Effects of Acidic Fibroblast Growth Factor on the Development of Experimental Parkinsonism and Striatal Level of Dopamine and Its Metabolites in Mice of Different Ages

V. G. Kucheryanu, G. N. Kryzhanovskii, V. S. Kudrin, V. V. Yurasov, E. V. Nikushkin, and I. V. Zhigal'tsev

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 127, No. 5, pp. 502-505, May, 1999
Original article submitted January 22, 1999

Intranasal administration of acidic fibroblast growth factor suppressed the development of oligokinesia and muscular rigidity and elevated the content of dopamine and its metabolites (dihydroxyphenylacetic acid and homovanillic acid) in the striatum of 7-month-old mice with MPTP-induced Parkinsonian syndrome. In 14-month-old mice the antiparkinsonic effect of acidic fibroblast growth factor was weakened or absent. It is suggested that this factor is able to attenuate or postpone degeneration and death of nigral dopaminergic neurons.

Key Words: *Parkinsonian syndrome; 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP); acidic fibroblast growth factor; dopamine; striatum; mice*

It is known that dopaminergic neurons of the substantia nigra in rats, monkeys, and humans are sensitive to acidic and basic fibroblast growth factors (aFGF and bFGF, respectively), which exert a neurotrophic effect and protect these cells from toxic effects of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), 1-methyl-4-phenylpyridine and 6-OHDA (6-hydroxydopamine) [3,4,8,13,15,16]. We have previously shown that aFGF and bFGF on intranasal administration weaken oligokinesia, muscular rigidity, and tremor and reduce neuronal degeneration in the substantia nigra of middle-aged (7-month-old) mice with MPTP-induced Parkinsonian syndrome [1,9]. It was reported that intrastriatal administration of aFGF to young mice pretreated with MPTP increased the striatal level of dopamine and tyrosine hydroxylase activity [6]. In old animals aFGF produced no such effects [6]. In the present study we investigated the effect of intranasal administration of aFGF on the development of Parkinsonian syndrome in MPTP-treated middle-aged and old mice and on the striatal level of dopamine and its metabolites.

Laboratory of General Pathology of the Nervous System, Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow

MATERIALS AND METHODS

Experiments were carried out on male C57B1/6 mice aged 7 and 14 months. Intraperitoneal injections of MPTP were given for 10 days (20 mg/kg, twice a day with 12-h intervals) to develop Parkinsonian syndrome. Bilateral intranasal administrations of aFGF in a dose of 3 μg per animal were performed with a Hamilton syringe 30 min prior to the first MPTP injection and then on days 3 and 5 of MPTP treatment. Immediately before administration, heparin was added to aFGF solution (1 μg/ml) to increase its activity [5]. The aFGF (R & D Systems Inc.) was kindly given by Prof. Y. Oomura. The mice were divided into 3 groups: group 1 received intraperitoneal MPTP and intranasal aFGF; group 2 received intraperitoneal MPTP and intranasal saline; group 3 (control) received saline

0007-4888/99/0005-0454$22.00

both intraperitoneally and intranasally. The development of Parkinsonian syndrome was assessed by oligokinesia and muscular rigidity score [1]. Oligokinesia was quantified by measuring locomotor activity and rearings. Locomotor activity was measured in a Opto-Varimex-3 system (Columbus Instr.) before and 5 and 10 days after treatment and expressed in percents of control (locomotor activity of control mice was taken as 100%). The animals were decapitated on the 11th day between 10.00 and 12.00. The brain was removed and striatum was dissected at 0-4°C. The contents of dopamine, dihydroxyphenylacetic acid (DOPA), and homovanillic acid (HVA) were measured by high-performance liquid chromatography (HPLC) with electrochemical detection [2]. Statistical analysis was performed using Student's *t* test and by analysis of variance (ANOVA) with post hoc Newman—Keuls test.

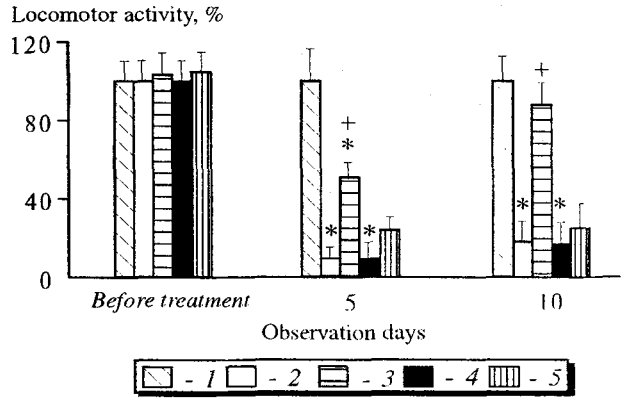

Fig. 1. Motor activity in mice of different ages after systemic administration of MPTP and intranasal administration of acidic fibroblast growth factor (aFGF). *1*) saline; *2*) MPTP, 7-month-old mice; *3*) MPTP+aFGF, 7-month-old mice; *4*) MPTP, 14-month-old mice; *5*) MPTP+aFGF, 14-month-old mice; Here and in Fig. 2: $p<0.05$: *in comparison with the control; +in comparison with MPTP-treated animals.

RESULTS

On the 5th day of MPTP treatment locomotor activity of the middle-aged and old mice decreased by 8-10% and remained lowered until the end of the treatment (Fig. 1). Administration of aFGF increased the locomotor activity of the middle-aged mice on the 5th day and completely restored it on the 10th day. In old mice, aFGF insignificantly increased locomotor activity on both test days. The number of rearings in MPTP-treated middle-aged mice became as low as 18% of the control value on the 5th day of treatment, and practically no rearings were recorded on the 10th day. Although intranasal aFGF did not prevent the drop in rearings, their number was higher than in the control group on both test days. Intranasal aFGF did not affect the number of rearings in MPTP-treated old mice. Muscular rigidity was observed in 87% of MPTP-treated 7-month-old mice and in all 14-month-old mice. Intranasal aFGF administration significantly attenuated rigidity in the middle-aged mice: it was observed in only 25% animals and its score decreased from 2 to 1.2 (day 5) and then to 1.1 points (day 10). In MPTP-treated old mice, aFGF slightly alleviated the symptom (rigidity was observed in 90% of the cases), but rigidity score remained unaffected (2.3-2.4 points) (Fig. 2).

HPLC data showed that the content of dopamine, DOPAC and HVA in the striatum of the middle-aged mice with Parkinsonian syndrome was reduced by 77, 82, and 86%, respectively, compared to the control. The rate of dopamine turnover (HVA/dopamine ratio) decreased 2-fold and the total content of dopamine, DOPAC and HVA decreased 4.8-fold in comparison with the control. In MPTP-treated middle-aged mice, intranasal aFGF induced a 2-fold elevation of striatal dopamine. It also increased the contents of DOPAC (2.5-fold) and HVA (5-fold), so that the total level of dopamine, DOPAC and HVA became 2.1 times higher (Fig. 3). aFGF increased the rate of dopamine turnover: the DOPAC/dopamine and HVA/dopamine ratios increased by 36% and 2.8-fold, respectively. In 14-month-old mice, MPTP considerably reduced the content of dopamine, DOPAC and HVA in the striatum, while combined administration of MPTP and aFGF had no effect on these parameters.

Thus, preliminary and repeated treatment with aFGF weakened oligokinesia and rigidity and elevated striatal content of dopamine and its metabolites in middle-aged mice with Parkinsonian syndrome induced by 10-day MPTP treatment. It is noteworthy that these effects were observed after intranasal administration which suggests ready penetration of the peptide administered by this route to the brain tissue. It is pos-

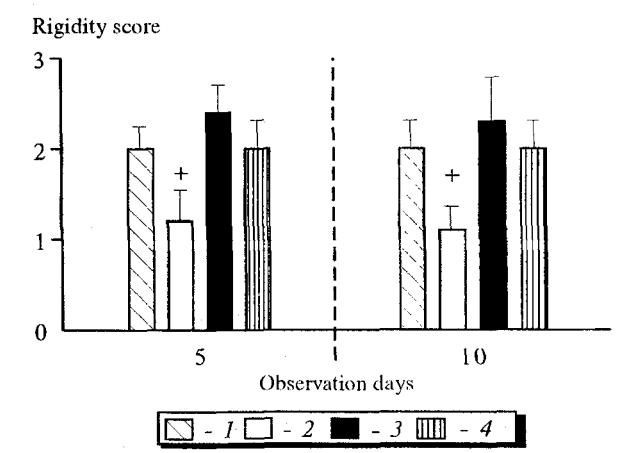

Fig. 2. Muscular rigidity in 7- (*1,2*) and 14- (*3,4*) month-old mice after systemic administration of MPTP alone (*1,3*) or in combination with acidic fibrobrast growth factor (*2,4*).

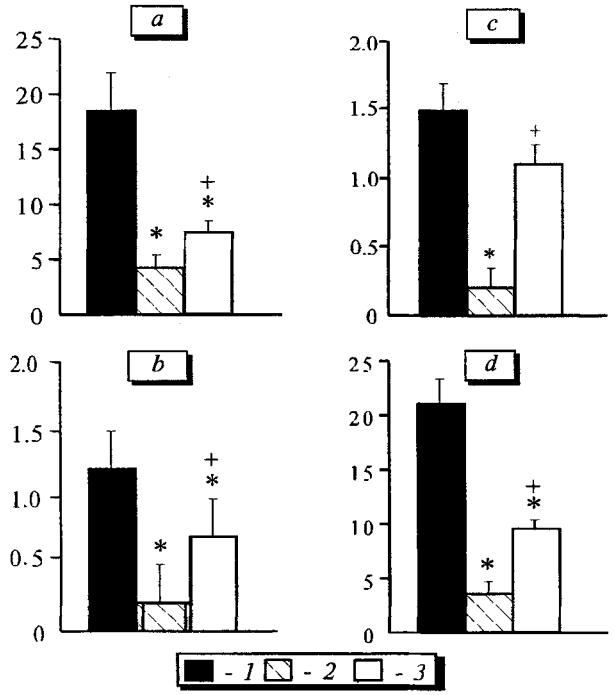

Fig. 3. Striatal content of dopamine (*a*) DOPAC (*b*), and homovanillic acid (*c*) and their total content (*d*) in middle-aged mice after treatment with saline (*1*), MPTP (*2*), and MPTP+acidic fibroblast growth factor (*3*). Ordinates: concentration, nmol/g tissue.

sible that long-term administration of MPTP affected permeability of the blood-brain barrier. Another way of reaching the brain from the nasal cavity bypassing the blood-brain barrier is the olfactory tract. Intranasal aFGF seemed to intensify dopamine synthesis in the preserved neurons of the nigrostriatal system, as evidenced by the elevated content of dopamine, DOPAC, and HVA and accelerated dopamine turnover in the striatum of the middle-aged mice with Parkinsonian syndrome treated with aFGF. Judging from the increased level of the two dopamine metabolites, aFGF intensified both the intracellular (DOPAC) and extracellular (HVA) metabolism of dopamine.

It has been shown that intrastriatal administration of aFGF to 6-OHDA- or MPTP-treated animals reduced the number of amphetamine-induced rotations and increased tyrosine hydroxylase activity and the striatal level of dopamine and DOPAC [6,7]. Intracerebroventricular administration of bFGF partially restored the striatal content of dopamine and DOPAC in MPTP-treated mice. It should be noted that these effects were observed only in young (2-3-month-old) or middle-aged (7-month-old) animals, which is in line with our results. Weak or absent neuroprotective effects of aFGF in old animals can be explained by the age-related decrease in the density of aFGF receptors in the substantia nigra pars compacta [16].

The mechanisms of aFGF neuroprotective effects in Parkinsonism remain obscure. It has been suggested that FGFs are involved in the cascade of cell reactions providing postimpairment repair of nigrostriate dopaminergic neurons. It has been shown that bFGF inhibited Ca^{2+}-induced apoptosis of rat cortical neurons in culture [14]. In gerbil hippocampal slices, aFGF prevented the ischemia-induced increase in intracellular Ca^{2+} [10]. The FGF-induced reduction in intraneuronal Ca^{2+} accumulation is thought to be due to activation of the Ca^{2+}-pump or protein kinase C [11,12].

The protective action of aFGF on the dopaminergic neurons can be attributed to its ability to delay or attenuate their degeneration. Our previous studies showed that aFGF reduced the number of impaired nigral neurons in mice with MPTP-induced Parkinsonism by 63% [9].

The study was supported by the Russian Foundation for Basic Research (grant No. 98-04-48275).

REFERENCES

1. G. N. Kryzhanovskii, V. G. Kucheryanu, E. V. Nikushkin, and N. A. Krupina, *Byull. Eksp. Biol. Med.*, **120,** No. 9, 260-262 (1995).
2. A. Yu. Shemanov, I. I. Miroshnichenko, V. S. Kudrin, and K. S. Raevskii, *Neurokhimiya*, **7,** No. 3, 134-138 (1988).
3. A. J. Bean, R. Elde, Y. H. Cao, *et al.*, *Proc. Natl. Acad. Sci. USA*, **88,** 10237-10241 (1991).
4. G. Chadi, A. Moller, L. Rosen, *et al.*, *Exp. Brain Res.*, **97,** 145-158 (1993).
5. D. H. Damon, R. R. Lobb, P. A. D'Amore, and J. A. Wagner, *J. Cell Physiol.*, **138,** 221-226 (1989).
6. I. Date, M. Notter, S. Felten, and D. Felten, *Brain Res.*, **526,** 156-160 (1990).
7. B. K. Jin and L. D. Iacovitti, *Neurobiol. Dis.*, **1,** 1-12 (1995).
8. P. B. Kirschner, R. Henshaw, J. Weise, *et al.*, *J. Cereb. Blood Flow Metab.*, **15,** 619-623 (1995).
9. G. N. Kryzhanovsky, V. G. Kucheryanu, O. M. Pozdnyakov, *et al.*, *Pathophysiology*, **4,** 59-67 (1997).
10. A. Mitani, Y. Oomura, H. Yanase, and K. Kataoka, *Neurochem. Int.*, **21,** 337-341 (1992).
11. M. Radhakrishna and G. Almazan, *Brain Res. Mol. Brain Res.*, **24,** 118-128 (1994).
12. K. Sasaki, Y. Oomura, K. Suzuki, *et al.*, *Neurochem. Int.*, **21,** 397-402 (1992).
13. H. W. M. Steinbusch, R. J. Vermeulen, and J. A. D. M. Tonnaer, *Prog. Brain Res.*, **82,** 81-86 (1990).
14. N. Takei, H. Ogaki, and Y. Endo, *Neurosci. Lett.*, **192,** 124-126 (1995).
15. I. Tooyama, E. G. McGeer, T. Kawamata, *et al.*, *Brain Res.*, **656,** 165-168 (1994).
16. D. G. Walker, K. Terai, A. Matsuo, *et al.*, *Ibid.*, **794,** No. 2, 181-187 (1998).