Antiparkinsonian Properties of a Nerve Growth Factor Dipeptide Mimetic GK-2 in *in Vivo* Experiments

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An intraperitoneal injection of GK-2 (dipeptide mimetic of nerve growth factor, 0.01-5.00 mg/kg) 24 h before the adverse exposure reduced the severity of haloperidol-induced catalepsy in rats. This agent retained the activity after oral administration in a dose of 10 mg/kg. An intraperitoneal injection of GK-2 in a dose of 1 mg/kg reduced the severity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced parkinsonian syndrome in mice. Administration of GK-2 45 min after haloperidol treatment was also followed by a decrease in the degree of catalepsy. The repeated intraperitoneal treatment with GK-2 in a dose of 1 mg/kg after intrastriatal injection of 6-hydroxydopamine was shown to prevent the development of apomorphine-induced rotations in rats.

Key Words: *antiparkinsonian activity; low-molecular-weight dipeptide mimetic of nerve growth factor GK-2; 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; 6-hydroxydopamine; haloperidol*

Parkinson's disease (PD) is a common neurodegenerative disease, whose pathogenesis involves nigrostriatal dopaminergic neurons.

Pharmacotherapy for PD is focused on the compensation of dopamine deficiency with a dopamine precursor levodopa, monoamine oxidase (MAO) inhibitors, or dopamine receptor agonists. These drugs improve the locomotor function, but do not prevent neurodegeneration. Hence, the development of new therapeutic approaches to PD is associated with the search for novel neuroprotective drugs. The activity of novel drugs should exceed that of adamantane derivatives (himantane and amantadine) and MAO inhibitor selegiline [4].

Published data indicate that nerve growth factors are involved in the pathogenesis of neurodegenerative diseases. PD is accompanied by a significant decrease in the content of nerve growth factor in nigrostriatal dopaminergic neurons [9]. The content of nerve growth factor is also reduced in rats with parkinsonian syndrome due to 6-hydroxydopamine (8-OHDA)-induced destruction of the striatum [8]. *In vitro* experiments showed that nerve growth factor protects the cells from oxidative stress and 6-OHDA-induced apoptosis [13]. Moreover, this agent prevents cell death induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [14].

A group of N-acyl-dipeptide mimetics of nerve growth factor was developed at the V. V. Zakusov Institute of Pharmacology. The substance GK-2 was selected *in vitro* due to high neuroprotective activity (*e.g.*, on the cellular model of PD) [2].

Here we studied the antiparkinsonian properties of GK-2 in *in vivo* experiments.

MATERIALS AND METHODS

Experiments were performed on 240 male outbred rats (250-450 g) and 30 male C57Bl/6 mice (22-25 g). The animals received water and pelleted feed *ad libitum* and were kept under standard vivarium conditions.

GK-2 (synthesized at the Department of Chemistry, V. V. Zakusov Institute of Pharmacology), chloral hydrate (Pancreac Qimica SA), haloperidol

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(SUN Pharmaceutical Industries LTD.), apomorphine, MPTP, Tween 80, and 6-OHDA (Sigma) were used. GK-2, haloperidol, and NPTP were dissolved in distilled water. Apomorphine was dissolved in distilled water and 0.01% ascorbic acid. 6-OHDA was dissolved in physiological saline and 0.02% ascorbic acid. All solutions were prepared *ex tempore*.

Antiparkinsonian properties of GK-2 were evaluated on the standard experimental models according to methodical recommendations on studying the antiparkinsonian activity of pharmacological agents [1].

GK-2 in various doses was injected intraperitoneally before and after treatment with haloperidol (1 mg/kg intraperitoneally). Control animals received distilled water. Catalepsy was recorded 60 min after haloperidol injection. The forelimbs of each animal were placed on a plastic support (10 cm in height). The time of immobility was measured for 120 sec.

GK-2 in a dose of 1 mg/kg was injected intraperitoneally 24 h before MPTP treatment (30 mg/kg intraperitoneally). Group 2 animals received distilled water and MPTP. Distilled water was injected twice to group 3 animals. The horizontal and vertical activity and latency of locomotor activity were recorded in the open-field test (450-500 lx, 4-min period) 60 min and 7 days after MPTP injection. The open-field behavior of mice was recorded using a video camera and analyzed with RealTimer software (Otkrytaya Nauka).

6-OHDA was administered unilaterally into the striatum of rats to induce parkinsonian syndrome [12]. The animals were intraperitoneally anesthetized with chloral hydrate in a dose of 350 mg/kg. They were placed in a stereotactic device. 6-OHDA (8 μg/2 μl) or 2 μl physiological saline containing 0.02% ascorbic acid was infused into the right striatum (AP +0.5; L +2.8; DV +4.8) [10] for 6 min. The needle was leaved for 5 min and then removed. Twelve rats were anesthetized without surgical treatment. Distilled water or GK-2 was injected intraperitoneally (8 times) 1 h after surgery (or anesthesia) and in the follow-up period (at 48-h intervals). Apomorphine-induced rotations in rats were recorded 24 h after the last injection (day 16 after surgery). The rat was placed in a cylinder with sawdust. The cylinder (height 30 cm, diameter 22 cm) was made from a non-transparent white plastic. The animal was maintained in this cylinder for 10 min (adaptation period). Video filming was started 5 min after apomorphine injection and continued for 20 min. The video image was processed with RealTimer software.

The results were analyzed using Statistica 7.0 software. Because data did not conform to the normal distribution, nonparametric statistics was used. Statistically significant differences between the experimental and control groups were evaluated by nonparametric Mann-Whitney *U* test and Fisher's exact test. The differences were significant at $p<0.05$. The data in graphs and tables are presented as the medians. Data scattering is presented as the lower and upper quartiles.

RESULTS

Intraperitoneal injection of GK-2 in doses of 0.1-10 mg/kg (before 24 h) was not followed by the development of catalepsy or stereotypy and had no effect on the horizontal and vertical activity of mice and rats in the open-field test.

Haloperidol caused catalepsy in rats, which was most pronounced 60 min after injection. Administration of the dipeptide GK-2 (0.01, 0.1, 0.5, 1.0, and 5.0 mg/kg) 24 h before haloperidol treatment significantly decreased the duration of catalepsy (by 1.5-15.0 times; Table 1).

The anticataleptic effect of GK-2 persisted after oral administration of this agent. The duration of catalepsy was significantly reduced in animals receiving haloperidol in a dose of 10 mg/kg (39% of the control level). Further experiments were performed with GK-2 in a dose of 1 mg/kg (intraperitoneally), which had a strong effect on the model of haloperidol-induced catalepsy. Experiments with various schemes of GK-2 treatment (48, 24, 16, and 1 h before haloperidol injection; and 30 and 45 min after haloperidol injection) showed that this agent exhibits anticataleptic activity only under conditions of administration 24 h before or 45 min after haloperidol injection.

The model of MPTP-induced parkinsonian syndrome is well studied and extensively used [1]. MPTP oxidation by MAO B is followed by neurotoxin formation (ion of 1-methyl-4-phenylpyridinium, MPP^+), which has a selective effect on dopaminergic neurons and causes neuronal degeneration. An intraperitoneal injection of MPTP to mice caused locomotor disorders, which is typical of PD. The horizontal and vertical activity of mice in the open field decreased significantly, while the movement latency increased 1 h after MPTP injection. These disturbances persisted, but were less pronounced after 7 days. Administration of the dipeptide GK-2 was shown to prevent the development of locomotor disturbances 60 min after MPTP treatment. This agent was most potent in preventing the decrease in horizontal activity of animals (Table 2).

By the 60th minute after MPTP injection, horizontal activity of mice receiving GK-2 was 5-fold greater compared to animals of the distilled water group. The latency of movements in GK-2-receiving mice was lower than that in animals of the distilled water group (by 3 times). Horizontal activity and movement latency of GK-2-receiving mice returned to the control level on day 7 after MPTP injection.

The therapeutic effect of chronic treatment with GK-2 was studied on the model of 6-OHDA-induced

TABLE 1. Effect of Intraperitoneal Injection of GK-2 24 h before Haloperidol Treatment on the Duration of Catalepsy

Group	*n*	Duration of catalepsy, sec	Duration of catalepsy, % of the control
Water+haloperidol	15	45 (21-120)	100
GK-2 (0.01 mg/kg)+haloperidol	10	6 (4-15)*	14*
GK-2 (0.1 mg/kg)+haloperidol	10	30 (19-75)*	67*
GK-2 (0.5 mg/kg)+haloperidol	10	3 (2-6)*	7*
GK-2 (1.0 mg/kg)+haloperidol	10	4 (1-6)*	9*
GK-2 (5.0 mg/kg)+haloperidol	10	8 (5-120)*	18*

Note. *$p<0.05$ compared to the control (Fisher's exact test).

parkinsonian syndrome. Intrastriatal administration of 6-OHDA was followed by progressive degeneration of dopaminergic neuron axons and terminals. Administration of a dopamine receptor agonist apomorphine to rats after striatal injury caused the contralateral rotation of animals. It was associated with hypersensitivity of postsynaptic dopamine receptors due to presynaptic denervation (Fig. 1). Apomorphine caused the contralateral (left-side) rotation of rats that received 6-OHDA into the right striatum. The rotational movement was not typical of sham-operated animals. Chronic treatment with the dipeptide GK-2 for 2 weeks after surgery was followed by a significant decrease in the number of rotational movements (by 94% compared to operated animals).

Hence, chronic treatment with GK-2 abolishes the rotational behavior in rats after degeneration of nigrostriatal dopaminergic neurons.

Our results suggest that GK-2 has antiparkinsonian activity. It may be related to the neuroprotective properties of GK-2 for dopaminergic neurons and modulatory effect of this agent on the brain dopaminergic system. Previous studies revealed that the same antiparkinsonian effect is typical of nerve growth factor. Experiments on rats with parkinsonian syndrome due to unilateral infusion of 6-OHDA into the substantia nigra demonstrated that intrastriatal administration of nerve growth factor decreases the number of amphetamine-induced rotations by 26% [6]. Our experiments on the same model of parkinsonian syndrome showed that GK-2 decreases the number of rotational movements by 94%. An intraperitoneal injection of nerve growth factor (sorbed on the surface of polybutylcyanoacrylate nanoparticles) decreased significantly the rigidity, but increased the locomotor activity of mice with MPTP-induced parkinsonian syndrome [3]. On this model of parkinsonian syndrome, the most significant effect of GK-2 and nerve growth factor sorbed on the surface of nanoparticles was manifested in the prevention of a decrease in horizontal activity 60-90 min and 7 days after MPTP treatment. We showed that administration of GK-2 is followed by an increase in horizontal locomotor activity of mice 60 min and 7 days after MPTP injection (by 5 and 2.5 times, respectively). Nerve growth factor sorbed on the surface of nanoparticles had the same effect. This agent increased the horizontal locomotor activity of mice 90 min and 7 days after MPTP injection (by 5-6 and 2.5 times, respectively). *In vivo* experiments showed that nerve growth factor low-molecular-weight mimetics can alleviate neuropathic pain [7], exhibit anti-stroke activity [15], improve cognitive functions in old rats, and have therapeutic properties in rats with experimental glaucoma [5]. However, little attention was paid to studying the antiparkinsonian properties of nerve growth factor low-molecular-weight mimetics.

We conclude that GK-2 is a nerve growth factor low-molecular-weight mimetic, which has antipar-

TABLE 2. Effect of Intraperitoneal Injection of GK-2 (1 mg/kg) 24 h before MPTP Treatment on Akinesia in Mice (Open-Field Test)

Experimental conditions	*n*	Number of crossed squares	Number of crossed squares, % of the control
Control (water)	10	24 (17-31)	100
MPTP	10	2 (0-5)*	8*
GK-2+MPTP	10	9 (5-13)$^{+}$	38^{+}

Note. *$p<0.05$: *compared to passive control; $^{+}$compared to active control (Mann–Whitney *U* test).

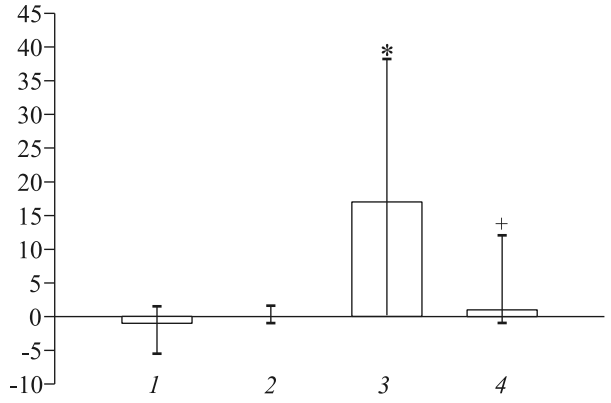

Fig. 1. Apomorphine-induced rotational behavior of rats. Data are presented as the medians of the difference between the total number of left-side and right-side rotations by 180°. $p<0.05$: *compared to sham-operated animals; +compared to operated animals (Fisher's exact test). Anesthesia (*1*); sham operation (*2*); surgery (*3*); surgery+GK-2 (*4*).

kinsonian activity. The antiparkinsonian agent GK-2, which has neuroprotective properties and is effective under conditions of systemic administration, should be studied in further researches.

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