Differential expression of FosB, c-Fos, and Zif268 in forebrain regions after acute or chronic l-DOPA treatment in a rat model of Parkinson’s disease

Kosuke Ebihara a, Yasushi Ishida a,∗, Ryuichiro Takeda a, Hiroshi Abe a, Hisae Matsuo a, Keiichi Kawaji b, Yasuhiro Magata c, Toshikazu Nishimori d

a Department of Psychiatry, Faculty of Medicine, University of Miyazaki, 5200 Kihara, Kiyotake-cho, Miyazaki 889-1692, Japan
b Department of Quantum Medical Technology, Faculty of Health Sciences, Institute of Medical, Pharmaceutical and Health Sciences, Kanazawa University, 5-11-80 Kodatsuno, Kanazawa 922-8542, Japan
c Laboratory of Genome Bio-Photonics, Photon Medical Research Center, Hamamatsu University School of Medicine, 1-20-1 Handayama, Hamamatsu 431-1192, Japan
d Division of Neurobiology, Faculty of Medicine, University of Miyazaki, 5200 Kihara, Kiyotake-cho, Miyazaki 889-1692, Japan

Abstract

A study was carried out to examine the effects of acute and chronic l-DOPA treatment on the distribution of the immediate-early gene (IEG) proteins (FosB, c-Fos, and Zif268) in forebrain regions in a unilateral 6-hydroxydopamine (6-OHDA) rat model of Parkinson’s disease. During a course of chronic l-DOPA treatment (15 mg/day, 15 days), rats with a 6-OHDA lesion developed abnormal involuntary movements. Compared with the rats in the acute l-DOPA treatment group, those in the chronic treatment group had significantly more FosB-immunopositive cells in the anterior cingulate (Cg) and the dorsolateral caudate-putamen ipsilateral to the lesion. No significant difference was observed in the number of Zif268-immunopositive cells between the acute and chronic l-DOPA groups. In summary, differential expression of three IEG proteins was observed in the forebrain regions during a course of chronic l-DOPA treatment of 6-OHDA-treated hemiparkinsonian rats.

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Pharmacological dopamine (DA) replacement therapy using l-DOPA remains the most effective treatment for Parkinson’s disease (PD). However, long-term l-DOPA treatment of patients with PD often induces not only motor complications such as fluctuation and dyskinesia but also psychotic conditions such as hallucinations, delusions, and impulse control problems [14,24]. The mechanisms underlying the l-DOPA-induced motor complications have been investigated in a number of neuropharmacological studies [7]. However, few studies have investigated the mechanisms underlying the l-DOPA-induced psychotic conditions.

Induction of cellular immediate-early genes (IEGs) may be a critical signal transduction step in neuronal plasticity induced by neurotransmitters and drugs, with the protein products of the IEGs functioning to either activate or repress genes that encode the proteins involved in the differentiated functions of the target neurons. This study examined the distribution of the IEG proteins (FosB, c-Fos, and Zif268) after acute and after chronic l-DOPA treatment in the forebrain regions including the striatal and limbic structures (anterior cingulate (Cg), nucleus accumbens shell (AcbSh), caudate-putamen (CPu), and basolateral nucleus of amygdala (BLA)) in a unilateral 6-hydroxydopamine (6-OHDA) rat model of Parkinson’s disease.

The 6-OHDA lesioned rat model of Parkinson’s disease has been instrumental in studies using a variety of approaches to investigate l-DOPA-induced plasticity at multiple levels. Among the postsynaptic changes examined, the striatal expression of prodynorphin (preproenkephalin-B) mRNA and FosB/ΔFosB-like transcription factors has shown a particularly strong positive correlation with the severity of l-DOPA-induced dyskinesia [2,6,25]. Cenci found that acute injection of l-DOPA increased the levels of FosB and c-Fos in the 6-OHDA-lesioned striatum, whereas repeated administration of the drug caused downregulation of c-Fos and upregulation of FosB [5,8]. Carta et al. found that zif268 mRNA levels increased in the 6-OHDA-lesioned striatum after either acute or subchronic l-DOPA treatment [4]. However, there have been few investigations of the l-DOPA-induced IEG proteins in the brain structures other than the striatum [10].

The study reported here examined the effects of acute and chronic l-DOPA treatment on the distribution of the IEG proteins in forebrain regions in a unilateral 6-OHDA rat model of Parkinson’s disease. The experimental protocols used were approved by the ethical committees of animal experimentation at the University of Miyazaki. Anesthetized male 120–130 g Wistar rats (Charles River, Japan) received stereotaxic injections of 8-μg 6-OHDA hydrobro-

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The injections of 6-OHDA immediately and almost completely destroyed the DA neurons of the substantia nigra and of the ventral tegmental area, resulting in the near total depletion (2% of normal) of DA in the ipsilateral striatum [16].

Two weeks later, motor disturbance was assessed by counting the full rotations per min in a cylindrical container (30-cm diameter) at 10-min intervals for the first 60 min after methamphetamine (3 mg/kg, i.p.) administration. The animals that turned no less than seven times/min were included in the study.

The rats that were included were allocated into three well-matched groups (in accordance with the methamphetamine-induced rotation): (1) chronic l-DOPA treatment (n = 9), (2) acute l-DOPA treatment (n = 7), and (3) saline treatment (n = 8). Those in the first group received a daily injection of l-DOPA methyl ester hydrochloride (15 mg/kg, i.p.; Sigma, MA, USA) plus an injection of benserazide hydrochloride (15 mg/kg, i.p.; Sigma) for 15 days. Those in the second group received a daily injection of an equivalent volume of saline (i.p.) for 14 days and then an injection of l-DOPA plus benserazide on the 15th day. Those in the third received a daily injection of an equivalent volume of saline for 15 days. This pattern of l-DOPA administration was based on previous findings that circling is significantly enhanced after 7 or 8 daily injections of l-DOPA (13 mg/kg, i.p.), and that this potentiated behavioral response reaches a plateau by day 14 [15]. This enhanced response is thought to be a useful model for the l-DOPA-induced dyskinesia seen in human patients with PD [15].

The l-DOPA-induced abnormal involuntary movements (AIMs) of the rats in the chronic treatment group were scored on the 1st and 14th days in accordance with a rat dyskinesia scale [6]. For each animal and each IEG protein, three sections through the Cg, AcbSh, and dorsolateral CPu were selected for quantitative analysis [23]. FosB, c-Fos, or Zif268 immunoreactivity in the Cg, AcbSh, and dorsolateral CPu approximately 1.2 mm rostral to the bregma and three sections through the BLA approximately 2.3 mm caudal to the bregma were selected for quantitative analysis [23]. FosB, c-Fos, or Zif268 immunoreactivity in the Cg, AcbSh, CPu, and BLA was quantified by counting the number of cells immunopositive for FosB, c-Fos, or Zif268, respectively, in 0.25 mm × 0.25 mm (0.0625 mm²) squares using a 20× microscope.
Fig. 2. Photomicrographs of FosB (A–C), c-Fos (D–F), and Zif268 (G–I) expression in dorsolateral CPu ipsilateral to the 6-OHDA lesion for rats in saline (A, D, and G), acute \(\text{l}-\text{DOPA} \text{ (B, E, and H)}

objective. The numbers obtained for the three sections in each area (Cg, AcbSh, CPu, and BLA) were averaged to give the results for each animal and each IEG protein. The data are therefore presented as the number of cells immunopositive for FosB, c-Fos, or Zif268 per 0.0625 mm\(^2\) of striatal or amygdalar tissue.

The behavioral data for the AIMS were analyzed nonparametrically using the Wilcoxon signed rank test. Two-way ANOVA with repeated measures was used to statistically analyze the histological data followed by Bonferroni post hoc tests. \(P\) values < 0.05 were regarded as being statistically significant.

Of the eight rats that received chronic \(\text{l}-\text{DOPA} \text{ treatment, six reached maximal dyskinesia severity \text{(grade 4) in at least one of the four AIMS subtypes, and the other two reached grade 3 severity in at least one subtype by the end of the treatment. As shown in Fig. 1, the score for each subtype (locomotive, axial, forelimb, and oro}l{ingual} showed a significant increase (about 7-, 4-, 22-, and 14-fold, respectively) on the 14th day compared to on the 1st day of \(\text{l}-\text{DOPA} \text{ administration.}

In the saline-treated control animals, the number of FosB-immunopositive cells was significantly greater in the dorsolateral CPu on the lesion side than on the intact side (Table 1), and the number of c-Fos- and Zif268-immunopositive cells was significantly smaller in the Cg on the lesion side than on the intact side (Tables 2 and 3). In the acute \(\text{l}-\text{DOPA} \text{ treated animals, the number of FosB-immunopositive cells was significantly greater in the AcbSh and CPu (Table 1), the number of c-Fos-immunopositive cells was significantly greater in the Cg, AcbSh, CPu, and BLA (Table 2), and the number of Zif268-immunopositive cells was significantly greater in the AcbSh and CPu on the lesion side than in the corresponding regions on the lesion side of the control animals (Table 3). In the chronic \(\text{l}-\text{DOPA} \text{ treated animals, the number of FosB-immunopositive cells was significantly greater in the Cg and CPu on the lesion side than in the corresponding regions on the lesion side of the acute \(\text{l}-\text{DOPA} \text{ treated animals (Table 1 and Fig. 2), and the number of c-Fos-immunopositive cells was significantly smaller in the Cg, AcbSh, and BLA on the lesion side than in controls.}

<table>
<thead>
<tr>
<th>Structure</th>
<th>Saline Mean ± SEM of number of FosB-immunopositive cells/0.0625 mm(^2).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cg</td>
<td>7.6 ± 1.2(8)</td>
</tr>
<tr>
<td>AcbSh</td>
<td>37.3 ± 3.4(8)</td>
</tr>
<tr>
<td>CPu</td>
<td>33 ± 6.2(8)</td>
</tr>
<tr>
<td>BLA</td>
<td>6.0 ± 1.0(8)</td>
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<tr>
<th>Structure</th>
<th>Acute (\text{l}-\text{DOPA} \text{ Mean ± SEM of number of FosB-immunopositive cells/0.0625 mm(^2).}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cg</td>
<td>6.4 ± 0.4(7)</td>
</tr>
<tr>
<td>AcbSh</td>
<td>62.5 ± 6.3(5)</td>
</tr>
<tr>
<td>CPu</td>
<td>76.9 ± 7.4(5)</td>
</tr>
<tr>
<td>BLA</td>
<td>5.9 ± 1.1(5)</td>
</tr>
</tbody>
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<tr>
<th>Structure</th>
<th>Chronic (\text{l}-\text{DOPA} \text{ Mean ± SEM of number of FosB-immunopositive cells/0.0625 mm(^2).}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cg</td>
<td>13.5 ± 1.0(8)</td>
</tr>
<tr>
<td>AcbSh</td>
<td>78.2 ± 7.6(7)</td>
</tr>
<tr>
<td>CPu</td>
<td>102.1 ± 4.4(7)</td>
</tr>
<tr>
<td>BLA</td>
<td>17.2 ± 7.0(5)</td>
</tr>
</tbody>
</table>

\(1\) \(P<0.05\) vs. corresponding side of saline group. \(2\) \(P<0.01\) vs. corresponding side of saline group. \(3\) \(P<0.05\) vs. corresponding side of acute \(\text{l}-\text{DOPA} \text{ group.}

\(4\) \(P<0.05\) vs. lesion side of each structure/group. \(5\) \(P<0.05\) vs. lesion side of each structure/group. \(6\) \(P<0.01\) vs. lesion side of each structure/group.
than in the corresponding regions on the lesion side of the acute l-DOPA treated animals (Table 2). Statistical analysis of the number of FosB-immunopositive cells in the AcbSh showed that the main effects of group (saline, acute l-DOPA, chronic l-DOPA), of latency (lesion side, intact side), and of group × latency were significant [group: F(2,19) = 35.0, P < 0.01; latency: F(1,19) = 31.2, P < 0.01; group × latency: F(2,19) = 7.8, P < 0.01]. However, subsequent post-hoc Bonferroni tests showed no significant difference between the acute and chronic l-DOPA groups (P = 0.28) (Table 1).

A significant difference was not found for the number of FosB-immunopositive cells in the BLA among the three groups and between the two sides [group: F(2,15) = 3.8, P = 0.05; latency: F(1,15) = 3.3, P = 0.09; group × latency: F(2,15) = 2.6, P = 0.11].

In summary, comparison of the results of the acute and chronic l-DOPA treatment revealed that the chronic treatment resulted in a significant upregulation of FosB in the Cg and CPu and in a significant downregulation of c-Fos in the Cg, AcbSh, and BLA on the lesion side.

The results of this study show that chronic l-DOPA treatment of 6-OHDA rats causes an increase in the number of FosB-immunopositive cells not only in the dorsolateral CPu but also in the Cg on the lesion side. It also causes a decrease in the number of c-Fos-immunopositive cells in the Cg, AcbSh, and BLA on the lesion side. These results are the first to demonstrate that chronic l-DOPA treatment of 6-OHDA-lesioned rats can alter the FosB and c-Fos expressions in forebrain regions other than the CPu or nucleus accumbens (Acb).

The FosB/ΔFosB-like transcription factors in the striatum of dyskinetic animals account for a pronounced increase in protein-DNA binding activities at both AP-1 enhancers and cyclic AMP response elements [3]. These transcription factors have been implicated in the dyskinesia priming process by a study using antisense oligonucleotides against fosB/ΔfosB mRNA [2]. Similar to the findings of a previous study [11], this study found enhanced FosB expression in the dorsolateral CPu on the lesion side of 6-OHDA rats without l-DOPA treatment. This enhanced expression was also found in the CPu and AchSh of both acute- and chronic-treated l-DOPA treated and in the Cg of the chronic l-DOPA treated rats.

The BLA has been implicated in the response to affective stimuli and in the process of conditioned reinforcement [12]. Furthermore, the BLA has a substantial projection directly to the Acb [1] and indirectly via the dorsal prefrontal cortex including the Cg [19]. The Acb area is thought to be integral to the reinforcing actions of drugs of abuse and to drug-induced psychosis. Thus, the BLA may be, at least in part, involved functionally in the mechanisms underlying l-DOPA-induced psychotic conditions. In agreement with a previous study [10], c-Fos expression was found to be induced by l-DOPA administration in the BLA ipsilateral to the lesion, especially for the rats in the acute l-DOPA treatment group. Additional findings, especially for FosB expression, were not observed in the immunohistochemical study.

Numerous studies of patients with schizophrenia or drug-induced psychosis have demonstrated functional or morphological alterations in the forebrain regions, including the Cg and Acb [13,18]. The Cg area is crucial for integrating cognitive and emotional processes in support of goal-directed behavior [13,22]. Therefore, the results of this study’s immunohistochemical examination may be related to the pathogenesis of not only motor complications but also psychotic conditions induced by chronic l-DOPA treatment, implicated in schizophrenia and methamphetamine- or other drug-induced psychosis. Clearly, further work is needed to define the causal mechanisms of l-DOPA-related adverse events.

In conclusion, differential expression of FosB, c-Fos, and Zif268 was observed in four forebrain regions (Cg, AcbSh, CPu, and BLA) during a course of chronic l-DOPA treatment of 6-OHDA-treated hemiparkinsonian rats.

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