109±11
I	R^1:-CH_3	10×2	7	115±21	—	99±20
	MPP	15×2*	8	111±16	97±7	89±11
		19×2*	7	98±11	89±13	83±4
II	R^1:-C_2H_5	18	5	104±26	100±7	90±8
		18×2	6	115±25	100±15	89±11
		20×2	6	100±14	83±14	94±11
III	R^1:-CH_2Ph	10	4	105±23	112±10	112±11
		10×2	4	112±20	105±21	94±13
		10×2	7	99±19	94±9	99±24
IV	R^1-H	80×2	4	125±17	98±7	114±6
		80×2	5	109±13	100±3	96±8
V	R^2:-H	100×2	3	103±6	92±12	107±11
	R^3:-H	100×2	5	98±4	98±4	97±7
VI	R^2:-CH_3	100×2	5	98±7	106±7	109±14
	R^3:-H	100×2	7	91±7	98±5	85±15
VII	R^2:-CH_3	6×2	2	92±18	—	97±13
	R^3:-CH_3	6×3	7	82±22	105±14	106±17
		20×2	5	105±12	111±5	105±20
VIII	R^2:-CH_2Ph	6	4	100±22	96±13	—
	R^3:-CH_2Ph	4×2	5	99±10	103±13	99±9

Legend. *) Injections every other day, **) significant differences from control.

centration; the small decrease in NA concentration observed under these circumstances was not statistically significant. Evident behavioral symptoms of parkinsonism were observed in groups of animals left for 20-28 days after receiving an injection of MPTP, in good agreement with data in the literature [7, 9, 11, 13]. On the basis of our own data showing that the fall in the brain DA level is independent of the number of near-toxic doses of MPTP, when studying the MPTP-like action of pyridine derivatives we limited ourselves to two or three injections of the test substances in doses close to LD_{50} for acute toxicity (Table 2). The well-known foreign herbicides paraquat (compound VII) and cyperquat (compound I) and their analogs (compounds II-IV, V-VIII) had no MPTP-like action on the brain DA level of the mice. It was shown previously that the absence of such an effect following intraperitoneal injection of MPP^+ is due to the difficulty with which it passes through the blood-brain barrier (BBB) [11]. The absence of an MPTP-like effect of paraquat and of substance VI can be explained similarly (Table 2). Injections of paraquat into frogs lead to lowering of brain DA concentration and to the development of symptoms of parkinsonism [4]. In our own experiments neither a dose close to LD_{50} (20 mg/kg) nor the minimal dose (4-6 mg/kg), inducing neurological symptoms (hyperactivity, tremor of the hind limbs) immediately after injection, had any effect on DA, NA, and 5-HT levels. The remaining compounds possess much greater hydrophobicity and can pass through the BBB. Contradictory data can be found in the literature on the MPTP-like action of 4-phenylpyridine (Table 2, compound IV), which can pass through the BBB and then by methylated through the action of the brain N-methyltransferase [2, 8]. Biotransformations of this kind can also be undergone by other N-unsubstituted MPP^+ analogs. The high toxicity of benzylviologen (Table 2, compound VIII), a hydrophobic analog of paraquat, must be noted. In the doses indicated in Table 2, benzylviologen caused hypokinesia and tremor on the 3rd-4th day after injection, but did not affect the DA, NA, and 5-HT levels. The action of benzylviologen may perhaps extend to other mediator systems [3] or it may be the result of lesions of the kidneys, liver, or lungs characteristic of paraquat and its analogs. In the animals left for 2 months after receiving the test compounds in the doses indicated in Table 2, no evident behavioral symptoms of parkinsonism were observed.

None of the 4-phenylpyridine and 4,4'-dipyridyl derivatives tested could include an MPTP-like effect on C57BL/6 mice. The immediate cause of this could be either their difficulty in passing through BBB in the case of polar compounds or certain other factors responsible for the action of MPTP and, in particular, the low affinity of interactions with the DA re-uptake system [3].

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