Brief Communication

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# Changes in Dopamine $D_2$ Receptors and 6-[$^{18}$F]Fluoro-*L*-3,4-Dihydroxyphenylalanine Uptake in the Brain of 6-Hydroxydopamine-Lesioned Rats

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**Key Words**
6-[$^{18}$F]Fluoro-*L*-3,4-dihydroxyphenylalanine · 6-Hydroxydopamine · Cerebral cortex · Dopamine receptors · Rat · Striatum

**Abstract**
We studied tracer distributions in positron emission tomography of ligands for dopamine $D_1$ receptors ([$^{11}$C]SCH23390) and $D_2$ receptors ([$^{11}$C]raclopride) and the dopamine precursor analog 6-[$^{18}$F]fluoro-*L*-3,4-dihydroxyphenylalanine ([$^{18}$F]FDOPA), as a measurement of presynaptic dopaminergic function, in the brain after 6-hydroxydopamine lesioning of the medial forebrain bundle in rats. The unilateral lesions were confirmed behaviorally by methamphetamine-induced rotation 2 weeks after lesioning, and the brains were analyzed by tissue dissection following an intravenous bolus of each tracer 3 weeks after lesioning. [$^{11}$C]Raclopride, but not [$^{11}$C]SCH23390, showed a higher accumulation in the striatum on the lesion side compared with that on the non-lesioned (intact) side. On the other hand, a lower accumulation of [$^{18}$F]FDOPA was found in the striatum and cerebral cortex on the lesion side. Our studies demonstrate upregulation of dopamine $D_2$ receptors in the striatum and a decrease in FDOPA uptake in both the striatum and cerebral cortex ipsilateral to the 6-hydroxydopamine lesions. Therefore, the combination of a $D_2$ antagonist and FDOPA may provide a potentially useful method for assessing the effects of dopamine depletion in Parkinson's disease.



## Introduction

6-[$^{18}$F]Fluoro-*L*-3,4-dihydroxyphenylalanine ([$^{18}$F]FDOPA) is a positron-emitting analog of the dopamine precursor *L*-DOPA and one of the earliest [$^{18}$F]-labeled compounds proposed as imaging agents for use in the external in vivo examination of the central nervous dopaminergic system [1]. Therefore, [$^{18}$F]FDOPA has been used as an imaging agent in the study of dopamine terminals in the living brain using positron emission tomography [2, 3].

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Experimental animals in which the nigrostriatal pathway is destroyed are considered to be useful models of Parkinson's disease. One popular model in rats is produced by the unilateral stereotaxic injection of the neurotoxin 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle or substantia nigra [4]. In this model, there is an immediate and almost complete destruction of the dopamine neurons of the substantia nigra and of the ventral tegmental area, resulting in near total depletion (2% of normal) of dopamine in the ipsilateral striatum to the 6-OHDA injections [5]. Immunohistochemical study showed there is no evident damage of dopaminergic neurons and fibers in the brain contralateral to the 6-OHDA injections [5, 6]. There have been many reports of enhanced behavioral (rotational behavior) effects of directly acting dopamine agonists such as apomorphine after unilateral 6-OHDA lesioning. The rotational behavior induced by dopamine agonists has been attributed to postsynaptic dopamine receptor supersensitivity in the striatum that occurs following the unilateral destruction of mesostriatal dopamine fibers [7]. From these observations, it is conceivable that the 6-OHDA-lesioned rat model may be useful for evaluating brain functions in Parkinson's disease. However, little is known about changes in dopamine receptors and FDOPA uptake in the brain following 6-OHDA lesioning under the same experimental conditions.

In the present study, therefore, we studied the tracer distributions of ligands for dopamine $D_1$ receptors ([$^{11}$C]SCH23390), $D_2$ receptors ([$^{11}$C]raclopride) and of [$^{18}$F]FDOPA in the brain after 6-OHDA lesioning of the medial forebrain bundle in rats.

## Materials and Methods

The subjects were male Wistar rats (Kiwa Laboratory Animals, Kaisou, Japan) weighing 120–130 g at the beginning of the experiment. The experimental protocols used in this study were approved by the ethical committees of animal experimentation at the Miyazaki Medical College and Kyoto University. Rats were anesthetized with pentobarbital (40 mg/kg, i.p.), and unilateral lesions of the left medial forebrain bundle were made by injecting 8 μg of 6-OHDA hydrobromide (Sigma, St. Louis, Mo., USA) in 4 μl of sterile saline containing 0.01% ascorbic acid. Stereotaxic coordinates for the lesions were as follows: anteroposterior 3.2 mm rostral to the interaural line, 1.3 mm to the left of the midline, and 6.7 mm ventral to the dural surface, with the incisor bar set 2.4 mm below the level of the ear bars [8]. Two weeks after the 6-OHDA treatments, the relative completeness of the lesions was confirmed by testing for rotation following intraperitoneal injection of methamphetamine (3 mg/kg) [5, 6]. Animals accomplishing no less than 7 turns/min on the methamphetamine challenge were included in the study.

[$^{11}$C]SCH23390 was synthesized by the [$^{11}$C]methylation reaction of SCH24518 in an automated synthesis apparatus (CUPID C-100, Sumitomo Heavy Industries, Tokyo, Japan), according to the method reported by Halldin et al. [9], with a slight modification. The radiochemical purity of [$^{11}$C]SCH23390 was >97.4%. The specific activity of the product was 38.5–81.4 GBq/μmol. [$^{11}$C]Raclopride was synthesized by [$^{11}$C]methylation of *O*-desmethylraclopride in the CUPID C-100. The radiochemical purity of [$^{11}$C]raclopride was >98.4%. The specific activity of the product was 32.6–88.9 GBq/μmol. [$^{18}$F]FDOPA was synthesized in a multipurpose synthetic system [10], according to the method reported by Namavari et al. [11], with a slight modification. The radiochemical purity of [$^{18}$F]FDOPA was >98.5%. The specific activity of the product was 33.0–33.7 MBq/μmol.

The regional distribution of radioactivity in the rat brain was examined after intravenous injection of [$^{11}$C]SCH23390, [$^{11}$C]raclopride or [$^{18}$F]FDOPA 3 weeks after the 6-OHDA lesions. In previous studies, many investigators reported that both degeneration of nigral dopaminergic neurons and dopamine receptor supersensitivity in the denervated striatum have been established at this time point after 6-OHDA lesioning [12, 13]. One hour ([$^{11}$C]SCH23390 and [$^{11}$C]raclopride) or 1.5 h ([$^{18}$F]FDOPA) after the intravenous injection, the rats were killed by decapitation, and the brains (each side) were dissected on ice into the different brain areas (striatum, cerebral cortex and cerebellum) as described by Glowinski and Iversen [14] for the rat brain. Brain samples were weighed, and radioactivity accumulation of each tracer was determined for each brain region bilaterally. Radioactivity was measured with a scintillation counter (model 5003, Packard, Downers Grove, Ill., USA). Firstly, results were expressed as radioactivity per unit wet tissue weight (cpm/g). Then, the biodistribution of [$^{11}$C]SCH23390, [$^{11}$C]raclopride or [$^{18}$F]FDOPA was computed as a ratio of the lesion side to the intact side of each brain region of the 6-OHDA rats.

Data of the lesion side/intact side ratio of each brain region were analyzed nonparametrically using the Wilcoxon signed rank test. p values $<0.05$ were regarded as significant.

## Results

Table 1 shows the biodistribution of [$^{11}$C]SCH23390, [$^{11}$C]raclopride and [$^{18}$F]FDOPA in three brain regions (cerebral cortex, striatum, and cerebellum) on the lesion side of the 6-OHDA rats, represented as the ratio to the intact side of the brain. In the striatum on the lesion side, the biodistribution of [$^{11}$C]raclopride was significantly higher ($p < 0.01$; 191% of the intact side) than that of the striatum on the intact side of the 6-OHDA rats. On the other hand, the biodistribution of [$^{18}$F]FDOPA was lower in both the striatum ($p < 0.05$; 80% of intact side) and cerebral cortex ($p < 0.05$; 89% of intact side) on the lesion side compared with the intact side. Regarding the three regions, there was no significant difference in the biodistribution of [$^{11}$C]SCH23390 between the lesion and intact sides in any of the three regions of the brain.

**Table 1.** Biodistribution of each tracer in three brain regions of the 6-OHDA rats

| Tracer | Region | Lesion side/intact side, cpm/g |
|---|---|---|
| e[$^{11}$C]SCH23390 (n = 7) | cerebral cortex | 1.04 ± 0.03 |
| | striatum | 1.11 ± 0.02 |
| | cerebellum | 1.00 ± 0.00 |
| [$^{11}$C]Raclopride (n = 9) | cerebral cortex | 1.14 ± 0.02 |
| | striatum | 1.91 ± 0.04** |
| | cerebellum | 1.01 ± 0.01 |
| [$^{18}$F]FDOPA (n = 9) | cerebral cortex | 0.89 ± 0.02* |
| | striatum | 0.80 ± 0.01* |
| | cerebellum | 0.99 ± 0.00 |

* $p < 0.05$, ** $p < 0.01$, vs. corresponding values of the intact side (Wilcoxon signed rank tests).

## Discussion

In the present study, the biodistribution of [$^{11}$C]SCH23390 did not change in any brain region of the 6-OHDA animals. An upregulation of dopamine $D_1$ receptors after lesioning of the medial forebrain bundle has been demonstrated in receptor autoradiographic studies [15, 16]. Furthermore, supersensitivity of dopamine $D_1$ receptors has been reported in striatal tissue homogenates after medial forebrain bundle lesioning with 6-OHDA [17, 18]. In contrast, there are other contradicting results showing no change or a decrease in the density of striatal dopamine $D_1$ receptors after denervation of the nigrostriatal dopaminergic pathway [19, 20]. The precise reason for these discrepancies is unknown. However, the discrepancies may be related to different methods (e.g. radioligand binding assay, autoradiography) used and/or different time points after 6-OHDA lesioning in the earlier studies.

On the other hand, denervation of the nigrostriatal pathway can cause the postsynaptic population of dopamine $D_2$ receptors to become supersensitive [15, 16]. These observations are consistent with our present findings. Furthermore, consistent with the present findings, Araki et al. also observed that the upregulation in $D_2$ receptors was more pronounced than that in $D_1$ receptors in the striatum after 6-OHDA lesioning [21, 22].

The present study also showed that [$^{18}$F]FDOPA uptake significantly decreased in both the striatum and cerebral cortex ipsilaterally after unilateral 6-OHDA lesioning. The degree of the decrease in the cerebral cortex, however, was relatively slight compared with that in the striatum. A lowered presynaptic dopamine function was found not only in the striatum but also in the cerebral cortex on the lesion side. The [$^{18}$F]FDOPA uptake was reported to increase [23] or decrease [24] in the cerebral cortex in patients with Parkinson's disease. Taken together with the evidence that the degree of dopamine denervation in the 6-OHDA animals was almost complete [5], the present findings suggest that the [$^{18}$F]FDOPA uptake may decrease in the cerebral cortex in parkinsonian patients in an advanced stage. Further studies on the tracer distributions in rats with partial 6-OHDA lesions and/or in the acute stage (e.g. days 1–7) following the complete 6-OHDA lesioning may clarify the issue.

FDOPA is an exogenous substrate of DOPA decarboxylase which is the enzyme directly responsible for the synthesis of dopamine and serotonin, and indirectly of noradrenaline. In the central nervous system, DOPA decarboxylase is localized in both monoaminergic neurons [25] and a subset of non-monoaminergic neurons [26]. In the cerebral cortex, afferent fibers of serotonin and noradrenaline are distributed in most areas [27]. Previous cerebral dialysis and immunohistochemical studies showed a slight decrease in serotonin overflow and immunoreactivity, respectively, in the denervated striatum in 6-OHDA rats [5]. Thus at least some of the cortical FDOPA uptake could be accounted for serotonergic and/or noradrenergic neuron innervation.

In conclusion, the present study demonstrates upregulation in dopamine $D_2$ receptors in the striatum and decrease in FDOPA uptake in both the striatum and cerebral cortex ipsilateral to the 6-hydroxydopamine lesions. Therefore, the combination of a $D_2$ antagonist and FDOPA may provide a potentially useful method for assessing the effects of dopamine depletion in Parkinson's disease. However, we must be careful not to interpret the results illogically. In spite of many similarities, the characteristics of acute 6-OHDA lesions may be rather different from the slowly progressive course of Parkinson's disease in humans.

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