Dipeptide Analog of Neurotensin Active Site Prevents the Development of Experimental Parkinson's Syndrome in Mice

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Chronic administration of neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (30 mg/kg) to C57BL/6 mice caused death of all animals within 7 days. Dipeptide analog of neurotensin active site injected with this neurotoxin protected the mice from death even after 2-week intoxication. When younger mice and lower dose of neurotoxin (25 mg/kg) were used, all animals survived, but after 2 weeks they developed parkinsonian syndrome with muscular rigidity, akinesia, decrease in motor and explorative activities. In mice treated with dipeptide analog of neurotensin active site these manifestations of oligokinesia caused by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine were less pronounced and the corresponding parameters approximated the control values. Possible mechanisms of neuroprotective action of neurotensin active site analog are discussed.

Key Words: *1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine*; *parkinsonism; neurotensin; dipeptide analog of neurotensin active site*; *neuroprotection*

Modern replacement therapy of parkinsonism with L-DOPA has no effect on the cause of this disease, *i.e.* progressive degeneration of dopaminergic (DA-ergic) nigrostriatal neurons [12]. The use of neurotrophic factors and peptides seems to be promising for direct inhibition of neural degeneration processes [1,2]. Of particular interest in this context is neurotensin (NT), a natural modulator of nigrostriatal and mesolimbic dopamine systems [6-8]. There are data that NT activates DA-ergic function in the nigrostriatal system. Substantia nigra and DA-ergic nerve terminals are characterized by maximum density of high-affinity NT-receptors [10,14]. NT selectively stimulates DA-ergic neurons in the substantia nigra [6], activates tyrosine hydroxylase in striatum sections [11], and stimulates the release of DA from the substantia nigra and caudate nucleus *in vivo* and *in vitro* [5,11].

At the State Research Institute of Pharmacology, T. A. Gudasheva and co-workers synthesized a group of dipeptide analogs of NT active site with DA-negative (antipsychotic agents) and DA-positive properties. TGS-79, a dipeptide analog of NT active site, the most active among DA-agonists, potentiated the apomorphine-induced verticalization, markedly increased L-DOPA-induced motor activity, and moderated haloperidol-induced catalepsy in rats. Hypothetically, DA-positive properties of TGS-79 can be used in the treatment of parkinsonism characterized by DA deficiency. In order to test this hypothesis, we used 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which destroys DA-ergic neurons in the substantia nigra [9,13], and examined the effect of TGS-79 on the development of Parkinson's syndrome in mice.

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MATERIALS AND METHODS

Two series of experiments were performed on male C57BL/6 mice aging 5 months (body weight 21±1 g) and 3 months (body weight 17±1 g). In series I, 5-month-old mice were intraperitoneally injected with MPTP (30 mg/kg) 2 times a day for 14 days (period between injections 12 h). In series II, 3-month-old mice received 25 mg/kg MPTP according to the same protocol. In each series, the mice of the same age and weight were subdivided into 3 groups (10 animals each): group 1 control mice were injected with physiological saline; group 2 mice were injected with MPTP and saline; and group 3 mice were injected with MPTP and TGS-79 (2 mg/kg).

The development of MPTP-induced parkinsonian syndrome was assessed by the degree of oligokinesia, muscle rigidity, and weight loss. Oligokinesia was assessed by the duration of immobility, total motor activity, number of rearings, and the number of small movements. Behavioral parameters were recorded automatically with Opto-Varimex-3 data acquisition system (Columbus Instr.) for 3 min before MPTP administration and on days 7 and 14 after the onset of injections. The developing muscle rigidity was scored by animal gibbosity. The data were analyzed statistically by Student's *t* test, Newman—Keuls post-hoc test, and Fisher's precise method.

In some experiments, the effects of examined agents on synaptoneurosome (SNS) membrane potential were studied. Conventional methods of SNS isolation on Millipore filters and measurement of SNS membrane potential with DiS-C_2-(5) (Molecular Probes) were described elsewhere [4].

RESULTS

In series I (higher MPTP dose), the behavioral changes in mice from control and experimental groups were compared with those in the group treated with TGS-79. The animals receiving MPTP alone after several days became extremely inert and moved slowly, and died on day 7 of treatment. Injections of physiological saline to control mice and MPTP in combination with TGS-79 to mice of group 3 were continued for one more week and all animals survived. Therefore, TGS-79 prevented death in mice injected with MPTP, although the period of MPTP exposure of these mice was 2 times longer than that producing 100% death of the mice receiving MPTP alone($p<0.025$).

In series II, the effect of TGS-79 on the development of parkinsonian syndrome was assessed in younger animals receiving lower MPTP doses (Fig. 1). In these mice, muscle rigidity appeared on day 7 of treatment, the rigidity score was the same in experimental and treatment group. Continuation of MPTP administration for the next 7 days produced a pronounced increase in muscle rigidity both in experimental mice and animals treated with TGS-79. However, rigidity score in mice treated with TGS-79 was almost 2-fold lower ($p<0.05$, Fig. 1, *a*).

On day 7 of the experiment, the effect of MPTP on motor activity was minimum, there were practically no differences between the experimental and treatment groups. However, on day 14 unambiguous signs of oligokinesia appeared in experimental mice receiving MPTP. In this group, the total motor activity (Fig. 1, *b*) and the number of rearings (orientation and exploratory activity, Fig. 1, *d*) were significantly lower than in the control group ($p<0.05$), while the time of immobility was longer than in control mice. On day 14 postinjection, the time of immobility was 129.14± 12.75 sec in the experimental group and 69.75±4.42 sec in the treatment groups. Similarly, the number of small movements in the experimental group was lower than in control mice ($p<0.05$, Fig. 1, *c*). TGS-79 injected simultaneously with MPTP prevented the appearance of all motor disturbances induced by 2-week administration of MPTP (Fig. 1). The corresponding indices in mice treated with TGS-79 almost did not differ from the control values. It should be noted that the mice receiving MPTP had lower body weight than control mice and animals receiving MPTP+TGS-79.

The death of nigrostriatal neurons induces denervation-type hypersensitivity of striatal neurons to DA. This hypothesis was tested in two experiments with SNS isolated from the striatum after 2-week MPTP treatment. DA (10^{-6} M) added to SNS isolated from animals with parkinsonian syndrome produced a 2-fold more pronounced shift in membrane potential compared to SNS from control animals. By contrast, in animals treated with TGS-79 the responses of SNS potential to DA did not surpass the control values (data not shown).

Muscle rigidity and oligokinesia reflect neural lesions in two different DA-ergic pathways: nigrostriatal and meso-cortico-limbic [3]. TGS-79 prevents the development of muscle rigidity and oligokinesia. A question arises about the nature of TGS-79 effect, which could be symptomatic or protective. Both variants are possible, but the latter seems to be more plausible. On day 7 of treatment, when the effect of MPTP becomes evident, the indices of motor activity in mice treated with TGS-79 did not surpass those in experimental animals, and on injection day 14, they did not surpass the control values. This suggest that TGS-79 prevents the toxic action of MPTP, rather than compensates the symptoms due to its own pharmacological effect. The increase in extracellular DA content caused by NT [5,11] can be determined by the block-

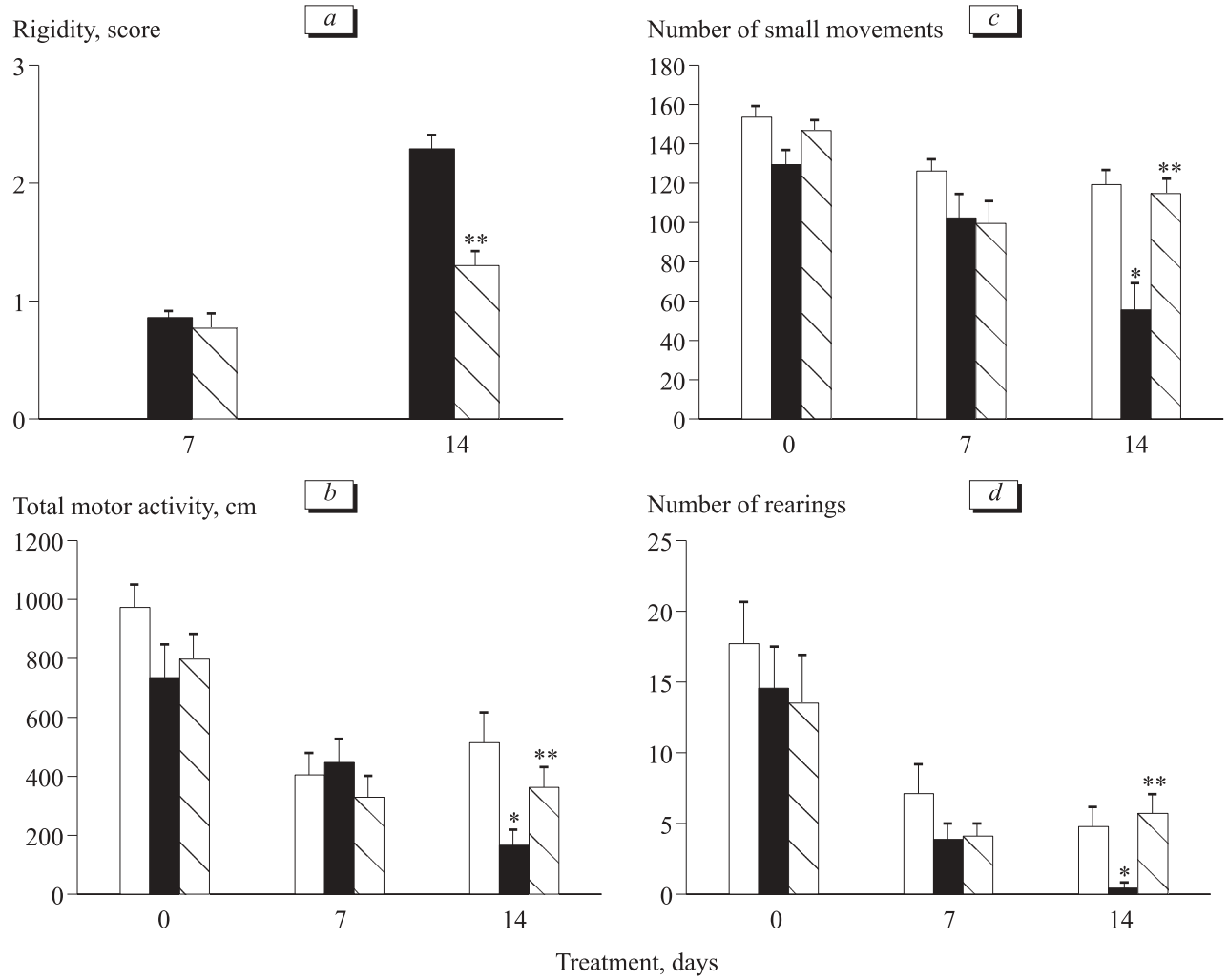

Fig. 1. Effect of dipeptide TGS-79 on experimental parkinsonian syndrome in C57BL/6 mice. *a*) muscle rigidity; *b*) total motor activity; *c*) number of small movements; *d*) orientation and exploratory activity. Light bars: physiological saline, solid bars: MPTP , and hatched bars: MPTP+TGS-79. $p<0.05$ compared to *control and **MPTP.

ade of its uptake mediated by DA transporter. It was shown that penetration of toxic MPTP metabolite into neurons is mediated by DA transporter [13]. Therefore, blockers of DA transporter prevent the toxic effect of MPTP towards neurons [9]. At present, there is no ground to explain the effect of TGS-79 by this mechanism. It looks more possible that TGS-79 interferes the mechanisms of neuronal damage.

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