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# EFFECT OF MPTP ON NEURONAL UPTAKE OF MONOAMINES

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**KEY WORDS:** MPTP; uptake; monoamines; synaptosomes

The substance 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) causes the development of clinical symptoms characteristic of parkinsonism in man and is used to create a model of experimental parkinsonism in monkeys and certain other laboratory animals [1, 6, 8, 9, 11]. The mechanism of the toxic effect of MPTP, leading to degeneration of dopamine-synthesizing neurons of the substantia nigra of the brain, is considered to be the formation of 1-methyl-4-phenylpyridine (MPP) from MPTP in the astroglia under the influence of monoamine oxidase. MPP, it is considered, is taken up by the neuronal dopamine uptake system and interacts with components of the cell to form toxic substances, which cause death of the neurons and, as a result, reduce production of dopamine and its metabolites in the striatum [5, 7, 10]. However, injection of L-dopa, which raises the dopamine level in the striatum, does not affect the development of symptoms of parkinsonism and manifestation of the neurotoxicity of MPTP [13]. Considering that the dopamine transport system is closely interconnected with other systems of monoamine transfer of high and low affinity [2-4], the aim of the present investigation was to study the effect of MPTP on the systems for synaptosomal reuptake of dopamine, noradrenalin, adrenalin, and serotonin.

## EXPERIMENTAL METHOD

Uptake of monoamines was determined by a modified method of Snyder and Coyle [14]. The coarse synaptosomal fraction was obtained by centrifugation of a 10% brain homogenate from male albino rats weighing 180-200 g, in 0.32 M sucrose at 1000*g* for 20 min. The supernatant was recentrifuged at 11,000*g* for 15 min. The residues containing synaptosomes, mitochondria, and myelin were resuspended in 0.32 M sucrose. For the experiments 50 $\mu$l of a suspension of synaptosomes was taken (on average 0.3 mg protein) and this was added to 1 ml of an incubation medium of the following composition: 100 mM NaCl, 6 mM KCl, 2 mM $CaCl_2$, 1.14 mM $MgCl_2$, 5 mM $Na_2PO_4$, 10 mM glucose, 10 mM sucrose, 0.125 mM pargyline, and 30 mM Tris-HCl, pH 7.4 The incubation medium also contained MPTP and the monoamine: H-D,L-noradrenalin (specific radioactivity 280 TBq/mole), $^3$H-D,L-adrenalin (93 TBq/mole), $^3$H-dopamine (492 TBq/mole, from Izotop Leningrad): $^3$H-serotonin (407 TBq/mole, from Amersham International UK), Incubation was carried out at 37°C for 3 min with continuous shaking. Binding of the mediator by synaptosomes was stopped by filtration of 0.5 ml of the incubation medium through "Millipore" membrane

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TABLE 1. Effect of MPTP on Synaptosomal Uptake of Neurotransmitters

| Neurotransmitters | Per cent inhibition of uptake with MPTP in a concentration of (μM) | | |
|---|---|---|---|
| | 500 | 50 | 5 |
| Dopamine | 88,9±2 | 75,5±3 | 40,8±5 |
| Noradrenalin | 68,0±4 | n/d | |
| Adrenalin | 40,2±6 | 43,4±6 | 7.9±9 |
| Serotonin | 83,3±2 | 85,8±2 | 79.9±3 |
| GABA | 32,6±6 | n/d | n/d |
| Glutamate | 24,9±7 | n/d | n/d |
| Aspartate | 0 | n/d | n/d |
| Glycine | 30,5±7 | n/d | n/d |

**Legend:** n/d) not determined; results obtained with concentration of ($\mu$M): dopamine 0.1, noradrenalin 0.5, adrenalin 4, serotonin 0,1, GABA 10, glutamate 30, aspartate 15, and glycine 15.

TABLE 2. Constants and Type of Inhibition by MPTP of Synaptosomal Uptake of Dopamine, Noradrenalin, and Serotonin with High and Low Affinity (M ± m)

| Neurotransmitters | High affinity (μM) | | Low affinity (μM) | |
|---|---|---|---|---|
| | $K_{M_1}$ | $K_{i1}$ | $K_{M_2}$ | $K_{i2}$ |
| Dopamine | 0,132±0,02 | 7,50±0,8 | 5,0±0,2 | 1712±200 |
| Noradrenalin | 0,84±0,01 | 484,5±31 | 13,5±0,8 | 1870±240* |
| Adrenalin | 2,0±0,2 | 167,9±18 | 49,8±5,0 | 3636±390* |
| Serotonin | 0,07±0,01 | 2,38±0,3 | 2,0±0,2 | 0,87±0,09 |

**Legend:** *) Competitive type of inhibition; in all other cases a noncompetitive type of inhibition.

filters 25 mm in diameter (pore size 0.45 $\mu$) followed by washing with 15 ml of incubation medium at 37°C. The washed filters were dried and dissolved in 10 ml of scintillation fluid containing 7 ml toluene, 3 ml methyl ester of ethylene-glycol, 0.5% 2,5-diphenyloxazole (PPO), and 0.01% bis-12-(5-phenyloxazolyl)-1-benzene (POPOP). Radioactivity was measured on a liquid scintillation beta-counter, with calculation of the mean number of fissions per minute. Each experiment was repeated 3 times. True uptake was determined as the difference between total binding and sorption at 0°C. Protein was measured by Lowry's method [12]. The results were subjected to statistical analysis with calculation of mean values and their confidence limits at the $p < 0.05$ level.

## EXPERIMENTAL RESULTS

The effect of MPTP on synaptosomal uptake of the neurotransmitters was studied in concentrations of the latter close to the value of $K_M$ (Table 1). As Table 1 shows, MPTP is a powerful inhibitor of reuptake of monoamines, especially dopamine and serotonin. MPTP in a concentration of 500 $\mu$M inhibits uptake of all monoamines by 40-90%, and of amino acids by 25-30%, except aspartate, uptake of which by nerve endings is unchanged.

Having regard to the dopamine hypothesis of development of experimental parkinsonism under the influence of MPTP and to our own results indicating marked inhibition by MPTP of synaptosomal dopamine transport, we undertook a kinetic analysis of the effect of MPTP on dopamine accumulation by nerve endings (Fig. 1). As will be clear from Fig. 1, dopamine uptake is effected by two systems – with high and low affinity. MPTP inhibits both uptake systems noncompetitively; the system of high affinity ($K_i$ = 7.5 $\mu$M), moreover, more than 200 times more strongly than the low affinity system ($K_i$ = 1712 $\mu$M). The value of $K_i$ (7-5 $\mu$M) which we obtained for high affinity of synaptosomal dopamine uptake is in satisfactory agreement with results obtained by other workers, who found that the value of $IC_{50}$ for inhibition of dopamine uptake by nerve endings by MPTP was 4.0 $\mu$M [1].

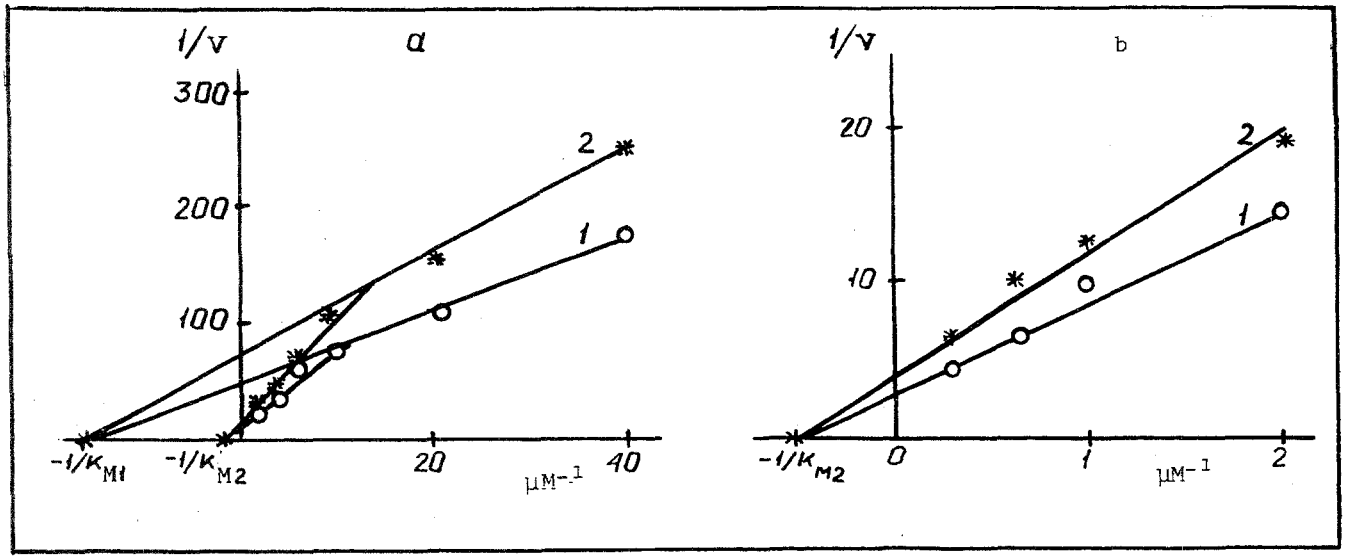


Fig 1. Effect of MPTP on synaptosomal high (a) and low (b) affinity uptake of dopamine. Abscissa, dopamine concentration ($\mu M^{-1}$); ordinate, (1/nmole)/mg protein/min). a) MPTP 10 $\mu$M; b) MPTP 2 $\mu$M.

It will be clear from Table 1 that MPTP affected synaptosomal uptake of serotonin by a greater degree than that of dopamine. Serotonin is known to inhibit transport of other monoamines competitively [2], and it was therefore important to study the effect of UPTP on the kinetics of transport of all monoamines. Values of $K_M$ and the constants of inhibition of uptake with high and low affinity by MPTP are given in Table 2.

The data given in Table 2 show that MPTP has an inhibitory action not only on the high-affinity monoamine uptake system, but also on the low-affinity system The essential difference is that MPTP inhibits high-affinity uptake of all monoamines noncompetitively, most strongly in the case of serotonin ($K_i$ = 2.38 $\mu$M), least in the case of noradrenalin ($K_i$ = 484.5 $\mu$M). Meanwhile, for the low-affinity system MPTP is a competitive inhibitor of synaptosomal uptake of noradrenalin ($K_i$ = 1870 $\mu$M) and adrenalin ($K_i$ = 3636 $\mu$M) and a noncompetitive inhibitor of serotonin ($K_i$ = 0.87 $\mu$M) and dopamine ($K_i$ = 1812 $\mu$M) (Table 2), which emphasizes the difference in the transfer sites in the presynaptic membrane for catecholamines and serotonin.

The results are evidence that each monoamine has its own high- and low-affinity transport systems, a result demonstrated previously only for the high-affinity system [2]. The noncompetitive character of inhibition of synaptosomal uptake of monoamines confirms the conclusions of Bachurin and co-workers [1], who showed that MPTP cannot be accumulated by nerve endings of the dopamine or other monoamine transport systems Comparison of values of $K_M$ for low affinity with $K_M$ of extraneuronal uptake by the heart muscle tissue [15] revealed a difference between them (values of $K_{M2}$ obtained were an order of magnitude lower than $K_M$ for extraneuronal uptake), in agreement with data [3, 4] on the existence of two transport systems in nerve endings for monoamines with high and low affinity, differing from systems of extraneuronal uptake.

Values of the inhibition constants showed (Table 2) that MPTP blocks most strongly the low-affinity synaptosomal system of serotonin uptake, and that its action on its high-affinity system is approximately only half as strong. High-affinity transport systems of dopamine, adrenalin, and noradrenalin are blocked 150-500 times less strongly than serotonin systems, and low-affinity systems 2000-4000 times less strongly. Thus low-affinity synaptosomal uptake of serotonin is most sensitive to the action of MPTP and may play a definite role in the development of symptoms of parkinsonism.

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# EFFECT OF AMIRIDINE ON MPTP-INDUCED PARKINSON'S SYNDROME IN MONKEYS

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**KEY WORDS:** N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; monkey; Parkinson's syndrome; amiridine

Antiparkinsonian drugs used in clinical practice are not sufficiently effective and give rise to many side effects. The search for new pharmacologic agents for the treatment of Parkinson's disease is thus extremely urgent and depends very much on the possibility of obtaining an adequate model of this pathological process. It has been found that the compound N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) gives rise to a symptom-complex typical of idiopathic parkinsonism both in man and in monkeys [7-9, 11, 12].

A new preparation for the treatment of nervous and mental diseases, amiridine (9-amino-2,3,5,6,7-hexahydro-1H-cyclopenta[b]quinoline monohydrate hydrochloride) has recently been developed at the All-Union Research Center for Safety of Biologically Active Substances [3-5]. The study of the mechanisms of action of this compound has shown that a shift of the region of activation of the $K^+$-channels of the excitable membrane in the direction of hyperpolarization. In vitro, this effect will evidently be equivalent to an increase in the membrane resting potential (RP), and it will consequently lead to more reliable generation and spread of the nervous impulse. Since RP of all cells, including those not electrically excitable, is maintained and regulated by potassium channels, amiridine can be expected to have a positive effect on impaired functions of nerve cells of all systems, whatever the factors causing their degenerative changes [9].

The aim of this investigation was to study the development of behavioral disturbances in monkeys receiving MPTP and to study the effect of amiridine on the symptoms thus produced.

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