Effect of subthalamic nucleus lesions in a 6-hydroxydopamine–induced rat parkinsonian model: behavioral and biochemical studies

YONG SUP HWANG, M.S., INSOP SHIM, PH.D., BOM BEE LEE, PH.D., AND JIN WOO CHANG, M.D., PH.D.

Department of Neurosurgery, Brain Korea 21 Project for Medical Science & Brain Research Institute, Yonsei University College of Medicine; and Department of Integrative Medicine, College of Medicine, The Catholic University of Korea, Seoul, South Korea

Object. The purpose of this study was to determine whether subthalamic nucleus (STN) ablation caused by kainic acid can restore dopaminergic neurotransmission and improve motor deficits in a 6-hydroxydopamine (6-OHDA)–induced hemiparkinsonian model.

Methods. The authors investigated behavioral changes in rats displaying parkinsonian symptoms (6-OHDA–lesioned rats) after an STN lesion was created using kainic acid. They also measured levels of dopamine and its metabolites following tissue dissection. The results of this study showed that STN ablation led to behavioral improvement in parkinsonian motor deficits. Increased levels of dopamine were also observed in the striatum and globus pallidus externus (GPE).

Conclusions. The results indicate that creation of an STN lesion in this hemiparkinsonian rat model may counteract some of the neurochemical changes within the striatum and GPE caused by the 6-OHDA, and influence striatal dopaminergic metabolism.

KEY WORDS • Parkinson disease • basal ganglia • subthalamic nucleus • 6-hydroxydopamine • kainic acid • rat

I
diopathic PD is a progressive neurodegenerative disorder caused by degeneration of dopamine-producing neurons in the SNc and a concomitant loss of dopamine in the striatum. The STN is an important structure in the basal ganglia circuitry and plays a critical role in regulating motor function. Abnormal activity in the STN, which sends hyperactivated glutamatergic neurotransmission to the SNr and GPI, has been considered a pivotal function in the expression of PD symptoms such as akinesia, rigidity, and tremor. During the past decades, ablation of the STN by creating lesions or by high-frequency stimulation has been shown to ameliorate motor dysfunction in a nonhuman primate PD model. The striatum is one of the fundamental components of the basal ganglia, and it plays an important role in motor function. The STN receives not only dopaminergic projections from the nigrostriatal pathway but also serotonergic projections from the raphestral pathway.

Thus far, the effects of STN lesions on the neurotransmission of dopamine have not been well studied in the 6-OHDA–induced rat PD model. The aim of the present study was to investigate the biochemical and behavioral effects of creating a lesion in the STN by using this model.

Materials and Methods

Animal Preparation

This study was conducted in a facility certified by the American Association for the Accreditation of Laboratory Animal Care. Twenty-six male Sprague–Dawley rats, each weighing between 200 and 250 g, were used in the experiments. They were divided into four groups: a control group of six rats; the PD group, which included six rats in which a lesion of dopaminergic neurons had been caused by 6-OHDA; the PD–STN lesion group, in which seven rats with a 6-OHDA–induced lesion of dopaminergic neurons also had STN ablation; and the STN lesion group, which included seven rats with kainic acid–induced STN lesions. All rats were housed in a temperature- and humidity-controlled room with a 12-hour light–12-hour dark cycle. The rats were anesthetized with a mixture of ketamine (75 mg/kg), acepromazine (0.75 mg/kg), and rompun (4 mg/kg), as previously described. Eight micrograms (free base weight) of 6-OHDA (Sigma Chemical Co., St. Louis, MO) in 0.2% ascorbic acid with 0.9% normal saline (2 μl total volume) was infused at a rate of 0.5 μl/minute at the following MFB sites: 4.4 mm anterior and 1.2 mm lateral to the bregma, and 7.5 mm ventral to the dura mater. All coordinates were obtained from the atlas of Paxinos and Wat...

Abbreviations used in this paper: ANOVA = analysis of variance; DOPAC = 3,4-dihydroxyphenylacetic acid; GABA = γ-amino-butyric acid; GPE = globus pallidus externus; GPI = GP internus; HVA = hemovanillic acid; LSD = least significant difference; MFB = medial forebrain bundle; PD = Parkinson disease; SEM = standard error of the mean; SNc = substantia nigra pars compacta; SNr = SN pars reticular; STN = subthalamic nucleus; 6-OHDA = 6-hydroxydopamine.
After the injection of 6-OHDA, the cannula was left in place for 30 seconds, briefly, while bearing weights on one forepaw, the SEM, p 4.6–mm HR-80 C-18 column (ODS, 3–0.05), but no forepaw, which the rats made to compensate for movement of their number of forepaw-adjusting steps involving the weight-bearing that moved at a rate of 90 cm/12 seconds. During this interval, the rats were held in a stationary position on the surface of a treadmill Co.) in 1 of saline solution; the solution was injected at a rate of 0.5 µl/minute (total 1 µl) into the STN (3.7 mm anterior and 2.5 mm lateral to the bregma, and 8 mm ventral to the dura). In the group of rats with paired lesions, the STN lesion was created 1 week after injection of 6-OHDA into the MFB.

Quantification of Dopamine and Its Metabolites

High-pressure liquid chromatography with electrochemical detection was used to quantify dopamine and its metabolites DOPAC and HVA. When the experiments were completed, the rats were killed by decapitation and rapidly dissected, as described in a previous study. The striatum and GPE were immediately removed, placed on dry ice, and their tissue weights while still wet were obtained. The tissue was homogenized in 1 ml of ice-cold 0.05-µM HClO, for 30 seconds, followed by centrifugation at 10,000 G and 4°C for 10 minutes. Twenty microliters of supernatant was injected at 0.7 ml/minute through an 80 × 1.7-mm 1-octanesulfonic acid–induced lesion in the STN was produced. The striatum and GPE control 6 75.611 

<table>
<thead>
<tr>
<th>Site</th>
<th>Group</th>
<th>No. of Rats</th>
<th>Mean Value (fmol/µl/mg wet tissue)</th>
<th>Metabolic/Monoamine Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dopamine</td>
<td>DOPAC</td>
</tr>
<tr>
<td>striatum</td>
<td>control</td>
<td>6</td>
<td>51.661 ± 4.858</td>
<td>42.197 ± 3.151</td>
</tr>
<tr>
<td>PD</td>
<td>6</td>
<td>17.570 ± 3.074</td>
<td>33.736 ± 3.241</td>
<td>66.043 ± 2.359</td>
</tr>
<tr>
<td>PD–STN lesion</td>
<td>7</td>
<td>37.711 ± 2.266</td>
<td>47.828 ± 4.449</td>
<td>94.127 ± 5.647†</td>
</tr>
<tr>
<td>GPE</td>
<td>control</td>
<td>6</td>
<td>75.611 ± 18.476</td>
<td>109.595 ± 15.303</td>
</tr>
<tr>
<td>PD</td>
<td>6</td>
<td>37.560 ± 2.827</td>
<td>62.297 ± 5.127†</td>
<td>67.482 ± 3.405†</td>
</tr>
<tr>
<td>PD–STN lesion</td>
<td>7</td>
<td>82.582 ± 3.852</td>
<td>111.051 ± 15.163‡</td>
<td>177.707 ± 10.280†</td>
</tr>
<tr>
<td>STN lesion</td>
<td>7</td>
<td>101.107 ± 18.460</td>
<td>124.986 ± 8.561</td>
<td>207.977 ± 12.114‡</td>
</tr>
</tbody>
</table>

* Values are expressed as means ± SEMs. Statistical significance was determined using one-way ANOVA with LSD post hoc comparisons.
† p < 0.05 compared with control group.
‡ p < 0.05 compared with PD group.

Results

Levels of dopamine and its metabolites were measured in the striatum and GPE for each group of rats. Table 1 shows changes in neurotransmitter levels in the striatum and GPE. There was a decrease in the levels of dopamine and its metabolites DOPAC and HVA in these two regions in the 6-OHDA–induced rat PD model, whereas the STN lesion group demonstrated a significant increase in these levels. After induction of the STN lesion, the amounts of dopamine in the striatum and GPE were significantly increased to 20.14 ± 3.21 fmol/µl/mg and 45.02 ± 1.03 fmol/µl/mg, respectively (seven rats, mean ± SEM, p < 0.05). The PD group followed by the STN lesion group demonstrated a significant increase in GPE DOPAC levels (from 62.279 ± 5.127 to 111.051 ± 15.163 fmol/µl/mg, p < 0.05), but no statistically significant difference was identified in the striatum. After a lesion was created in the STN in the rat PD model, HVA levels in the striatum and GPE increased significantly: 28.08 ± 3.29 fmol/µl/mg and 50.23 ± 6.88 fmol/µl/mg, respectively.

In 6-OHDA–induced lesions in the MFB and the STN lesions, both the DOPAC/dopamine and HVA/dopamine ratios in the striatum tended to decrease but did not show any significant difference from ratios in the PD group.

The mean number of rotations in the rat PD model (six animals) for a period of 1 hour was 452.8 ± 45.82 (p < 0.01 compared with values in the control group; Fig. 1). After an STN lesion had been created in the rat PD model, the mean number of rotations for a period of 1 hour was significantly
Interestingly, STN lesions induced by kainic acid in the rat hemiparkinsonian 6-OHDA model produced increased dopamine neurotransmission in the striatum. The results of a previous study indicated that high-frequency stimulation of the STN produced an increased level of extracellular dopamine metabolites without an increase in extracellular dopamine in rats with 6-OHDA–induced lesions in the striatum. Therefore, an alteration in dopamine neurotransmission may reflect changes in nigrostriatal dopaminergic neuronal firing or dopamine release. Moreover, we observed increased levels of dopamine and its metabolites after creation of an STN lesion in the GPE, a finding that suggests that output neurons from the STN project to the GPE, GPI, and SNr. Disinhibition of the STN neurons might increase, thereby restoring the dopamine deficit. Interestingly, STN lesions in the rat PD model produced decreased DOPAC/dopamine and HVA/dopamine ratios in the striatum. These findings prompt the speculation that increased dopamine turnover and dopamine receptor sensitization (a compensatory mechanism in surviving neurons in the parkinsonian condition) were normalized. Our finding that dopamine levels increased in the striatum after the creation of the STN lesion might explain the disinhibition of the thalamocortical projection. The striatum is known to be influenced along nigrostriatal thalamocortical connections. The decreased basal ganglia output would lead to disinhibition of the thalamocortical projection, which could result in an increased glutamatergic input from the cortical efferent projection to the striatum. The resulting increment of cortical afferent activation would account for some mediation of parkinsonian symptoms. Alternatively, such a strong improvement in the dopamine level may be the consequence of reduced STN output.

Our findings may prompt speculation that a kainic acid–induced lesion of the STN could provoke an increase in the activity of nigral dopamine neurons. The SNc neurons are activated as a result of STN ablation in the rat hemiparkinsonian 6-OHDA model. This is supported by deep brain stimulation studies in which high-frequency stimulation of the STN has been found to increase the activity of nigral dopaminergic neurons. In the present study, the results provide the first evidence that STN lesions induced by kainic acid in the rat hemiparkinsonian 6-OHDA model produced increased dopamine neurotransmission in the striatum. The results of a previous study indicated that high-frequency stimulation of the STN produced an increased level of extracellular dopamine metabolites without an increase in extracellular dopamine in rats with 6-OHDA–induced lesions in the striatum. Therefore, an alteration in dopamine neurotransmission may reflect changes in nigrostriatal dopaminergic neuronal firing or dopamine release. Moreover, we observed increased levels of dopamine and its metabolites after creation of an STN lesion in the GPE, a finding that suggests that output neurons from the STN project to the GPE, GPI, and SNr. Disinhibition of the STN neurons might increase, thereby restoring the dopamine deficit. Interestingly, STN lesions in the rat PD model produced decreased DOPAC/dopamine and HVA/dopamine ratios in the striatum. These findings prompt the speculation that increased dopamine turnover and dopamine receptor sensitization (a compensatory mechanism in surviving neurons in the parkinsonian condition) were normalized.

Our finding that dopamine levels increased in the striatum after the creation of the STN lesion might explain the disinhibition of the thalamocortical projection. The striatum is known to be influenced along nigrostriatal thalamocortical connections. The decreased basal ganglia output would lead to disinhibition of the thalamocortical projection, which could result in an increased glutamatergic input from the cortical efferent projection to the striatum. The resulting increment of cortical afferent activation would account for some mediation of parkinsonian symptoms. Alternatively, such a strong improvement in the dopamine level may be the consequence of reduced STN output.

Our findings may prompt speculation that a kainic acid–induced lesion of the STN could provoke an increase in the activity of nigral dopamine neurons. The SNc neurons are activated as a result of STN ablation in the rat hemiparkinsonian 6-OHDA model. This is supported by deep brain stimulation studies in which high-frequency stimulation of the STN has been found to increase the activity of nigral dopaminergic neurons. 

In the present study, the results provide the first evidence that STN lesions induced by kainic acid in the rat hemiparkinsonian 6-OHDA model produced increased dopamine neurotransmission in the striatum. The results of a previous study indicated that high-frequency stimulation of the STN produced an increased level of extracellular dopamine metabolites without an increase in extracellular dopamine in rats with 6-OHDA–induced lesions in the striatum. Therefore, an alteration in dopamine neurotransmission may reflect changes in nigrostriatal dopaminergic neuronal firing or dopamine release. Moreover, we observed increased levels of dopamine and its metabolites after creation of an STN lesion in the GPE, a finding that suggests that output neurons from the STN project to the GPE, GPI, and SNr. Disinhibition of the STN neurons might increase, thereby restoring the dopamine deficit. Interestingly, STN lesions in the rat PD model produced decreased DOPAC/dopamine and HVA/dopamine ratios in the striatum. These findings prompt the speculation that increased dopamine turnover and dopamine receptor sensitization (a compensatory mechanism in surviving neurons in the parkinsonian condition) were normalized.

Our finding that dopamine levels increased in the striatum after the creation of the STN lesion might explain the disinhibition of the thalamocortical projection. The striatum is known to be influenced along nigrostriatal thalamocortical connections. The decreased basal ganglia output would lead to disinhibition of the thalamocortical projection, which could result in an increased glutamatergic input from the cortical efferent projection to the striatum. The resulting increment of cortical afferent activation would account for some mediation of parkinsonian symptoms. Alternatively, such a strong improvement in the dopamine level may be the consequence of reduced STN output.

Our findings may prompt speculation that a kainic acid–induced lesion of the STN could provoke an increase in the activity of nigral dopamine neurons. The SNc neurons are activated as a result of STN ablation in the rat hemiparkinsonian 6-OHDA model. This is supported by deep brain stimulation studies in which high-frequency stimulation of the STN has been found to increase the activity of nigral dopaminergic neurons. 

In the present study, the results provide the first evidence that STN lesions induced by kainic acid in the rat hemiparkinsonian 6-OHDA model produced increased dopamine neurotransmission in the striatum. The results of a previous study indicated that high-frequency stimulation of the STN produced an increased level of extracellular dopamine metabolites without an increase in extracellular dopamine in rats with 6-OHDA–induced lesions in the striatum. Therefore, an alteration in dopamine neurotransmission may reflect changes in nigrostriatal dopaminergic neuronal firing or dopamine release. Moreover, we observed increased levels of dopamine and its metabolites after creation of an STN lesion in the GPE, a finding that suggests that output neurons from the STN project to the GPE, GPI, and SNr. Disinhibition of the STN neurons might increase, thereby restoring the dopamine deficit. Interestingly, STN lesions in the rat PD model produced decreased DOPAC/dopamine and HVA/dopamine ratios in the striatum. These findings prompt the speculation that increased dopamine turnover and dopamine receptor sensitization (a compensatory mechanism in surviving neurons in the parkinsonian condition) were normalized.

Our finding that dopamine levels increased in the striatum after the creation of the STN lesion might explain the disinhibition of the thalamocortical projection. The striatum is known to be influenced along nigrostriatal thalamocortical connections. The decreased basal ganglia output would lead to disinhibition of the thalamocortical projection, which could result in an increased glutamatergic input from the cortical efferent projection to the striatum. The resulting increment of cortical afferent activation would account for some mediation of parkinsonian symptoms. Alternatively, such a strong improvement in the dopamine level may be the consequence of reduced STN output.

Our findings may prompt speculation that a kainic acid–induced lesion of the STN could provoke an increase in the activity of nigral dopamine neurons. The SNc neurons are activated as a result of STN ablation in the rat hemiparkinsonian 6-OHDA model. This is supported by deep brain stimulation studies in which high-frequency stimulation of the STN has been found to increase the activity of nigral dopaminergic neurons. 
Effect of STN lesions in a rat PD model

Conclusions

We conclude that STN lesions significantly influence the striatal dopamine system. The results indicate that STN ablation can mediate the pathophysiology of PD. Nevertheless, the entire PD mechanism cannot be determined by the results of the present experimental study. Further studies are needed to characterize SNr and GPI modulation by using glutamate and GABA immunohistochemical analyses. This would present further evidence that an STN lesion may restore dopamine neurotransmission in the SNc.

Acknowledgments

Yong Sup Hwang, M.S., and Insoo Shim, Ph.D., equally contributed to this study. All authors gratefully appreciate the technical assistance of Mrs. M. Jeon.

References


Manuscript received June 1, 2005. Accepted in final form April 1, 2006.

This work was supported by Grant No. R11-2000-075-01006-0 from the SRC/ERC program of MOST/KOSEF and by a CMB-YUHAN research grant, No. 6-2005-0121, from the Yonsei University College of Medicine in 2005.

Address reprint requests to: Jin Woo Chang, M.D., Ph.D., Department of Neurosurgery, Yonsei University College of Medicine, C.P.O. Box 8044, Seoul, Korea. email: jchang@yumc.yonsei.ac.kr.