Influence of Basic Fibroblast Growth Factor on the Development of Parkinsonian Syndrome in Mice

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Intranasal administration of basic fibroblast growth factor prevented or reduced oligokinesia, muscular rigidity, and weight loss and also prevented mortality in mice with parkinsonism induced by repetitive intraperitoneal injections of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. These beneficial effects are probably due to trophic actions of the factor, whereby the degeneration of nigrostriatal neurons is weakened and delayed and their survival is prolonged.

Key Words: *parkinsonian syndrome; basic fibroblast growth factor; MPTP; mice*

The parkinsonian syndrome is known to be associated with damage to the dopaminergic nigrostriatal system. In animal experiments, this syndrome has been produced with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a neurotoxin causing degeneration of nigrostriatal neurons [7]. Recent studies show that the polypeptide designated basic fibroblast growth factor (bFGF) exerts strong trophic effects [4-6,12], is involved in maintaining the viability of central nervous system (CNS) neurons and prolongs their survival following damage caused by ischemia [12], hypoglycemic shock [2], or NMDA receptor agonists [6], and is capable of stimulating the growth of nerve cells and their processes [5,12]. Elevated levels of this factor have been detected in damaged CNS structures [5,9]. Intrastriatal injection of acid bFGF to young mice administered the MPTP neurotoxin (4 doses of 20 mg/kg each) led to partial restoration of the dopamine level and tyrosine hydroxylase activity in the striatum [4].

Intrastriatal administration of bFGF to mice with MPTP-damaged dopaminergic neurons resulted in biochemical and morphological improvements in the nigrostriatal system [10]. Being a potent trophic agent, bFGF can probably prevent or lessen the degeneration of nigrostriatal neurons and thus hinder the development of experimental parkinsonism. We found earlier that intranasal instillation of substance P suppressed the oligokinesia, rigidity, and tremor in rats with an MPTP-induced parkinsonian syndrome [1]. In the present study we explored the possibility of preventing the development of this syndrome in C57Bl/6 mice with intranasally administered bFGF.

MATERIALS AND METHODS

Male C57Bl/6 mice aged 7 months were used. A parkinsonian syndrome was induced by systemic (intraperitoneal) injection of MPTP in a dose of 20 mg/kg twice daily at 12-h intervals for 10 days. In the test group of mice, bFGF was injected from a Hamilton microsyringe into both nostrils in a dose of 3 μg per mouse before and 3 and 5 days after the first MPTP dose. Before each injection, bFGF was treated with heparin (1 μg per ml of bFGF solution) to increase its activity [3]. Control mice were administered MPTP alone as described above (control group 1) or only physiological saline in the same volumes and by the same routes (control group 2).

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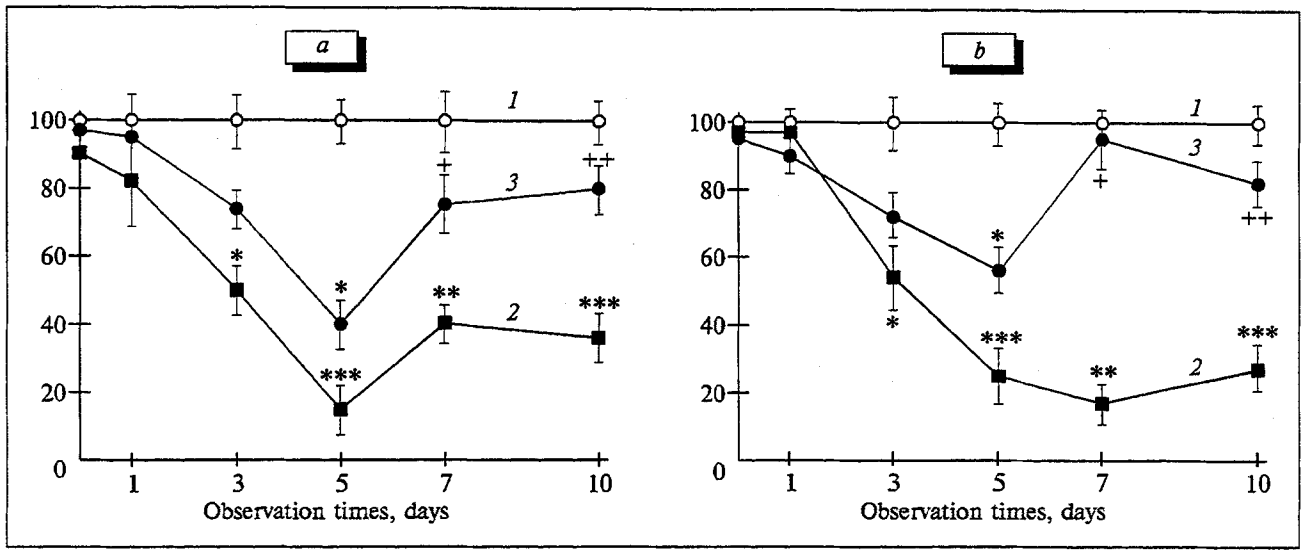

Fig. 1. Effects of bFGF on total motor activity and large movements of mice given systemic MPTP injections. *Ordinates*: total motor activity (*a*) and large movements (*b*), both expressed in percent of their control values (in the group given only saline) taken as 100%. Here and in fig. 2: *1*) mice administered only saline (control group 2); *2*) mice administered MPTP (control group 1); *3*) mice administered MPTP and bFGF. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ in comparison with control group 2; $^{+}p<0.05$, $^{++}p<0.01$ in comparison with control group 1.

The severity of the parkinsonian syndrome was evaluated by noting the degree of oligokinesia and rigidity. The degree of oligokinesia was estimated from changes in motor activity and in the number of rearings. Motor activity was measured either by the number of squares crossed in an open-field test or by using a device (small animal movement monitor manufactured by Coulbourn Instruments) with which the number of "small" movements, the number of "large" movements, and total motor activity can be determined over a specified period. Motor activity was recorded before and 1, 3, 5, 7, and 10 days after the start of MPTP dosing and expressed in percent of the motor activity shown by the control mice. Muscular rigidity was scored in points.

The results were statistically analyzed using Student's *t* test and the nonparametric Wilcoxon-Mann-Whitney test.

RESULTS

In the mice given MPTP alone (control group 1), the total motor activity (Fig. 1, *a*, *2*) and the number of large movements (Fig. 1, *b*, *2*) were decreased on day 3 of MPTP dosing and even more so later; the levels of motor activity were lowest on days 5 and 7 and remained significantly below their values in control group 2 on day 10. In the test group, which received MPTP intraperitoneally and bFGF intranasally, the total motor activity and the number of large movements were decreased only on day 5 and differed little from their values in control group 2 later (Fig. 1, *a*, *3* and *b*, *3*).

The number of rearings in the MPTP-dosed group were reduced to 19% of the control group 2 value on day 5 and virtually no rearings were observed on day 10. In the MPTP-dosed group treated with bFGF, the number of rearings was significantly lower than in control group 2 on day 5 but remained higher than in control group 1 given MPTP alone and did not differ from that in control group 2 on day 10 (Fig. 2).

Muscular rigidity developed in 87% of the mice in the MPTP-dosed group but only in 25% of those in the group given MPTP plus bFGF. These two groups also differed in the degree of rigidity, which scored 1.75 points on day 5 and 2 points on day 10 in the MPTP-dosed group *vs.* 0.25 point in the group given bFGF in addition to MPTP.

The daily exposure to MPTP alone for 10 days also resulted in weight losses (with a decrease in the mean body weight in this group from 29.7±0.9 g before MPTP dosing to 23.6±1.4 g) and in a 50% mortality rate. In sharp contrast, neither weight losses nor deaths were recorded among the MPTP-exposed mice treated with bFGF.

Intranasal treatment of mice with bFGF before and during their twice-daily 10-day dosing with MPTP thus prevented them from developing oligokinesia and muscular regidity and from losing weight as well as averting their death, i.e., this treatment hindered the development of parkinsonism caused by chronic systemic administration of MPTP. The observed beneficial effects of bFGF may be

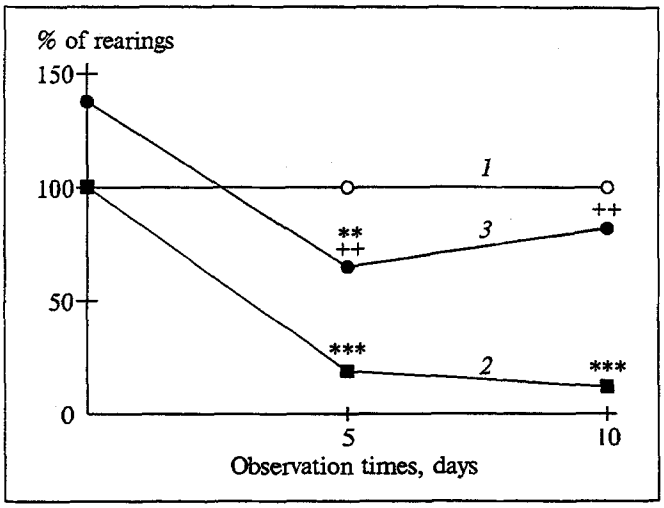

Fig. 2. Effect of intranasally adminstered bFGF on the number of rearings by mice given systemic MPTP injections; the number of rearings in control group 2 (the one given only saline) was taken as 100%.

attributed to its trophic action, whereby the degeneration of nigrostriatal neurons is weakened and delayed and their survival prolonged. It is significant that this factor produced such effects after intranasal administration, suggesting that some peptides, including bFGF, penetrate into the brain more readily by this route.

bFGF is thought to participate in the cascade of cellular reactions taking place during regenerative processes in the CNS after its injury. With histochemical methods, bFGF and its receptors were detected in the neostriatum [11]. The addition of this factor to cultured cells from the mesencephalon resulted in the release of dopamine from dopaminergic neurons and in elevated tyrosine hydroxylase activity [8]. In a culture of striatal cells, bFGF prolonged the survival of neurons damaged by the NMDA receptor agonists glutamate and quinolinic acid [6]. When added to a striatal cell culture containing glutamatergic neurons, bFGF increased the growth and density of nerve processes. The factor did not exert trophic effects in a culture containing glial cells instead of glutamatergic neurons [13]. Intranasal administration of acid bFGF to young mice aged 3 months after their neurons were damaged by MPTP was followed by rises in the dopamine level and in the immunoreactivity of tyrosine hydroxylase in the striatum. Neither of these effects was observed in older mice aged 12 months [4].

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