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Enhancement of Rotational Behavior Induced by Repeated Administration of SKF38393 in Rats with Unilateral Nigrostriatal 6-OHDA Lesions

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MATSUDA, H., Y. HIYAMA, K. TERASAWA, H. WATANABE AND K. MATSUMOTO. *Enhancement of rotational behavior induced by repeated administration of SKF38393 in rats with unilateral nigrostriatal 6-OHDA lesions.* PHARMACOL BIOCHEM BEHAV **42**(2) 213–218, 1992.—To clarify if the enhancement of rotational behavior induced by repeated administration of SKF38393 is mediated by upregulation of D_1 and/or D_2 receptors in the striatum, we investigated effects of SCH23390 and sulpiride on SKF38393-induced rotational behavior and the changes in striatal dopamine receptors in rats with unilateral nigrostriatal 6-hydroxydopamine lesions (1). Repeated weekly administration of SKF38393 markedly enhanced the number of rotations and shortened the latency of rotational behavior depending on the number of SKF38393 administrations 1 or 6 weeks after the treatment with 6-OHDA (2). A selective D_1 antagonist, SCH23390, but not a selective D_2 antagonist, sulpiride, suppressed SKF38393-induced rotation and inhibited the enhancement by the repeated administration (3). Repeated administration of SKF38393 did not modify the density and the affinity of either the striatal D_1 or D_2 receptors in the striatum. These results suggest that the enhancement of SKF38393-induced rotational behavior by the repeated administration is not associated with the upregulation of striatal D_1 and D_2 receptors.

6-OHDA Nigrostriatal pathway Rotational behavior SKF38393 SCH23390 Sulpiride
D_1 and D_2 dopaminergic receptors

INTRODUCTION

RATS with unilateral nigrostriatal dopaminergic lesions made by 6-hydroxydopamine (6-OHDA) show a contralateral rotation when direct stimulants of either dopamine (DA) D_1 or D_2 receptors were administered.

A selective D_1 agonist, SKF38393, produced lasting contralateral rotation in naive 6-OHDA-lesioned rats (6), and the rotational behavior was enhanced by a single previous exposure to a stimulant of D_1 and/or D_2 receptors (7,8). However, SKF38393-induced rotation was completely stopped by pretreated continuous infusion of SKF38393 into the striatum for 3–7 days in mice with unilateral 6-OHDA-induced lesions (18). These results suggest that a single previous stimulation of D_1 and/or D_2 receptors may sensitize the receptors to the agonists, and continuous infusion of SKF38393 may desensitize the receptors in the striatum to the agonists.

However, the mechanism of sensitization and desensitization due to D_1-receptor stimulation are not fully elucidated. To clarify a mechanism of enhancement of rotational behavior induced by repeated administration of SKF38393, we investigated effects of SCH23390 and sulpiride on SKF38393-induced rotational behavior and changes in striatal DA receptors in rats with unilateral nigrostriatal 6-OHDA lesions.

METHOD

Animals

Male Wistar rats (Japan SLC corp., Hamamatsu, Japan), weighing 160–180 g at the time of surgery, were used. Animals were housed four to a cage at 22°C room temperature, had free access to food and water, and were maintained in a 12 L: 12 D cycle (lights on 0720–1920).

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6-OHDA Treatment

Sixty min after administration of desipramine hydrochloride (25 mg/kg, IP), animals were anesthetized with sodium pentobarbital (50 mg/kg, IP) and placed in a stereotaxic apparatus with the incisor bar 2.5 mm below the interaural line. A burr hole was drilled to the left of the midline at the following coordinates relative to the interaural line: anterior +2.8 mm, lateral +1.7 mm. A stainless steel guide cannula (diam. 0.5 mm) was positioned vertically with the tip at a depth of 7.7 mm below the dura. 6-OHDA HBr (8 μg/4 μl 0.05% ascorbic acid in 0.9% NaCl) was injected at the rate of 1 μl/min. After the 6-OHDA treatment the animals were left for 1, 3, and 6 weeks until the behavioral study.

Behavioral Study

Each animal was placed in a rotational chamber and habituated for at least 10 min. SKF38393 (2.5 mg/kg) was injected SC. The rotational chamber consists of a hemispherical bowl with a flat floor, surrounded by cylindrical walls with an average height of 30 cm. The bowl has a 35-cm top diam., a 30-cm bottom diam., and is 20 cm high. The harness is made of a Velcro waistband with a plastic plate connected to the counter switch with a 0.35-mm diam. steel wire. Rotation was counted by our computerized counter system that is a modified "Rotometer" (16). When testing effects of DA antagonists on SKF38393-induced rotational behavior, SCH23390 (5–500 μg/kg) and sulpiride (5–50 mg/kg) were SC injected 30 and 60 min before SKF38393 administration, respectively.

Dopamine Analysis

The tissue samples (wet weight from 2 to 5 mg) from sacrificed rats were divided at the center of each individual striatum to measure the DA contents. The tissue samples were homogenized in 1 ml of 0.15 N perchloric acid solution containing 1.7 mM L-cysteine monohydrochloride monohydrate at 4°C. The homogenates were centrifuged 10,000 *g* × 10 min at 4°C. The supernatants, to which 1 ml Tris HCl buffer (1.5 M, pH 8.5) was added, were absorbed to alumina. Catecholamines were eluted from alumina with 300 μl of 0.15 N perchloric acid solution with 0.2% L-cysteine and quantitated using HPLC with a TSK-GEL (TOSOH, Tokyo, Japan) column and an electrochemical detector (Eicom ECD-100, Kyoto, Japan) (17).

Radiolabeled Receptor Assay

DA-receptor binding assays were by a modification of the method of Sibley et al. (12). Striatal tissue was homogenized in 5 ml ice-cold buffer (Tris HCl 50 mM; KCl 5 mM; NaCl 120 mM; $CaCl_2$ 1 mM; $MgCl_2$ 1 mM, pH 7.4) for 20 min at 4°C. The homogenates were centrifuged 50,000 *g* for 20 min at 4°C. The pellet was suspended in 5 ml buffer and recentrifuged under the same condition. This process was repeated three times. The final pellet was resuspended in 5 ml buffer and diluted to 0.1 mg protein/ml. [^{3}H]SCH23390 (AMERSHAM, Tokyo, Japan) and [^{3}H]spiperone (AMERSHAM, Tokyo, Japan) were used to assay D_1 and D_2 DA receptors. One μM SCH23390 and 10 μM sulpiride were also used as competitors to define the nonspecific binding for D_1 and D_2 receptors, respectively. To prevent the cross reaction between ligands and serotonergic receptors, 100 nM ketanserine was added to the tubes. Five different concentrations of [^{3}H]SCH23390 ranging from 0.1–2.0 nM and [^{3}H]spiperone ranging from 0.1–1.0 nM were used to assay binding in a final volume of 1 ml. The samples were incubated in the 25°C water bath for 90 min and diluted with 3 ml ice-cold buffer (50 mM Tris HCl, pH 7.7) and rapidly filtered through Whatman (GF/B) glass fiber filters under vacuum followed by three rapid (4-ml) washes with the same buffer. The radioactivity was measured by liquid scintillation counter in 4 ml Clear-sol 1 (Nacalai Tesque Inc., Kyoto, Japan). Scatchard plots were constructed from saturation data and least-square linear regression analysis was performed to calculate the dissociation constant (K_d) and maximum number of binding sites (B_{max}).

Drugs

The drugs used in this study included sodium pentobarbital (Abbott Laboratories, North Chicago, IL); 6-hydroxydopamine hydrobromide, sulpiride, and desipramine HCl (Sigma Chemical Co., St. Louis, MO); (±)SKF38393 and R(+)SCH233390 (Research Biochemicals Inc., MA, USA). (±)Sulpiride that was dissolved in 0.1 N HCl and adjusted to pH 7.4 with 0.5 M Tris HCl was diluted with saline.

RESULTS

Single Administration of SKF38393

The representative rotational behaviors that were induced by repeated administration of SKF38393 once a week, 3 weeks after 6-OHDA treatment, are shown in Fig. 1. Although the duration of each rotational behavior was almost the same, the number of rotations per 10 min was remarkably increased on and after a second application of SKF38393 (Fig. 1A). Similarly, the number of rotations that were accumulated for 60 min after rotation was induced increased remarkably (Fig. 1B), while latency of rotational behavior decreased (Fig. 1C).

Various Times Following 6-OHDA Treatment

The relationship between the enhancement of rotational behavior and the various times following 6-OHDA treatment is shown in Fig. 2. Experiments began 1 week after 6-OHDA in group A and 6 weeks after 6-OHDA in group B. Although the rotational behavior was not induced at the first application of the drug in the group A, induction occurred at the second and third application of SKF38393 (Fig. 2 I-A). Similarly, group B also showed a remarkable increase in the number of rotations (Fig. 2 I-B). In contrast, the latency of rotational behavior in each group was shortened on and after the second administration of the drug (Fig. 2 II-A,II-B).

Influence of D_1 and D_2 Antagonists

Treatment of SCH23390 applied 30 min before administration of SKF38393 decreased the rotation rate in a dose-dependent manner (Fig. 3A), but sulpiride applied 60 min before the administration did not affect the rotational behavior at any dose (Fig. 3B). Pretreatment with 500 μg/kg SCH23390 completely suppressed the SKF38393-induced contralateral rotation in the first and second applications but rotational behavior was enhanced after a third administration of SKF38393 without SCH23390 pretreatment (Fig. 4).

The Change of Receptors

The DA contents, and the D_1 and D_2 DA receptor densities and affinities in the striatum of rats, are shown in Table 1.

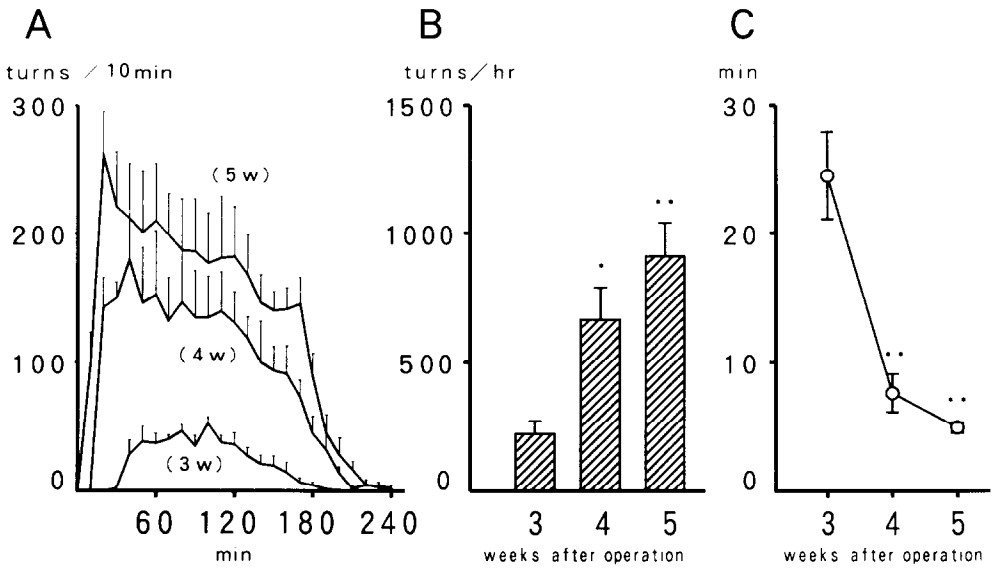

FIG. 1. Effects of repeated administration of SKF38393 on the rotational behavior in rats with unilateral nigrostriatal dopaminergic lesions. SKF38393 was administered 3 weeks after 6-OHDA treatment and repeated once a week for 2 more weeks. (A), (B), and (C) represent the time course of rotational behavior, number of rotations, and latency of rotational behavior, respectively. Number of rotations was accumulated for 60 min after the rotational behavior was induced. Each value [(A) $n = 5$, (B, C) $n = 9$] represents the mean ± SEM. $^*p < 0.05$ and $^{**}p < 0.01$ ANOVA with Dunnett test, comparing with the rotation produced by a single administration of SKF38393.

Group A was killed 48 h after a single administration of SKF38393 that was given 3 weeks after 6-OHDA treatment. Group B was also killed 48 h after two administrations of the drug injected 3 and 4 weeks after 6-OHDA treatment. DA contents were reduced in the lesioned side of both groups (group A: 98.9%, group B: 99.8%). DA contents in each lesioned and intact side did not differ between the two groups, respectively. [^{3}H]spiperone binding assay for striatal tissues showed an increase of the B_{max} of D_2 receptors on the lesioned side compared with intact side (group A: 20.2%, group B: 22.0%). However, affinity was not significantly altered. In contrast, [^{3}H]SCH23390 binding assay for striatal tissues showed no significant difference of the B_{max} and K_d of D_1 receptors between the intact and lesioned sides in each group.

DISCUSSION

Repeated administration of SKF38393 starting 1, 3, and 6 weeks after 6-OHDA treatment increased the number of

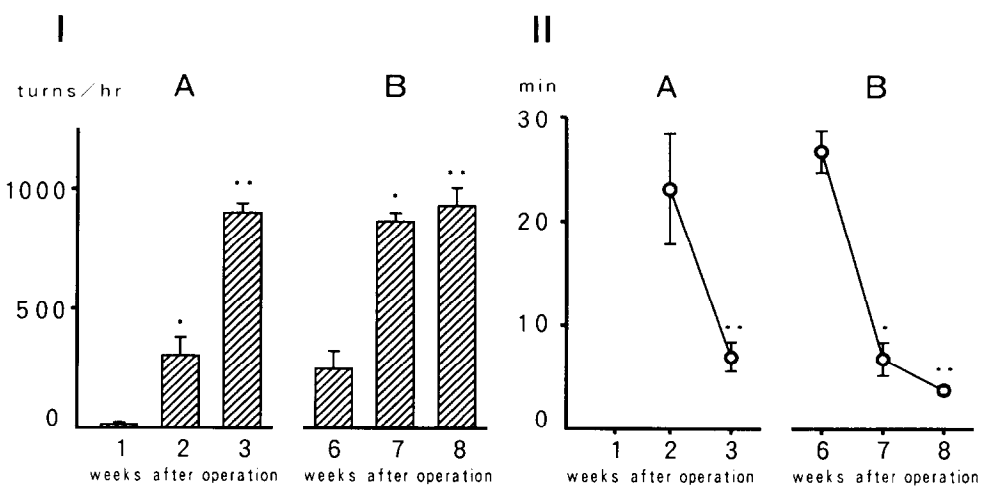

FIG. 2. SKF38393-induced rotations at different periods of time after the 6-OHDA treatment. SKF38393 was administered either 1 [(A) $n = 10$] or 6 [(B) $n = 6$] weeks after the 6-OHDA treatment and repeated once a week for 2 more weeks. (I-A, I-B) Number of rotations accumulated for 60 min after the rotational behavior was induced. (II-A, II-B) The latency of each group. $^*p < 0.05$ and $^{**}p < 0.01$ as described in Fig. 1.

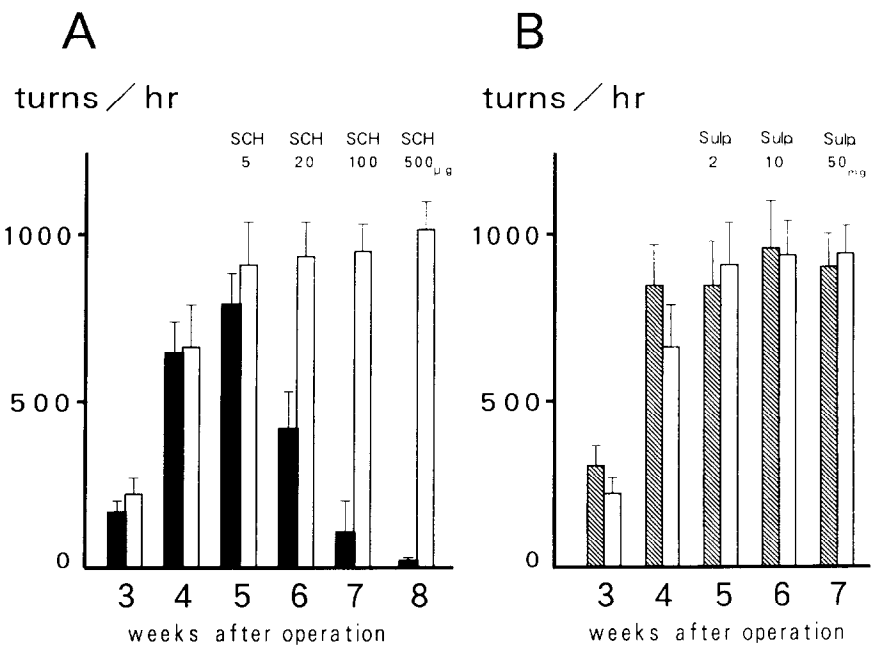

FIG. 3. Effect of (A) SCH23390 and (B) sulpiride on rotational behavior induced by repeated administration of SKF38393. Repeated administrations of SKF38393 alone and SKF38393 plus DA antagonists [SCH23390 (5–500 μg/kg); sulpiride (2–50 mg/kg)] were started 3 and 5 weeks after the 6-OHDA treatment, respectively. Number of rotations was accumulated for 60 min after the rotational behavior was observed. □ = SKF38393 (n = 9); ■ = SCH23390 + SKF38393 (n = 7); ▨ = sulpiride + SKF38393 (n = 8).

rotations and decreased the latency. These results suggest that enhanced rotational behavior can be induced by repeated stimulation of the D_1 receptor.

Degeneration of nigrostriatal dopaminergic neurons is histochemically and biochemically completed 12 days after 6-OHDA treatment (10,13–15). Simultaneously, rotational behavior induced by apomorphine is enhanced in this period (13,15). Striatal D_2-receptor density using in vitro [^{3}H]spiperone binding assay increases over a 2–3 week period (10,13) and peaks on and after 25 days the 6-OHDA treatment (5). However, there are conflicting results on the changes in striatal D_1-receptor density and affinity after the destruction of nigrostriatal pathway. One report shows no changes in striatal D_1 receptor concentration analysis using ^{125}I-SCH23982 (1) and others show the upregulation of the striatal D_1 receptor (4,11). These findings suggest that neuronal degeneration, decrease of DA, and change of DA receptor in the striatum are still incomplete 3 weeks after the treatment with 6-OHDA into the ipsilateral substantia nigra. In our studies, we may not exclude a possibility that enhancement of rotational behavior that was observed 1–3 weeks after the 6-OHDA treatment may include the process of denervation supersensitivity. However, enhancement of rotations was also observed 3–6 weeks after the 6-OHDA treatment. These results indicate that enhancement of rotations is not due to the process of the denervation supersensitivity but the repeated administration of D_1 agonist.

The enhancement of SKF38393-induced rotational behavior by repeated administrations of SKF38393 was inhibited by D_1 antagonist SCH23390 and not by D_2 antagonist sulpiride, suggesting the possibility that the enhancement of rotations is mediated by D_1 but not by D_2 receptors. This possibility is also supported by the present data that repeated administrations of

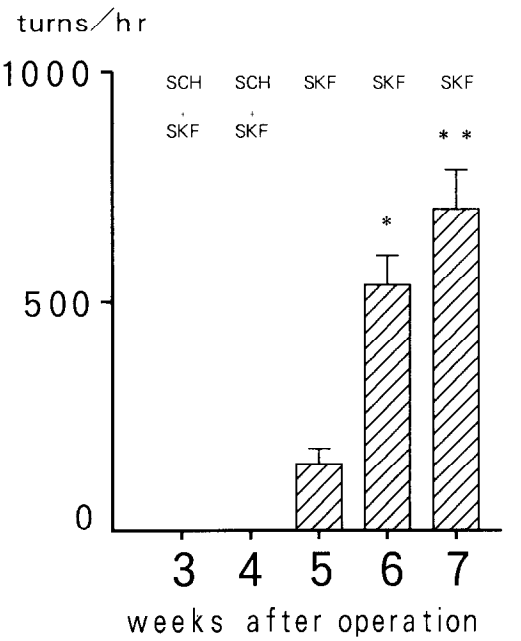

FIG. 4. Preventive effect of SCH23390 on the enhancement of SKF38393-induced rotational behavior by repeated administration. Animals were treated with 500 μg/kg SCH23390 prior to SKF38393 in the first and second trial. Number of rotations (n = 7, mean ± SEM) was accumulated for 60 min after the rotational behavior was induced. *$p < 0.05$ and **$p < 0.01$ ANOVA with Dunnett test, comparing with rotation at the third administration of SKF38393.

TABLE 1

DOPAMINE CONTENTS AND B_{max} AND K_d VALUES OF D_1 AND D_2 RECEPTORS IN THE STRIATUM

		Group A	Group B
Dopamine content		(n = 8)	(n = 7)
(ng/mg tissue)	intact	7.19 ± 0.59	8.62 ± 0.88
	lesioned	0.08 ± 0.012†	0.06 ± 0.002†
	ratio	0.011 ± 0.002	0.002 ± 0.001
[^{3}H]spiperone		(n = 7)	(n = 6)
B_{max}	intact	343.9 ± 11.9	338.8 ± 25.6
(fmol/mg protein)	lesioned	413.4 ± 14.5*	413.5 ± 9.10*
K_d	intact	0.0448 ± 0.0037	0.0491 ± 0.0032
(nM)	lesioned	0.0453 ± 0.0037	0.0429 ± 0.0035
[^{3}H]SCH23390		(n = 8)	(n = 6)
B_{max}	intact	825.3 ± 96.8	874.5 ± 84.3
(fmol/mg protein)	lesioned	873.6 ± 102.6	914.2 ± 99.4
K_d	intact	0.4667 ± 0.0458	0.4329 ± 0.0785
(nM)	lesioned	0.4246 ± 0.0318	0.4055 ± 0.0507

Rats were sacrificed 48 h after the first (group A) and second (group B) application of SKF38393. Each value represents the means ± SEM.

*$p < 0.01$ and †$p < 0.001$ compared with intact side (paired t-test).

a large dose of SCH23390 prior to SKF38393 delayed the enhancement of SKF38393-induced rotation. However, there were no significant differences in D_1-receptor density and affinity in the striatum between the two groups of rats. Thus, our results indicate that the enhancement of SKF38393-induced rotational behavior is directly mediated by the D_1-receptor stimulation but does not relate to the change of DA-receptor density and affinity in the striatum.

Several reports describe the relationship between D_1 receptor and adenyl cyclase activity or cyclic AMP in the striatum. Some reports show that the specific association of the D_1 receptor with cyclic AMP-stained neurons is abolished following chemical interruption of the nigrostriatal pathway (2,3). Moreover, there are reports that apomorphine and/or quinpirole given 3 days prior to the application of SKF38393 facilitate the SKF38393-induced rotational behavior (6–8), and such a priming elicits changes at the level of the transduction mechanism of D_1 receptors (9).

Taking our results into consideration, the enhancement induced by repeated administration of SKF38393 may be mediated by an intramembranous and/or intracellular transduction mechanism that is common to D_1 and D_2 receptors.

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