Characteristic Upregulation of Dopamine D₁-Receptor in Rat Striatum after 6-Hydroxydopamine Treatment

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ABSTRACT—We measured the number of D₁-receptors ($B_{\max}$) in the striatum whose dopaminergic terminals were destroyed to various degrees by 6-hydroxydopamine (6-OHDA) to clarify the relationship between the degree of denervation and the change in $B_{\max}$ for D₁-receptor. Rats were sacrificed 28 days after 6-OHDA treatment, and [³H]SCH23390 (*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine) binding capacity and monoamine content were measured in striatal homogenate. $B_{\max}$ was significantly upregulated when dopamine content was reduced to less than 5% of that in the vehicle-treated striatum. In contrast, it was significantly decreased when dopamine content was 5–25% of that in the vehicle-treated striatum. The D₁-receptors showed either upregulation or downregulation depending on the degree of denervation.

Keywords: [³H]SCH23390, 6-Hydroxydopamine, Upregulation

The cause of Parkinson's disease is thought to be degeneration of dopaminergic neurons in the substantia nigra. Consequently, dopamine content in the striatum is severely reduced in the disease. 6-Hydroxydopamine (6-OHDA) destroys catecholaminergic terminals after uptake into the terminals. Thus, 6-OHDA has been used to make an animal model of Parkinson's disease.

In general, postsynaptic receptors are upregulated in compensation for decreased release of neurotransmitter when nerve terminals controlling the receptors are damaged. It is well-documented that the D₂-receptor is upregulated after denervation of dopaminergic terminals by 6-OHDA. On the contrary, there are controversial results about the 6-OHDA-induced alteration in the number of D₁-receptors, as to whether it is decreased (1), increased (2, 3) or unchanged (4). Furthermore, using an autoradiographic assay for D₁-receptors, researchers also found an inconsistent alteration in the number of D₁-receptors (5–9). There are some reports that attempted to attribute this inconsistency to variations in the time periods used after 6-OHDA treatment (6, 8). Fornaretto et al. (8) indicated that upregulation of D₁-receptors exhibited a longer lag-time than D₂-receptors. However, Marshall et al. (6) observed no upregulation of D₁-receptors 2 weeks, 8 weeks or 11 months after 6-OHDA treatment. We believe that the degree of destruction is another important factor in the upregulation of D₁-receptors.

Various doses of 6-OHDA were administered into the striatum to destroy dopaminergic terminals to different degrees to determine if there is a relationship between the degree of destruction of D₁-receptors and the alteration in their number.

Male 8–9-week-old Wistar rats (Japan SLC Co., Hamamatsu), weighing 240–260 g, were used. The animals were housed with free access to standard food in an air-conditioned room under a constant 12-hr light and dark cycle (lights on at 7:00 a.m.) at a temperature of 22–24°C and 60–70% relative humidity. Rats were anesthetized with 50 mg/kg pentobarbital (Nembutal; Abbott Laboratories, North Chicago, IL, USA), s.c. and ether. 6-OHDA (Sigma Chemical Co., St. Louis, MO, USA) was dissolved in 0.9% saline with 0.1% ascorbic acid. Various doses of 6-OHDA (2.5–60 µg) were administered into two separate regions of the left striatum using a 10-µl microsyringe (Hamilton Co., Reno, NV, USA) and an infusion pump (Natume Seisakujo Co., Ltd., Tokyo) at a rate of 1.0 µl/min. The tip of the syringe was inserted vertically using a stereotaxic apparatus (Narishige Scientific Instrument Lab., Tokyo) through a small hole in the skull 3-mm lateral to the bregma and 5-mm-deep as measured from the surface of the skull. The second injection was 2-mm rostral to the first lesion. The syringe remained in place for 2 min following the infusion to minimize the spread of the drug.

through injection tracks. The volume of the 6-OHDA solution was 2 μ l per lesion, and the concentration of 6-OHDA solution was 0.625–15 μ g/ μ l. Vehicle was administered into the right striatum, but otherwise its injection was performed similarly to the 6-OHDA injection.

Rats were decapitated 28 days after the drug infusion. The striatum of each rat was rapidly removed on an ice-cold glass plate and then homogenized by a Polytron (Kinematica Co., Lucerne, Switzerland) at setting 6 for 20 sec in 1 ml of 50 mM Tris/HCl (pH 7.4), 120 mM NaCl and 5 mM KCl (Tris buffer). Part of the homogenate was used for the protein assay and part for the monoamine assay. The rest of the homogenate was centrifuged at 30,000 $\times g$ for 10 min at 4°C, and the pellet was used for the binding assays.

[³H]SCH23390 (*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine) (specific activity 316.5 GBq/mmol; Du Pont Co., Boston, MA, USA) was employed as a ligand for specific D₁-receptor binding. Striatal membrane (0.1 mg protein; protein concentration, 0.1 mg/ml) was incubated with various concentrations (0.1–2.0 nM) of [³H]SCH23390 at 37°C for 15 min. The reaction was initiated by adding the membranes to tubes containing [³H]SCH23390, terminated by the addition of 4 ml ice-cold Tris buffer, and rapidly

filtrated through Whatman GF/B glass-fiber filters (Whatman International, Ltd., Maidstone, England). The filters were washed twice with 4 ml ice-cold Tris buffer, dried and radioactivity determined by liquid scintillation counting (LSC-903; Aloka Co., Ltd., Tokyo) at 40% efficiency. Specific [³H]SCH23390 binding was defined as that displaced by incubation with 30 μ M *R*(+)-SKF38393 HCl (Research Biochemicals, Inc., Natick, MA, USA).

[³H]Spiperone (specific activity of 888 GBq/mmol, Du Pont Co.) was used as a specific ligand for D₂-receptor binding at various concentrations (0.025–0.3 nM) of [³H]spiperone, and non-specific binding was determined using 1 μ M (+)butaclamol (Research Biochemicals, Inc.). Otherwise, the procedure was the same as that for the [³H]SCH23390 binding assay. A 5-HT₂ receptor blocker, such as ketanserin, was not used in the [³H]spiperone binding assay because we already reported that the specific [³H]spiperone binding curve is monophasic when the concentration of [³H]spiperone is less than 0.5 nM (10).

The striatal homogenate was mixed with 1 ml 0.1 M perchloric acid containing 5 mM ethylenediaminetetraacetic acid (EDTA) and 3,4-dihydroxybenzylamine (DHBA) (Sigma) and stored at –40°C. Monoamine content was measured by high-performance liquid chro-

Fig. 1. Relationship between the loss of dopaminergic terminals in the striatum and B_{max} of [³H]SCH23390 binding after 6-OHDA treatment. The abscissa is the percent of dopamine (DA) content in 6-OHDA-treated striatum compared with contralateral vehicle-treated striatum. The ordinate is the change in B_{max} $\pm$ S.E. in 6-OHDA-treated striatum compared with contralateral vehicle-treated striatum. The number of rats in the groups in which dopamine contents were less than 5%, 5–15%, 15–25% and more than 25% of the control level was 5, 11, 6 and 4, respectively. *Statistically different from vehicle-treated contralateral striatum as determined by Student's *t*-test (*P* < 0.05).

Fig. 2. Relationship between the loss of dopaminergic terminals in the striatum and B_{max} of [³H]spiperone binding after treatment with 6-OHDA. The abscissa is the percent of dopamine (DA) content in 6-OHDA-treated striatum compared with vehicle-treated striatum. The ordinate is the change of B_{max} $\pm$ S.E. in 6-OHDA-treated striatum compared with vehicle-treated striatum. The number of rats whose dopamine content was less than 10% and 10–20% as compared with the vehicle-treated contralateral striatum was 6 and 4, respectively. *Statistically different from vehicle-treated contralateral striatum by Student's *t*-test (*P* < 0.05).

matography (HPLC) with an electrochemical detector (ECD) as described previously (10).

Protein concentration in striatal homogenate was determined by the Bradford protein assay using dye reagent purchased from Bio-Rad Laboratories (Hercules, CA, USA). Bovine serum albumin was used as the standard.

There was a significant alteration in the $B_{\max}$ for D_1 -receptors in the striatum whose dopamine content was less than 5% compared with contralateral vehicle-treated striatum (Fig. 1). The $B_{\max}$ was decreased by 16–17% when the dopamine content was 5–25% of the control. When the dopamine content was greater than 25% of the control, there was no change in the number of D_1 -receptors. Dopamine content (mean $\pm$ S.E.) in the vehicle-treated striatum ($n=26$) was 129 ± 5.5 ng/mg protein, and $B_{\max}$ (mean $\pm$ S.E.) of the vehicle-treated controls ($n=26$) was $1,362 \pm 66$ fmol/mg protein. The K_d for [3 H]-SCH23390 binding (mean $\pm$ S.E.) in 6-OHDA ($n=26$)- and vehicle ($n=26$)-treated striata were 350 ± 25 and 360 ± 31 pM, respectively.

On the other hand, D_2 -receptors were upregulated when the dopamine content was decreased to approximately 10% of control level (Fig. 2). Dopamine content (mean $\pm$ S.E.) in the vehicle-treated striatum ($n=10$) was 139 ± 5.4 ng/mg protein, and the $B_{\max}$ (mean $\pm$ S.E.) for the vehicle-treated striatum was 615 ± 47 fmol/mg protein ($n=10$). The K_d for 6-OHDA ($n=10$)- and vehicle ($n=10$)-treated striata were 30.8 ± 2.3 and 30.6 ± 1.9 pM, respectively.

The [3 H]spiperone binding assay showed upregulation of D_2 -receptors induced by the denervation of dopaminergic terminals. However, the relationship between the degree of denervation in dopaminergic terminals and upregulation of D_1 -receptors is not simple. The $B_{\max}$ of [3 H]SCH23390 binding was not increased until dopamine content was reduced by more than 95%. Furthermore, the $B_{\max}$ was decreased when the dopamine content was between 5% and 25% of the control level. Previous studies indicated that the D_1 -receptor was upregulated after denervation by 6-OHDA when more than 97% of the dopaminergic terminals were destroyed (11). Therefore, prominent dopaminergic terminal denervation is required in order for D_1 -receptors to be upregulated.

As mentioned earlier, time is an important factor in D_1 -receptor upregulation. Fornaretto et al. (8) showed that D_1 -receptors were upregulated 16 to 90 days after 6-OHDA treatment, while D_2 -receptors were already increased after 15 days. We sacrificed rats 28 days after treatment, since this should be enough time to observe D_2 -receptor upregulation (10). It is possible that upregulated D_1 -receptors return to the control level after a longer lag-time, e.g., one year as Fornaretto et al. suggested (8). However, we are uncertain if upregulation in moder-

ately denervated striatum (whose dopamine content was 5–25% of the control level) can occur longer than 28 days after 6-OHDA.

The $B_{\max}$ for D_1 -receptors in rats whose dopamine content is less than 5% of the control showed a 42% increase compared with the $B_{\max}$ in a group of rats whose dopamine content was 5–15% of the control. However, the $B_{\max}$ for 6-OHDA-treated animals is only increased by 19% when expressed as a percentage of the vehicle-treated contralateral striatum. Therefore, if the slight decrease in the $B_{\max}$ for D_1 -receptors due to moderate denervation is neglected, only a small increase in $B_{\max}$ would be seen. That may be one reason other investigators reported no significant change in the number of D_1 -receptors after denervation by 6-OHDA. Recently, Marcotte et al. (12) suggested one possible explanation for why it is difficult to upregulate D_1 -receptors. They showed an increase in the D_1 -receptor-linked G-protein, G_s and G_{olf} , with only a small increase in the D_2 -receptor-linked G-protein, G_i and G_o . They attributed difficulty in upregulation of D_1 -receptors to the increase in G-proteins coupled to D_1 -receptors.

We observed a decrease in D_1 -receptors when the degree of denervation was moderate. This result coincides with several studies in which denervation of dopaminergic terminals were insufficient. We are unsure as to the mechanism. A decrease in the production of D_1 -receptors (9, 13), degeneration of dendrites that bear D_1 -receptors (2), and an alteration in the distribution pattern of D_1 -receptors (14) have been suggested. However, existence of D_1 -receptors on dopaminergic terminals is not possible, since a decrease in D_1 -receptors was not observed when dopamine content was greater than 25% of the control level.

In conclusion, we observed either a decrease or an increase in D_1 -receptors after 6-OHDA treatment depending on the dose administered. D_1 -receptors could be upregulated after 6-OHDA treatment as could D_2 -receptors. However, dopaminergic terminals must be mostly destroyed for upregulation to occur, and the number of D_1 -receptors was decreased if the denervation was insufficient.

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