GENERAL PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY

Effect of Glutamate and Antagonists of N-Methyl-D-Aspartate Receptors on Experimental Parkinsonian Syndrome in Rats

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Intranigral administration of glutamate to rats with parkinsonian syndrome induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine augmented the development of parkinsonian symptoms (oligokinesia and muscular rigidity), but did not affect motor activity of intact animals. Memantine administered intraperitoneally in parallel with induction of parkinsonian syndrome weakened the development of oligokinesia and muscular rigidity in a dose-dependent manner starting from 5 mg/kg and abolished toxic effect of glutamate. Ketamine (15 mg/kg) under the same conditions less potently prevented the development of oligokinesia, did not prevent the development of muscular rigidity, and did not antagonize glutamate toxicity. The data attest to an important role of glutamate and activation of N-methyl-D-aspartate receptors in the induction and development of parkinsonian syndrome.

Key Words: *parkinsonian syndrome; 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; ketamine; memantine; rats*

Parkinsonian syndrome (PS) results from degeneration and damage to nigral dopaminergic neurons, which drastically decrease dopamine content in the striatum [3,5,6]. An important role in the mechanisms of damage and death of the nigrostriatal neurons is played by disturbances in mitochondrial respiration leading to energy deficiency [12], activation of free radical lipid oxidation and generation of neurotoxic metabolites [4], production of NO, enhanced calcium permeability and calcium overload [1], and activation of N-methyl-D-aspartate (NMDA) glutamate receptors [7,11, 13]. The dominant mechanism responsible for degeneration and death of cerebral neurons in parkinsonism and the interplay between these processes are unknown. There is evidence that antagonists of NDMA-receptors protect cerebral neurons in various PS models. Memantine protects cultured cortical neurons from toxic effects of glutamate [10] and diminishes damage to the striatum induced by mitochondrial toxin malonate [14]. NDMA antagonists memantine and amantadine alleviate haloperidol-induced catalepsy in animals [11]. NMDA aggravated damage to cultured mesencephalic dopaminergic neurons caused by malonate, while NMDA receptor antagonist MK-801 prevented this damage. *In vivo* administration of NMDA into *substantia nigra* also potentiated damage to neurons induced by intranigral injection of malonate, which was also prevented by MK-801 [13]. Ifenprodil, an antagonist of NR2B-type NMDA receptors, increased motor activity in rats with reserpine-induced PS [9]. Amantadine moderates motor fluctuations and dyskinesia induced by L-DOPA in parkinsonian patients [15].

Our aim was to study the effect of intranigral administration of glutamate and NMDA antagonists ket-

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amine and memantine on the development of PS induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in rats.

MATERIALS AND METHODS

The study was carried out on 10-12-month-old male random-bred male rats. PS was induced by systemic intraperitoneal (i.p.) injection of neurotoxin MPTP (20 mg/kg) every 12 h for 6 days. NMDA antagonists ketamine (15 mg/kg, Gedeon Richter) and memantine (1-amine-3,5-dimethyladamantan, 5 and 10 mg/kg, Merz) were injected intraperitoneally 2 times a day 30 min prior to MPTP. Glutamate (10 μg/kg, Sigma) was injected into *substantia nigra pars compacta* (A 5.0; L 2.0; H 8.5 [13]) using a Hamilton syringe at a rate of 1 μl/min to control and PS rats fixed in a stereotaxic apparatus under hexenal narcosis (150 mg/kg). Each group comprised 7-9 rats. The development of PS was assessed by the degree of oligokinesia and muscular rigidity. Oligokinesia was assessed by motor activity and the number of rearings in the open field test before and 6 day after MPTP treatment. Both indices were expressed in percent of the control (NaCl i.p. and into *s. nigra*). Muscular rigidity was scored [2]. The results were analyzed statistically using Student's *t* and Newman—Keuls (ANOVA) tests.

RESULTS

MPTP 5-fold reduced motor activity in rats (to 20.0±1.5% of the control value). Intranigral glutamate further inhibited motor activity (to 5.8±1.3%). Preliminary intraperitoneal injection of ketamine enhanced motor activity in rats with PS to 30.0±3.0%. Intraperitoneal memantine, another NMDA antagonist, was more efficient: in doses of 5 and 10 mg/kg it enhanced motor activity in rats with PS to 53.0±2.5 and 68.0±3.1%, respectively (Fig. 1, *a*). Rearing in PS rats decreased to 23±5% on day 6 of neurotoxin treatment. Ketamine increased rearing to 34±6%, while memantine increased it to 65±7 and 80±7% in doses of 5 and 10 mg/kg, respectively. Muscular rigidity developed in 78% MPTP-treated rats and in 100% rats additionally treated with intranigral glutamate. The degree of muscular rigidity differed in these groups: in PS rats it was 2.25±0.14 points, while in glutamate-treated PS rats it was 3.0±0.2 points. Preliminary injection of memantine in doses of 5 and 10 mg/kg decreased muscular rigidity from 2.25±0.14 to 1.2±0.1 and 0.9±0.1 points, respectively ($p<0.05$). Intraperitoneal ketamine did not affect muscular rigidity in MPTP-treated rats (Fig. 2, *a*). Preliminary intraperitoneal injection of memantine (10 mg/kg) to rats treated with MPTP and intranigral glutamate increased motor activity from 8.6±2.3% to 49.2±2.5% ($p<0.05$) and weakened muscular rigidity from 2.95±0.13 to 1.2±0.1 points ($p<0.05$). Ketamine had no effect on motor activity and muscular rigidity in rats receiving intraperitoneal MPTP and intranigral glutamate (Figs. 1, *b* and 2, *b*).

Thus, intranigral glutamate augments oligokinesia and muscular rigidity in old rats induced by repeated injections of MPTP. Preliminary injection of NMDA-antagonist memantine attenuated oligokinesia and muscular rigidity and prevented aggravation of parkinsonian symptoms induced by intranigral administration

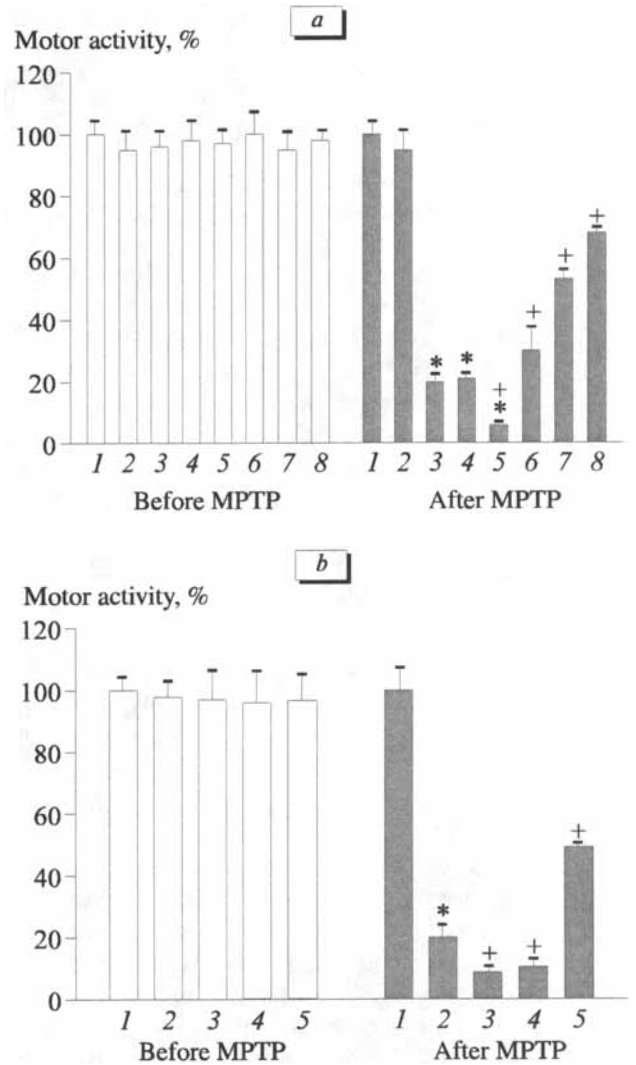

Fig. 1. Effect of glutamate, ketamine, and memantine on motor activity in rats before and on day 6 of treatment with MPTP alone (*a*) and in combination with intranigral glutamate (*b*). *a*: *1*) control; *2*) NaCl i.p. and intranigral glutamate; *3*) MPTP and NaCl i.p.; *4*) MPTP i.p. and intranigral NaCl; *5*) MPTP i.p. and intranigral glutamate; *6*) MPTP and ketamine (15 mg/kg) i.p.; *7*) MPTP and memantine (5 mg/kg) i.p.; *8*) MPTP and memantine (10 mg/kg) i.p.; *b*: *1*) control; *2*) MPTP i.p. and NaCl i.p. and intranigrally; *3*) MPTP i.p. and intranigral glutamate; *4*) MPTP and ketamine (15 mg/kg) i.p., intranigral glutamate; *5*) MPTP and memantine (10 mg/kg) i.p. and intranigral glutamate. $p<0.05$: *compared to the control, +compared to 2).

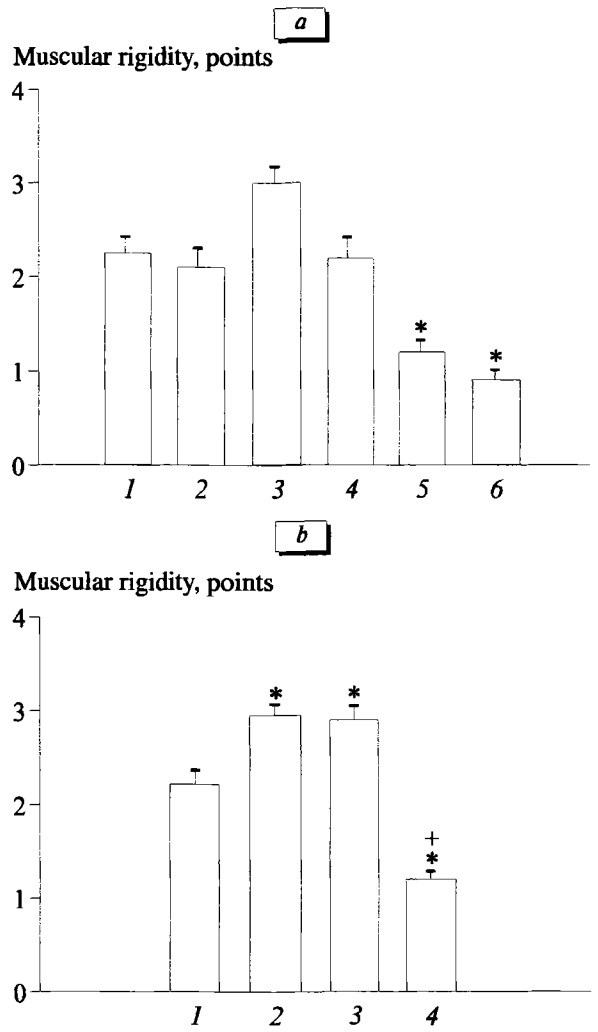

Fig. 2. Effect of glutamate, ketamine, and memantine on muscular rigidity in rats treated with intraperitoneal MPTP alone (*a*) or in combination with intranigral glutamate (*b*). *a*: *1*) MPTP and NaCl i.p.; *2*) MPTP i.p. and intranigral NaCl; *3*) MPTP i.p. and intranigral glutamate; *4*) MPTP and ketamine (15 mg/kg) i.p.; *5*) MPTP and memantine (5 mg/kg) i.p.; *6*) MPTP and memantine (10 mg/kg) i.p.; *$p<0.05$ compared to *1*). *b*: *1*) MPTP i.p. and NaCl i.p. and intranigrally; 2) MPTP i.p. and intranigral glutamate; 3) MPTP and ketamine (15 mg/kg) i.p., intranigral glutamate; *4*) MPTP and memantine (10 mg/kg) i.p. and intranigral glutamate. $p<0.05$: *compared to *1*), +compared to *2*).

of glutamate in rats with MPTP-induced PS. Ketamine injected under the same conditions enhanced motor activity to a lesser degree than memantine, had no effect on muscular rigidity, and did not abolish toxic effect of glutamate. Our data attest to participation of glutamate and NMDA receptors in degeneration and death of nigrostriatal neurons and the development of parkinsonism. An important role of activation of NMDA receptors in parkinsonism was demonstrated previously [7,10,11,13,15], but the mechanisms and the sequence of events going on with participation of NMDA-subtype glutamate receptors in the degeneration and death of nigrostriatal neurons during parkinsonism are still to be clarified. NMDA stimulated the release of acetylcholine from striatal slices and synaptosomes from rats treated with neurotoxin 6-hydroxydopamine, which indicates that during parkinsonism glutamate produced a more potent effect on acetylcholine release in the striatum mediated via pre- and postsynaptic NMDA receptors [8]. Treatment of cultured mesencephalic neurons with NMDA under conditions of 57% inhibition of ATP synthesis completely blocked dopamine reuptake in neurons [16]. Both our finding and published data suggest the following mechanism of degradation and death of nigrostriatal dopaminergic neurons during parkinsonism. Deficiency in the high-energy phosphates due to impaired mitochondrial oxidative phosphorylation in neurons triggers cascade events leading to activation of NMDA-subtype glutamate channels and a drastic increase in inward Ca^{2+} current. Accumulation of Ca^{2+} in neurons activates calmodulin, which activates NO-synthase catalyzing NO production from arginine. NO possessing free radical activity reacts with superoxide radicals produced during dopamine autooxidation and LPO activation. These processes lead to the production of neurotoxic pyroxynitrite [4]. There is evidence that the sharp increase of intracellular Ca^{2+} in dopaminergic neurons damages neuronal cytoskeleton and is responsible for neuronal degeneration [7].

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