Neuroprotective effect of BDNF in young and aged 6-OHDA treated rat model of Parkinson disease

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Received November 2005; revised 19 April 2006

Brain derived neurotrophic factor (BDNF) has been shown to exert trophic effects on dopaminergic neurons against 6-hydroxydopamine (6-OHDA) in young rat. Since the degeneration of substantia nigra dopaminergic neurons that occurs in Parkinson’s disease is more often than not confined to elderly individuals, it is of interest to determine whether the effects of BDNF against 6 hydroxydopamine (6-OHDA) in young rats can be extended to aged animals. 6-hydroxydopamine was stereotaxically injected into the striatum of young (3-months) and aged (24-months) rats, which were treated two hours earlier with BDNF. 6-OHDA results in almost complete destruction of substantia nigra pars compacta dopaminergic neurons. BDNF injection significantly changed apomorphine induced rotations from 132 ± 15 to 181 ± 10, staircase test from 73 ± 2% to 61 ± 3%, initiation time from 7 ± 2 to 12 ± 1 sec, and disengage time from 80 ± 7 to 90 ± 5 sec in young and aged animals, respectively. It is concluded that BDNF causes the limited behavior recovery of striatal DA systems from 6-OHDA toxicity in aged animals.

Keywords: Brain derived neurotrophic factor, Neuroprotection, Parkinson disease, Rat

Parkinson’s disease (PD) is a neurodegenerative disorder characterized by progressive loss of dopaminergic neurons in the substantia nigra pars compacta that results in a significant decrease of dopamine level in the striatum. In the last three decades, levodopa (L-dopa) has remained the best drug for PD. It dramatically improves morbidity and mortality. But various adverse reactions are seen with long term L-dopa therapy i.e., dyskinesia, on-off phenomenon and psychiatric effects (agitation, visual hallucination, psychosis, paronia, hyper sexuality etc). In addition, it actually speeds the progression of disease. This led to a search for alternative treatment. Attempts to overcome the limitations of therapy and to reverse disease progression have included infusion of brain-derived neurotrophic factors (BDNF) for the dopamine system1.

Brain-derived neurotrophic factor is a basic dimeric 28KDa protein of non-covalently linked 14 KDa subunits structurally related to NGF, which are responsible for neuron proliferation, survival and differentiation2-4. BDNF can protect nigrostriatal dopaminergic neurons from neurotoxins in rodent and monkey models of PD5. BDNF is required for the establishment of the proper number of dopaminergic neurons in the substantia nigra pars compacta6. BDNF induces striatal neurogenesis in adult rats with 6-hydroxydopamine lesions7. BDNF interact with dopaminergic transmission and dopamine receptor stimulation in the frontostriatal circuitry, with subsequent consequences on cognition in Parkinson's disease8. Reduced BDNF mRNA expression in Parkinson’s disease substantia nigra contributes directly to the death of nigral dopaminergic neurons and the development of Parkinson's disease9. BDNF synthesized by dopamine neurons is responsible for the appearance of the dopamine D3 receptor during development and maintains its expression in adults. Moreover, BDNF triggers behavioral sensitization to levodopa in hemiparkinsonian rats4.

The ultimate value of neurotrophic factors in clinical disorders lies in their ability to exert a beneficial effect at the behavioral level. While the use of neurotrophic factors to prevent the loss of damaged neurons or decreases in biochemical parameters has

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gained substantial attention, relatively little attention has been paid to their behavioral effects. Since the motor deficits observed in aged rodents appear to be mediated by alterations in striatal dopaminergic systems, the present study was undertaken to evaluate the influence that striatal injection of BDNF may have on motor functions mediated by dopaminergic systems. To further define the extent of possible protection offered by BDNF, the present study examined the 2 hours time point between BDNF and 6-OHDA administration in 3 and 24 months old Sprague Dawley rats. These experiments were designed to determine the scope of BDNF neuroprotection in aged animals against intrastriatal 6-OHDA lesions by evaluating various behaviour tests.

Materials and Methods

Animals—A total of sixty rats Sprague-Dawley, weighing 150-250 g at the beginning of the experiment, were used. Young (3 months) and aged (24 months) rats were used for this study. The rats were maintained in a temperature-controlled environment under a 12 hr of light/dark cycle with free access to food and water when not in experimental sessions. They were treated in compliance with the regulation, policies and principles of the Institutional animal ethical committee. All efforts were made to reduce the number of animals used and their suffering. These rats were divided into 6 groups having 10 animals in each group. They were grouped as—Gr 1: Sham-operated control (young animals); Gr 2: Sham-operated control (aged animals); Gr 3: Young animals with 8 µg 6-OHDA into lateral striatum only; Gr 4: Aged animals with 8 µg 6-OHDA into lateral striatum only; Gr 5: Young animals received an injection of 100 ng BDNF dissolved in 5 µl sterile Dulbecco'phosphate-buffered saline intrastrial plus 8µg 6-OHDA in to lateral striatum; and Gr 6: Aged animals received an injection of 100 ng BDNF dissolved in 5 µl sterile Dulbecco’phosphate-buffered saline intrastrial plus 8µg 6-OHDA into lateral striatum

Lesions by 6-hydroxy dopamine (6-OHDA)—The rats were anaesthetized with chloropent (Sigma, 3 ml/kg, ip) and placed in a Kopf stereotaxic frame (INCO). Unilateral nigrostriatal lesions were produced by stereotaxic injection of 8 µg of 6-OHDA (Sigma) into the striatum according to the atlas of Paxinos and Watson11. The injection coordinates were 0.0 mm anterior-posterior, 3.5 mm lateral and 5.5 mm dorsoventral with tothbar setting at 3.3 mm below the interaural line on the non-dominants side.

Quantitation of rotational behavior—At 4 weeks after lesion surgery, apomorphine (Sigma, 0.05mg/kg) induced rotations was observed. Testing started after 5 min of injection of the drug. The left and right full-body turns were monitored for 30 min. Net rotational asymmetry score was expressed as full-body turns per min in the direction ipsilateral or contra lateral to the lesion12.

Staircase test—A modified version of the staircase test described by Montoya et al.13 was used. For each test the animals were left in the test boxes for 15 min. The double staircase was filled with 30 chow pellets (45 mg) on each side. After each test the number of pellets taken and the number of pellet eaten were counted separately13.

Stepping test—For the test of akinesia, the experimenter gently held the rat so that it was standing with most of its body weight on one forelimb, and allowed to initiate steps with the forelimb while the other limbs were held off the surface. The numbers of steps taken with the forelimb were recorded separately during 30 sec trials for each forelimb14.

Initiation time—In initiation time, the time to actively initiate a forelimb movement was determined in the same test sessions as for the stepping test. The rats were pretrained for two days to turn up a wooden ramp (1.1 m) into their home cage. During the test, the rat was held as described for the stepping test and placed with its unrestrained paw (the one to be monitored) on the bottom of the ramp. The time elapsed before the rat actively initiated movement with the unrestrained forelimb and started to step forward along the ramp toward the home cage was recorded. The test was performed once a day for each forelimb on three consecutive days and the mean of the three test sessions was calculated15.

Postural test—In the same test sessions as for the stepping test, postural balance was examined in a side-falling test. The rat was held in the same position as described for the stepping test and then in a fast movement tilted toward the side of the paw touching the table, which caused a loss of balance. The animal’s attempt to regain balance with an adjusting step toward the side was monitored by a scoring system ranging from 0 to 3 — (0) no detectable muscle reaction, the rat falls onto the side; (1) clear forelimb reaction, but the rat cannot move limb under
the body toward the center of gravity and thus still falls onto the side; (2) incomplete recovery of balance i.e., the rat moves its limb under the body but not yet fully into the center of gravity, and thus the forelimb is not aligned vertically to the body; furthermore, the forepaw might not be placed in a plain position on the table and digits might be crossed over each other; (3) complete recovery of balance. The test was repeated six times every day on both sides giving a maximum daily score of 18. Final results are expressed as average of the three-test days score.

Disengage behaviour—Disengage behaviour was performed according to the protocol of Schallert and Hall. A blunt wooden probe touched the perioral region beneath the vibrissae of the rat repeatedly at 1 sec intervals when the rat was engaged in eating a piece of milk chocolate. The latency of the orienting response, i.e., a turning of the head toward the stimulus, was recorded. Stimulation was discontinued if the animal did not respond within a period of 180 sec. As for the stepping test, a blinded experimenter who was not aware of the identity of the animals performed this test. The test was performed once a day on each side over two days and the mean of the two subtests was calculated.

Statistical analysis—Results are expressed as mean ± S.E.M. of the different treatment groups. The significance of difference between pre-lesion and post-lesion within the group was determined by paired Student’s t test. Side differences (lesion versus normal) within groups were analyzed using paired Student’s t test. Intergroup comparisons were done using analysis of variance (ANOVA). A P value <0.05 was considered as statistically significant.

Results

Rats with unilateral (left striatum) 6-OHDA lesions exhibited statistical significant difference in various behavior tests i.e. apomorphine-induced rotational behavior, staircase test, stepping test, initiation time, disengage time and postural balance test (Table 1). 6-OHDA treated animals of group 3 exhibited statistical significant difference (paired Student’s t test, P<0.001) between pre-lesion and post-lesion values in apomorphine-induced rotational behavior, staircase test and disengage time and significance at P<0.05 in stepping test and postural balance test. The animals of group 4 demonstrated statistical significant difference (paired Student’s t test, P<0.001) between pre-lesion and post-lesion values in apomorphine-induced rotational behavior, staircase test, initiation time, postural balance test and disengage time; significance at P<0.05 in stepping test. Sham operated animals of group 1 or 2 failed to exhibit significant difference between pre-lesion and post-lesion values in group 1 or 2 (paired Student’s t test, P>0.1) in various behavior tests.

Young rats subjected to BDNF and receiving stereotaxic injection of 6-OHDA had significant effect in various behavior tests. Comparative analysis

<table>
<thead>
<tr>
<th>Behavior tests</th>
<th>Group 1 Pre-lesion</th>
<th>Post-lesion</th>
<th>Group 2 Pre-lesion</th>
<th>Post-lesion</th>
<th>Group 3 Pre-lesion</th>
<th>Post-lesion</th>
<th>Group 4 Pre-lesion</th>
<th>Post-lesion</th>
<th>Group 5 Pre-lesion</th>
<th>Post-lesion</th>
<th>Group 6 Pre-lesion</th>
<th>Post-lesion</th>
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<tr>
<td>Rotational behavior</td>
<td>10 ± 1</td>
<td>12 ± 1</td>
<td>13 ± 1</td>
<td>14 ± 1</td>
<td>11 ± 1</td>
<td>274 ± 21**</td>
<td>14 ± 2</td>
<td>286 ± 25**</td>
<td>13 ± 1</td>
<td>132 ± 15**</td>
<td>11 ± 1</td>
<td>181 ± 10**##</td>
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<td>Staircase test taken eaten</td>
<td>28 ± 2</td>
<td>29 ± 2</td>
<td>26 ± 2</td>
<td>27 ± 3</td>
<td>26 ± 2</td>
<td>13 ± 3</td>
<td>24 ± 3</td>
<td>11 ± 3**</td>
<td>28 ± 3</td>
<td>22 ± 2*</td>
<td>25 ± 2</td>
<td>18 ± 3 *##</td>
</tr>
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<td>Success rate</td>
<td>89 ± 5</td>
<td>90 ± 4</td>
<td>89 ± 5</td>
<td>93 ± 4</td>
<td>92 ± 3</td>
<td>54 ± 3**</td>
<td>92 ± 4</td>
<td>45 ± 3**</td>
<td>89 ± 4</td>
<td>73 ± 2*</td>
<td>92 ± 3</td>
<td>61 ± 3###</td>
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<tr>
<td>Stepping test in forehand direction in backhand direction</td>
<td>10 ± 2</td>
<td>12 ± 1</td>
<td>9 ± 1</td>
<td>10 ± 1</td>
<td>11 ± 1</td>
<td>4 ± 1*</td>
<td>9 ± 1</td>
<td>3 ± 1*</td>
<td>12 ± 2</td>
<td>9 ± 1*</td>
<td>12 ± 1</td>
<td>6 ± 1#</td>
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<td>Initiation time</td>
<td>14 ± 1</td>
<td>13 ± 1</td>
<td>12 ± 1</td>
<td>11 ± 1</td>
<td>12 ± 2</td>
<td>5 ± 2*</td>
<td>10 ± 1</td>
<td>4 ± 2*</td>
<td>14 ± 2</td>
<td>11 ± 1*</td>
<td>13 ± 2</td>
<td>7 ± 2*#</td>
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<td>Postural balance test score</td>
<td>2 ± 1</td>
<td>2 ± 1</td>
<td>4 ± 1</td>
<td>5 ± 1</td>
<td>3 ± 1</td>
<td>15 ± 1**</td>
<td>6 ± 1</td>
<td>18 ± 1**</td>
<td>2 ± 1</td>
<td>7 ± 2*</td>
<td>3 ± 1</td>
<td>12 ± 1###</td>
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<td>Disengage time</td>
<td>17 ± 1</td>
<td>16 ± 1</td>
<td>13 ± 1</td>
<td>12 ± 1</td>
<td>16 ± 2</td>
<td>7 ± 2*</td>
<td>18 ± 3</td>
<td>6 ± 2**</td>
<td>17 ± 2</td>
<td>12 ± 1*</td>
<td>15 ± 2</td>
<td>10 ± 2*</td>
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* paired t test, P<0.05 vs pre-lesion value of same group. ** paired t test, P<0.001 vs pre-lesion value of same group. #ANOVA, P<0.05 vs post-lesion value of different group 1-4. ## ANOVA, P<0.001 vs post-lesion value of different groups 1-6.
between pre-lesion value and post-lesion value of group 5 was found significant in apomorphine-induced rotational behavior and disengage time (paired Student’s t test, \( P<0.001 \)) and in staircase test, stepping test, initiation time and postural balance test (at \( P<0.05 \)).

However, when BDNF was administered 2 h prior to 6-OHDA injection a change in various behavior tests was observed in aged rats. Significant difference between pre-lesion and post-lesion values of group 6 was found in apomorphine-induced rotational behavior, staircase test (success rate), initiation time and disengage time (paired Student’s t test, \( P<0.001 \)) and in stepping Student’s t test and postural balance test (paired Student’s t test, \( P<0.05 \)). Intergroup comparison of post lesion values of group 1-6 was found significant in apomorphine-induced rotational behavior, staircase test, initiation time, disengage time (ANOVA, \( P<0.001 \)) and in stepping test, postural balance test (ANOVA, \( P<0.05 \)). There were no statistical significant differences between pre-lesion values of group 1 to 6 (ANOVA, \( P>0.1 \)).

**Discussion**

In the present study, the possible neuroprotective effect of BDNF was tested against 6-OHDA neurotoxicity for dopaminergic neurons in the young and aged rat. This study used the “partial lesion” rat model of PD, which involves 6-OHDA administration to striatal dopaminergic terminals. This induces rapid degeneration of striatal dopaminergic fibers, which is followed by the protracted death of their cell bodies in the SN, which begins after a delay of about 1 week and progresses over several weeks. This “partial lesion” is a more appropriate model of the early stages of PD, where neurodegeneration is still ongoing, compared to the “complete lesion” of the medial forebrain bundle (MFB), which is a good model of the end stages of the disease. This reflects more accurately the pathological process of neurodegeneration that occurs in PD than the complete “complete lesion” model does, making the intrastriatal lesion a suitable model in which to investigate the regenerative effects elicited by administration of exogenous BDNF on dying dopaminergic neurons19.

In the present study, the presence of lesion was confirmed by behavioral testing at 5 days after 6-OHDA administration, when all rats were found to rotate at a rate of more than five turns per min. Injection of BDNF into the striatum prior to lesion surgery led to a significant behavioral improvement in the rotational behavior, staircase test, stepping test, initiation time, postural balance test and disengage time tests. These findings suggest that BDNF is neuroprotective against 6-OHDA lesion. These results are consistent with previous work where protection of nigrostriatal dopaminergic neurons has been seen with BDNF19-22. Transplantation of modified fibroblasts that expresses BDNF into either striatum or midbrain attenuates 6-hydroxydopamine (6-OHDA)-induced losses of nigrostriatal neurons23,24. Also, BDNF can modulate dopaminergic neurotransmission in nigrostriatal neurons, as shown by elevated rotational behavior and increased turnover of dopamine in the striatum19. BDNF can promote functional recovery from 6-OHDA lesion following expression in striatal cells from an AAV vector25.

In the present study, extent of protection against 6-OHDA induced dopaminergic neurotoxicity was less in aged hemiparkinsonium animals. It might be due to neurochemical and cellular changes in the nigrostriatal dopaminergic changes during aging. There are reductions in striatal levels of DA as well as DA receptors26-28. Neuronal loss in substantia nigra reaches about 50 % by the ninth decade in humans29. Reduction in high affinity DA uptake sites30, DA transporter messenger RNA31 and TH messenger RNA32 also become evident as individual’s age.

It has been postulated that free radicals play a major role in the neuronal degeneration that occurs during aging as well as in many neurodegenerative diseases such as Parkinson’s disease. It is possible that BDNF is eliciting its protective effect by enhancing or maintaining the synthesis of free radical scavenging enzymes. Indeed, it has recently been reported that intrastriatal BDNF leads to an upregulation of the antioxidant enzymes. BDNF can increase the production of one or more antioxidant enzymes (Cu/Zn–superoxide dismutase (SOD), Mn-SOD, glutathione peroxidase and catalase) in hippocapal neurons32. BDNF can induce expression of anti-apoptotic Bcl-2 family members33 and inhibitor of apoptosis proteins (IAP)34. Neurotrophic factors can also modulate the expression and/or activity of subunits of glutamate receptors and voltage dependent calcium channels in ways that promote neuronal survival and synaptic plasticity35.

The results of present study conclude that the administration of BDNF prior to an intrastriatal 6-OHDA injection protects striatal DA neurons in
young and aged rats. However, there was less protection in aged rats compared to young rats. To elucidate the exact mechanism of action of FGF, further study is needed.

Acknowledgment

One of the author(SS) is grateful to ICMR, New Delhi for the financial support.

References


