British Journal of Pharmacology (1996) 118, 585-592

The effect of dopamine D_1 receptor stimulation on the up-regulation of histamine H_3-receptors following destruction of the ascending dopaminergic neurones

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1 The binding of [^{3}H]-(*R*)α-methylhistamine and [^{3}H]-N$^\alpha$-methylhistamine to histamine H_3-receptors, [^{3}H]-SCH23390 to dopamine D_1-receptors, and [^{3}H]-YM09151-2 to dopamine D_2-receptors was investigated by quantitative receptor autoradiography in the rat brain following 6-hydroxydopamine injection into the substantia nigra.

2 The levels of [^{3}H]-(*R*)α-methylhistamine binding sites in the denervated striatum and substantia nigra were significantly higher than those in the contralateral side from 1 week to 12 weeks after nigral lesions. The H_3-receptor binding was maximal at 3 weeks after nigral lesions and maintained until 12 weeks.

3 The increased number of histamine H_3-receptors was decreased to the level of the contralateral side by chronic treatment with a selective dopamine D_1 agonist, SKF38393, but not modified by a selective dopamine D_2 agonist, quinpirole.

4 Dopamine D_1- and D_2-receptors in the striatum were similarly up-regulated after unilateral nigral lesion. On the other hand, the number of dopamine D_2-receptors in the substantia nigra was markedly decreased after administration of 6-hydroxydopamine.

5 The treatment with *(S)*α-fluoromethylhistidine increased the H_3-receptor binding in both the ipsilateral and contralateral sides. As a result, the magnitude of the ratio of the H_3-receptor binding between ipsilateral and contralateral sides was partially attenuated by treatment with *(S)*-α-fluoromethylhistidine.

6 These results strongly suggest that the expression of histamine H_3-receptors in the striatum and substantia nigra is influenced through D_1-receptors by tonic nigrostriatal dopaminergic inputs.

Keywords: histamine H_3-receptor; dopamine D_1-receptors; dopamine D_2-receptors; 6-hydroxydopamine; denervation; striatum; substantia nigra; SKF38393; quinpirole; *(S)*-α-fluoromethylhistidine

Introduction

Histaminergic nerve fibres have varicosities and extend widely to the rostral and caudal brain regions from cell bodies present in the posterior hypothalamic regions (Panula *et al.*, 1984; Watanabe *et al.*, 1984). Three histamine receptors (H_1, H_2 and H_3) mediate diverse actions of the histaminergic neurone system. The histamine H_3-receptor binding sites labelled with [^{3}H]-(*R*)-α-methylhistamine ([^{3}H]-αMeHA) and [^{3}H]-N$^\alpha$-methylhistamine ([^{3}H]-NAMH) are widely and heterogeneously distributed in rat brain (Cumming *et al.*, 1991; Pollard *et al.*, 1993). The H_3-receptors are located presynaptically on histaminergic neurones and mediate feedback inhibition of histamine synthesis by histidine decarboxylase and depolarization-induced release of histamine (Arrang *et al.*, 1987). However, several lines of evidence have suggested that a proportion of the H_3-receptors in the cortical and striatonigral regions are not on histaminergic neurones but on intrinsic neurones (Cumming *et al.*, 1991; Pollard *et al.*, 1993).

There are several reports on the functional interaction between histaminergic and dopaminergic neurotransmission (Itoh *et al.*, 1984; Schlicker *et al.*, 1993; Clapham & Kilpatrick, 1994). Thioperamide, a selective histamine H_3-receptor antagonist, attenuates amphetamine- and apomorphine-induced locomotor activity in mice, suggesting that antagonism of the central histamine H_3-receptor inhibits the effects amphetamine and apomorphine on dopamine receptors (Clapham & Kilpatrick, 1994). Recently we showed that intrastriatal injection of quinolinic acid resulted in loss of H_3-receptors labelled with [^{3}H]-αMeHA in the striatum and substantia nigra simultaneously in parallel with that of dopamine D_1-receptors labelled with [^{11}C]-SCH 23390 (Ryu *et al.*, 1994a). We also revealed that nigrostriatal dopaminergic denervation induced marked up-regulation of H_3-receptors in the ipsilateral striatum and substantia nigra, although the mechanism of the increase in H_3-receptor number is unknown (Ryu *et al.*, 1994b; 1995).

In the present study, we examined the binding of [^{3}H]-αMeHA and [^{3}H]-N AMH to histamine H_3-receptors, [^{3}H]-SCH23390 to dopamine D_1-receptors and [^{3}H]-YM-09151-2 to D_2-receptors in rat brain following chronic lesions of the nigrostriatal dopaminergic neurones induced by 6-hydroxydopamine (6-OHDA) injection. In addition, we examined the effects of selective dopaminergic agonists and an inhibitor of L-histidine decarboxylase (HDC) on the H_3-receptor and dopamine D_1- and D_2-receptors caused by lesioning dopaminergic neurones.

Methods

6-OHDA lesion

Male Wistar rats weighing 270-300 g were used. To spare noradrenergic pathways from the neurotoxic effects of 6-hydroxydopamine (6-OHDA) animals were pretreated with the noradrenergic uptake inhibitor desmethylimipramine (25 mg kg^{-1}, i.p.) 20 min before administration of sodium pentobarbitone (50 mg kg^{-1}, i.p.). 6-OHDA·HCl (8 μg free base in 4 μl of saline with 0.1% ascorbic acid) was injected stereotaxically into the right substantia nigra (coordinates based on the bregma: AP-5.3, ML-2.3, DV-7.6 mm, ac-

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cording to the atlas of Paxinos and Watson (1982)). After the infusion over a period of 5 min, the cannula was left in place for an additional 10 min to allow diffusion, and then carefully removed. On designated days (1, 2, 3, 5, 8 and 12 weeks after 6-OHDA treatment), animals were decapitated, and their brains were quickly removed and stored at −80°C until use.

Drug treatment

Two weeks after 6-OHDA injection, circling behaviour was tested by the injection of apomorphine (1 mg kg^{-1}, i.p.). Animals showing an average of at least 7 contralateral turns min^{-1} over 60 min were then selected for this study (Cadet & Zhu, 1992). Three weeks after 6-OHDA injection, rats were randomly assigned to control or treatment groups. Animals received 10 daily injections (i.p.) of either SKF-38393 (10 mg kg^{-1}), quinpirole (1 mg kg^{-1}) or *(S)*-α-fluoromethylhistidine (FMH, 100 mg kg^{-1}). Rats were killed by decapitation 16 h after the last injection.

Autoradiographic studies

Tissue preparation Serial coronal sections (20 μm thickness) were prepared at −20°C and were thaw-mounted onto cover glasses coated with 3-aminopropyl-triethoxysilane and stored at −80°C until use. Sections were dried at room temperature for 30 min just before use.

[^{3}H]-MeHA binding to H_3-receptors Sections were incubated for 60 min at room temperature in a solution of 50 mM Na/K phosphate buffer (pH 7.4) containing 2 nM [^{3}H]-αMeHA. Then the sections were washed for 5 min at 4°C in the same solution, briefly rinsed at 4°C with water and dried under a stream of cold air. For examination of nonspecific binding, adjacent sections were incubated in the presence of 1 μM thioperamide.

[^{3}H]-NAMH binding to H_3-receptors Sections were incubated for 45 min at room temperature in a solution of 150 mM Na/K phosphate buffer (pH 7.5) containing 100 μM dithiothreitol, 2 mM $MgCl_2$, and 4 nM [^{3}H]-N AMH. Then they were washed 3 times for 20 s, each time with the same buffer at 4°C, rinsed briefly with water at 4°C, and dried under a stream of cold air. For examination of nonspecific binding, adjacent sections were incubated in the presence of 1 μM thioperamide.

Dopamine D_1-receptor binding For the binding assay of dopamine D_1-receptors by the autoradiographic method, incubation was performed for 60 min at room temperature in a 50 mM Tris-HCl buffer (pH 7.4), containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM ascorbic acid, and 1 nM [^{3}H]-SCH23390. Nonspecific binding was defined by unlabelled SCH23390 (2 μM). The incubation was terminated by rinsing sections twice for 5 min in cold 50 mM Tris-HCl (pH 7.4). Sections were then dipped briefly in ice cold water and dried rapidly under a stream of cold air.

Dopamine D_2-receptor binding The procedure for labelling D_2 sites with [^{3}H]-YM09151-2 (emonapride) was based on a previously published method (Ryu *et al.*, 1994a). Incubation was performed for 90 min at room temperature in 50 mM Tris-HCl buffer (pH 7.4), containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, and 0.2 nM [^{3}H]-YM09151-2. The sections were washed twice for 1 min in cold 50 mM Tris-HCl buffer (pH 7.4), rinsed briefly with water at 4°C, and dried under a stream of cold air. Non-specific binding was defined with 1 μM haloperidol.

Quantification of histamine H_3, dopamine D_1-, and D_2-receptor bindings The sections, together with ^{3}H microscales, were exposed to a tritium-sensitive imaging plate with no anti-scratch superficial layer (TR-IP) for 7 days to obtain the

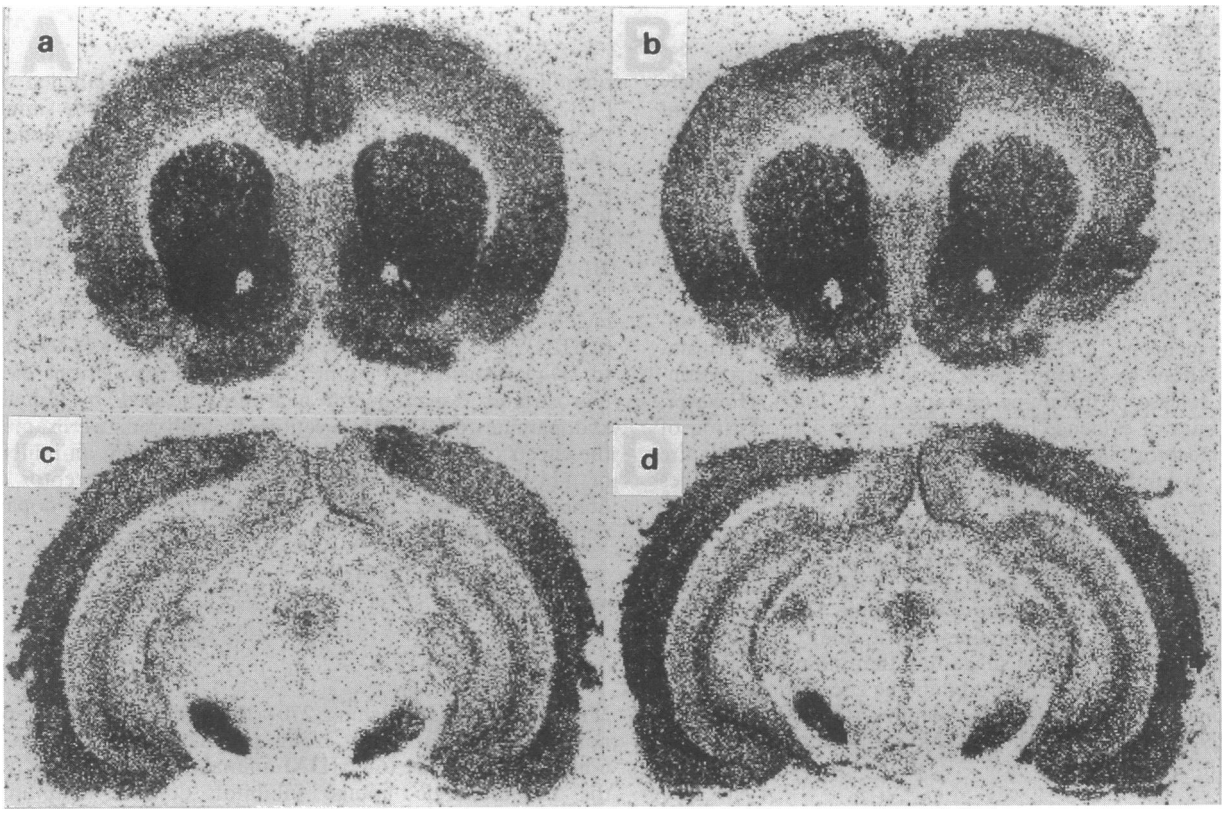

Figure 1 Autoradiograms of sections showing the localization and levels of [^{3}H]-(*R*)-α-methylhistamine (αMeHA) binding to the H_3-receptors at the levels of striatum (a, b) and substantia nigra (c, d). (a and c) Histamine H_3-receptors 1 week after 6-OHDA administration. (b and d) Histamine H_3-receptors 12 weeks after 6-OHDA administration. In the above autoradiograms, the left hand hemisphere is the denervated side. Note that the [^{3}H]-MeHA binding was increased in the substantia nigra and striatum.

images. The exposed imaging plates were inserted into an image reading unit and scanned with a fine laser beam. The imaging data were recorded as digitized values in an analyzing unit for further analysis (Fujix Bio-Imaging Analyzer BAS3000). The specific binding was calculated by subtracting the nonspecific binding from the total binding. For each determination, values in 3 to 4 brain sections were averaged. Calibration of tritiated polymer for tissue equivalent tritium concentration in the phosphor imaging plates was performed according to the method of Geary *et al.* (1985).

Drugs

Drugs were obtained from the following sources: desmethylimipramine, 6-OHDA·HCl (Sigma, St. Louis, MO, U.S.A.); SKF-38393 ((±)-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzapine-7,8-diol), quinpirole, thioperamide (RBI, Natick, MA, USA); (*S*)-α-fluoromethylhistidine (FMH a gift from Dr Kollonitsch, Merck Sharp & Dohme, Rahway, NJ, U.S.A.); 3-aminopropyl-triethoxysilane (Shinetsu Chemicals, Japan); [^{3}H]-NAMH (78.9 Ci mmol^{-1}), [^{3}H]-SCH23390 (R-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5,tetrahydro-1H-3-benzapine) (87 Ci mmol^{-1}), [^{3}H]YM09151-2 (cis-N-(1-benzyl-2-methylpyrrolidin-3-yl)-5-chloro-2-methoxy-4-methylaminobenzamide) (87 Ci mmol^{-1}) (New England Nuclear, Boston, MA, U.S.A.); [^{3}H]-αMeHA (39 Ci mmol^{-1}), Autoradiographic ^{3}H micro-scales (Amersham, Buckinghamshire, U.K.)

Results

Time-dependent changes of receptor bindings

Unilateral injection of 6-OHDA into the substantia nigra resulted in the degeneration of almost all the tyrosine hydroxylase (TH) immunoreactive neurones in the ipsilateral side of rats at 1 to 12 weeks (data not shown). Autoradiograms of [^{3}H]-αMeHA binding in rat brain 1 week and 12 weeks after lesions revealed a marked increase in the binding to the substantia nigra and caudate-putamen compared with that to the contralateral side (Figure 1). Considerable up-regulation of [^{3}H]-NA MH binding was also demonstrated in the substantia nigra and caudate-putamen (Figure 2). Similarly, there were considerable increases in [^{3}H]-SCH23390 and [^{3}H]-YM09151-2 binding in the ipsilateral striatum after dopaminergic denervation (Figure 2). However, the binding of [^{3}H]-SCH23390 to the dopamine D_1 receptors in the substantia nigra was not changed from 1 to 12 weeks, while the binding of [^{3}H]-YM09151-2 to dopamine D_2 receptors in the ipsilateral substantia nigra was markedly decreased.

The time-dependent changes of H_3, D_1, and D_2-receptor binding were compared and are presented in Figure 3. H_3-receptor binding increased from 1 week to 12 weeks after 6-OHDA injection both in the striatum and substantia nigra. The increase was higher in the substantia nigra (about 170%) than in the striatum (about 115%) as shown in Figure 3a. Dopamine D_1-receptor binding in the striatum was also up-regulated to 120–130%, but D_1-receptor binding was not significantly increased in the substantia nigra except 2 weeks after 6-OHDA injection (Figure 3b). Dopamine D_2-receptor binding in the striatum was also increased to approximately 130–140% of that in the contralateral side from 1 week to 12 weeks after dopaminergic denervation (Figure 3c), while D_2-receptor binding in the substantia nigra gradually decreased from 1 week to 40% of that in the contralateral side.

Histamine H_3-receptor binding using two different ligands of [^{3}H]-αMeHA and [^{3}H]-NAMH was compared in the rats subjected to nigrostriatal dopaminergic denervation (Figure 3a). The rate of increase in H_3-receptor binding measured with [^{3}H]-NAMH was similar to that measured with [^{3}H]-αMeHA at all times.

Effects of chronic treatment with selective dopamine agonists and FMH on H_3, D_1- and D_2-receptors

For each of the drug treatment groups, the changes in H_3-receptor binding with [^{3}H]-αMeHA are summarized in Table 1. The increased H_3-receptor density in the striatum (especially, dorsolateral and dorsomedial regions) after 6-OHDA-induced dopaminergic denervation was down-regulated by the treatment of SKF-38393, a selective D_1-agonist, resulting in no significant difference between ipsilateral and contralateral regions of the striatal regions. The treatment with SKF38393 significantly attenuated the up-regulated H_3-binding in the substantia nigra as well. However, quinpirole, a selective D_2-agonist, had no effect on the up-regulated H_3-receptor binding either in the striatum or in substantia nigra. Treatment with FMH, a selective inhibitor of HDC, increased significantly the binding to H_3-receptors in the striatum and substantia nigra.

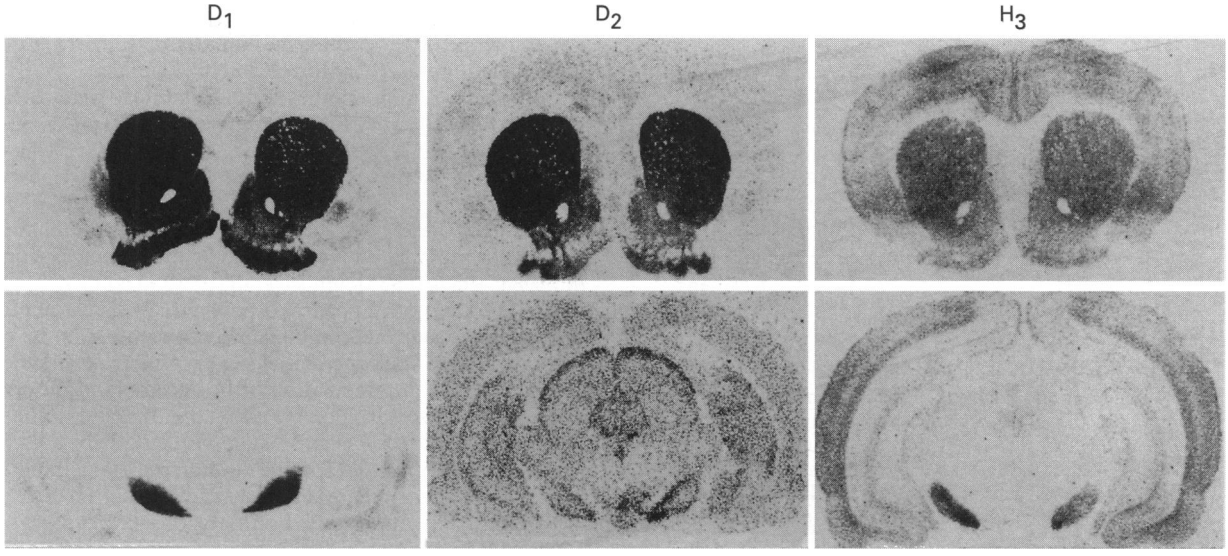

Figure 2 Autoradiograms of sections showing the localization and levels of [^{3}H]-SCH23390 binding to D_1-receptors (D_1), [^{3}H]-YM09151-2 binding to D_2-receptors (D_2) and [^{3}H]-N$^\alpha$-methylhistamine binding to H_3-receptors (H_3) 3 weeks after 6-OHDA administration. The upper and lower figures are the level of striatum and substantia nigra, respectively. The left hand hemisphere is the lesioned side. Note the apparently different changes of D_1, D_2, and H_3-receptor binding in the striatum and substantia nigra after 6-OHDA lesioning.

The increases in binding by FMH were approximately 27 and 20% in the contralateral and ipsilateral sides, respectively. Accordingly, the difference in binding of H_3-receptor between ipsilateral and contralateral sides was partially attenuated. The effects of the chronic drug treatments on H_3-receptor binding are summarized in Figure 4.

Treatment with SKF38393 significantly increased the binding of [^{3}H]-αMeHA to H_3-receptors in the substantia nigra when compared to the control group. In addition, the effects of SKF38393, quinpirole, and FMH on dopamine D_1- and D_2-receptor binding are shown in Tables 2 and 3, respectively. SKF38393 and quinpirole had no significant effects on D_1-binding labelled with [^{3}H]-SCH23390. On the other hand, D_1-receptor binding was slightly, but significantly, increased in some regions by treatment with FMH. D_2-receptor binding was significantly decreased by quinpirole, but was unaffected by SKF38393 and FMH.

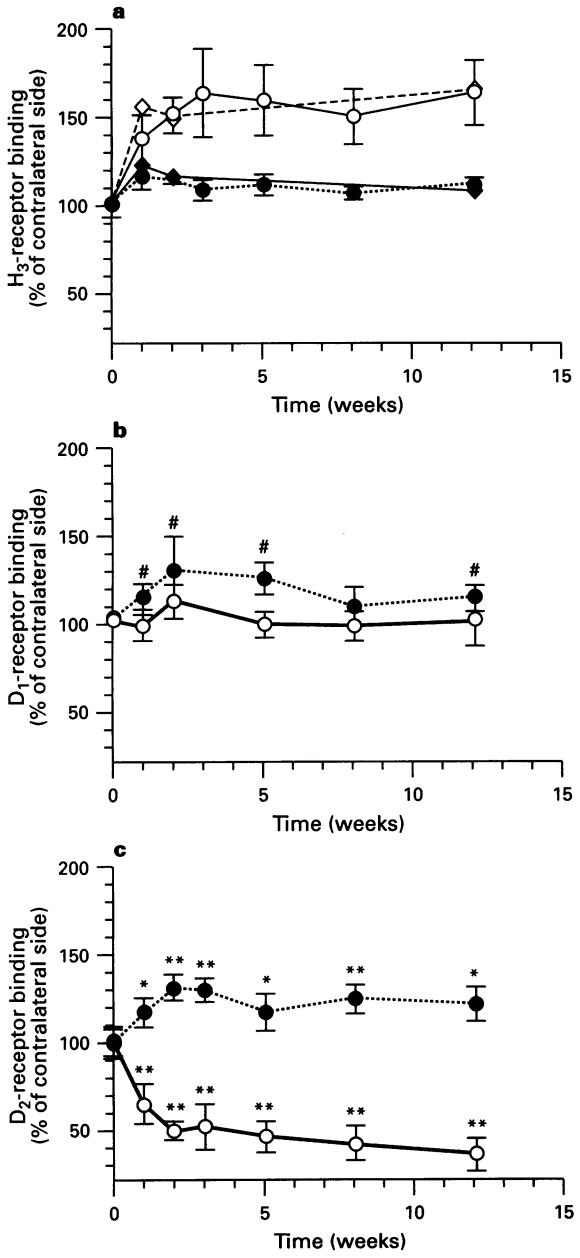

Figure 3 Effects of 6-OHDA-induced dopaminergic denervation on histamine H_3-receptor, dopamine D_1-receptor, and dopamine D_2-receptor binding in the striatum and substantia nigra. (a) Histamine H_3-receptor binding measured with [^{3}H](R)-α-methylhistamine (○, substantia nigra; ●, striatum) and [^{3}H]-N$^\alpha$-methylhistamine (◇, substantia nigra; ◆, striatum). H_3-receptor binding measured with both ligands was significantly increased in the substantia nigra ($P<0.01$) and striatum ($P<0.05$) at all time points. (b) Dopamine D_1-receptor binding in the substantia nigra (○) and striatum (●). (c) Dopamine D_2-receptor binding in the substantia nigra (○) and striatum (●). The mean±s.d. are shown, $n=6-21$. $^*P<0.05$, $^*P<0.01$, $^{**}P<0.001$, statistical significance of difference between the treated and control groups by ANOVA followed by Dunnett's multiple comparison test.

Discussion

The present study confirms that the H_3-receptor was up-regulated by 6-OHDA-induced dopaminergic denervation in the striatum and substantia nigra. Dopamine D_1- and D_2-receptors were also increased in the striatum after dopaminergic denervation, but the degree of increase in D_1- and D_2-receptor binding was not as high as that for the H_3-receptor. In addition, the up-regulation of the H_3-receptor was attenuated by treatment with a dopamine D_1-receptor agonist, but not by a D_2-agonist.

Cumming *et al.* (1991) reported that striatal injection of quinolinic acid reduced the binding of [^{3}H]-N AMH in the striatum and ipsilateral substantia nigra. We demonstrated that the decrease in H_3-receptor induced by quinolinic acid in the striatum was almost parallel with the loss of dopamine D_1-receptors (Ryu *et al.*, 1994a). From these results, it was postulated that histamine H_3-receptors are located on striatonigral neurones, and that they may be affected by nigrostriatal dopaminergic neurones. Recently, we demonstrated that H_3-receptor binding sites were highly up-regulated by the destruction of nigrostriatal dopaminergic neurones (Ryu *et al.*, 1994b). We revealed an increase in H_3-receptors in several brain regions after the injection of 6-OHDA by using a new technique of quantitative autoradiography with highly-sensitive imaging plates (Yanai *et al.*, 1992).

The increase of H_3-receptor both in the striatum and substantia nigra persisted from 1 week to 12 weeks after the administration of 6-OHDA. It was observed that H_3-receptor binding sites labelled either with [^{3}H]-αMeHA or [^{3}H]-N AMH were similarly up-regulated from 1 week to 12 weeks in the region of striatum and substantia nigra. And the degree of increase in the striatum and substantia nigra was almost the same using both ligands. We previously found that [^{3}H]-S-methylthioperamide could be used to label H_3-receptors in the brain as a tritium-labelled antagonist (Yanai *et al.*, 1994). In our preliminary data, the up-regulation of H_3-receptors by dopaminergic denervation was similarly detected by a tritium-labelled antagonist, [^{3}H]-S-methylthioperamide.

Net release and synthesis of histamine were originally shown to be regulated by H_3-receptors located presynaptically in histaminergic nerve terminals (Arrang *et al.*, 1987). However, several studies have suggested that H_3-receptors may have regulatory effects on the release of other neurotransmitters besides histamine, such as noradrenaline, 5-hydroxytryptamime, dopamine and acetylcholine (Schlicker *et al.*, 1988; 1989; 1993; Ichinose *et al.*, 1989; Arrang *et al.*, 1995). Thus, H_3-receptors appear not only to be autoreceptors of histaminergic neurones, but also heteroreceptors in the central nervous system. Lesions of the medial forebrain bundle were shown to induce up-regulation of H_3-receptors in the striatum and ipsilateral cortex (Pollard *et al.*, 1993). We also observed that the depletion of brain histamine caused by the chronic treatment of FMH, a specific and irreversible inhibitor of HDC, significantly increased the binding of [^{3}H]-αMeHA to H_3-receptors in almost all brain regions (Nakagawa *et al.*, 1994; Ryu *et al.*, 1995). These findings support the hypothesis that most of the H_3 receptors are located at the postsynaptic sites of histamine neurones.

Several lines of evidence have been obtained over the last 20 years that central histaminergic neurones may be involved in arousal mechanism, circadian rhythm and locomotor activity.

In accordance with these findings, we demonstrated that the administration of thioperamide, a potent H_3-receptor antagonist, increased the short term locomotor activity (exploratory behavior) of W/W^v mice dose-dependently 1–2 h after its administration (Sakai *et al.*, 1991). From our results on H_3-receptor binding, it is suggested that H_3-receptors in the striatum and substantia nigra are located on striatonigral projection neurones, and that their numbers can be modified through dopamine D_1-receptors by nigrostriatal dopaminergic neuronal activity. H_3-receptors were not changed in rats showing no contraversive turning behaviour induced by injection of apomorphine. Immunohistochemical studies also showed that dopamine neurones in such rats were not completely destroyed. Results suggested that histamine H_3-receptors might play an important role in the turning behaviour and also in the supersensitivity after dopaminergic denervation, although any effects of histamine on circling behaviours were not observed in our preliminary experiments. Pharmacological studies are needed to elucidate the exact roles of H_3-receptors in dopamine supersensitivity.

We previously found that H_3-receptor binding in the visually deprived superior colliculus, contralateral to the enucleated eye, was markedly increased 5–50 days after orbital enucleation of rats (Nakagawa *et al.*, 1994). However, H_3-receptors in the lateral geniculate nucleus were not changed by orbital enucleation. Denervation supersensitivity would be caused in the superior colliculus after orbital enucleation, but not in the dorsal lateral geniculate nucleus. The superior colliculus receives 65% of the retinal efferent from the con-

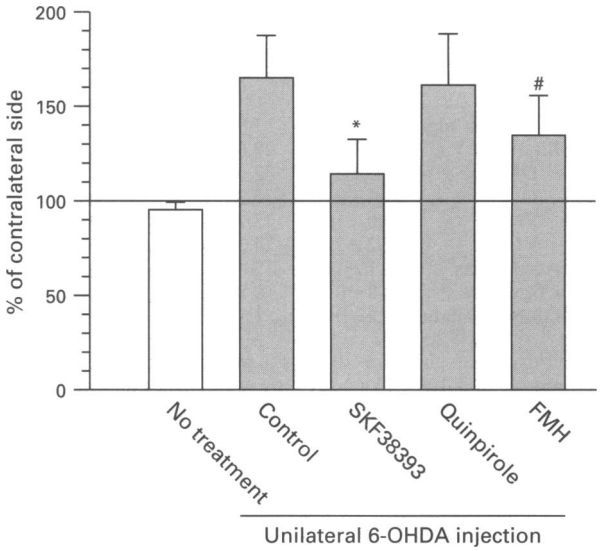

Figure 4 Effects of chronic treatment of SKF38393, quinpirole and (*S*)-α-fluoromethylhistidine (FMH) on the up-regulation of H_3-receptor binding in the substantia nigra induced by 6-OHDA administration. The mean and s.d. are shown, $n=6-21$. $^{\#}P<0.05$, $^{*}P<0.01$, statistical significance of difference between the treated and control groups by ANOVA followed by Dunnett's multiple comparison test.

Table 1 Effects of SKF38393, quinpirole, and FMH on histamine H_3-receptor binding labelled with [^{3}H]-(R) α-methylhistamine in rats with unilateral 6-OHDA lesions of substantia nigra

	Control		*SKF38393*		*Quinpirole*		*FMH*	
Brain regions	*Contralateral*	*Ipsilateral*	*Contralateral*	*Ipsilateral*	*Contralateral*	*Ipsilateral*	*Contralateral*	*Ipsilateral*
Substantia nigra	8.9±0.7	14.6±2.1**	10.4±1.0[a]	11.7±1.2[b]	10.0±1.4	15.8±1.1**	11.7±1.7[b]	15.4±0.9**
Striatum	11.7±1.1	12.9±1.2**	12.8±1.7	13.4±1.6	12.0±0.7	13.3±0.9*	14.2±0.9[b]	15.1±1.1[b]
DM	10.0±1.0	11.5±1.2**	11.1±1.5	12.1±0.6	10.2±0.7	11.9±1.2*	11.9±1.5[b]	13.1±1.4[b]
DL	9.9±1.0	11.2±0.9**	11.0±1.7	11.0±0.8	10.2±0.5	12.1±0.7**	12.1±0.9[b]	12.8±1.0[b]
VM	13.8±1.5	14.9±1.8**	15.3±2.2	17.1±2.1[a]	14.3±1 1	15.5±1.6	17.5±1.1[b]	18.4±1.6[b]
VL	13.4±1.5	14.3±1.6	14.9±1.8	15.6±1.9	13.8±0.8	14.6±0.7	16.5±0.8[b]	17.1±1.3[b]
Accumbens	10.3±1.7	10.6±1.7	11.1±2.2	12.5±3.2	10.2±1.0	10.4±0.6	14.6±1.0[b]	15.0±1.0[b]
Cortex	7.6±0.6	7.7±0.6	8.0±0.5	8.4±0.6[a]	7.4±0.7	7.6±0.6	8.6±0.6[b]	9.0±0.3[b]
Globus pallidus	6.3±1.3	6.2±1.3	6.4±1.2	8.9±1.0**[b]	7.6±1.0	7.3±1.4	8.9±1.0[a]	8.2±0.7

Each drug was given for 10 days 3 weeks after 6-OHDA injection, 10 mg kg^{-1} day^{-1} (i.p.) for SKF38393, 1 mg kg^{-1} day^{-1} (i.p.) for the quinpirole and 100 mg kg^{-1} day^{-1} (i.p.) for (*S*)-α-fluoromethylhistidine (FMH). In the control group, rats received the same volume of saline. Values (fmol mg^{-1} tissue) are the mean ± s.d. from 6 to 21 rats. $^{*}P<0.05$, $^{**}P<0.01$ versus the intact side (paired *t* test); $^{a}P<0.05$; $^{b}P<0.01$ versus the corresponding regions and sides of the control group (Dunnett's multiple range test). Abbreviations of regions of the striatum: DM, dorsomedial; DL, dorsolateral; VM, ventromedial; VL, ventrolateral.

Table 2 Effects of SKF38393, quinpirole, and FMH on dopamine D_1-receptor binding labelled with [^{3}H]-SCH23390 in rats with unilateral 6-OHDA lesions of substantia nigra

	Control		*SKF38393*		*Quinpirole*		*FMH*	
Brain regions	*Contralateral*	*Ipsilateral*	*Contralateral*	*Ipsilateral*	*Contralateral*	*Ipsilateral*	*Contralateral*	*Ipsilateral*
Substantia nigra	61.3±6.9	59.4±6.6	55.0±6.1	67.7±12.5	51.7±1.3	52.6±7.9	64.6±5.5	68.3±4.8
Striatum	67.3±6.5	83.5±8.3**	64.6±4.1	85.9±6.6**	62.5±3.9	75.7±7.1**	75.9±8.8	91.7±5.7**
DM	71.2±6.7	88.4±7.8**	69.2±5.5	90.8±9.3**	68.2±4.3	80.5±6.7**	78.9±8.8	96.1±7.8**
DL	70.0±7.5	84.1±10.4*	66.2±4.7	89.1±8.0**	65.4±5.3	77.3±6.2**	77.5±10.0	94.2±6.1*
VM	63.9±6.6	80.1±9.2**	61.7±5.9	81.4±6.2**	58.3±7.7	73.1±9.5*	75.2±9.0[a]	87.2±4.8*
VL	64.9±6.4	80.3±10.1**	61.6±2.8	83.6±6.1**	58.3±3.3	73.0±7.4**	72.7±9.1	89.9±6.1**
Accumbens	39.8±8.3	45.2±5.7	35.2±5.2	40.3±5.3	36.2±7.5	39.7±7.8	55.1±11.8[a]	58.7±9.8[a]
Cortex	4.2±0.6	4.5±0.4	3.9±0.4	4.4±0.4	4.1±0.3	4.2±0.3	4.2±0.3	4.3±0.2
Globus pallidus	10.3±2.1	12.9±2.0	10.6±1.7	15.7±2.3**	9.3±1.5	10.7±1.3	12.9±1.9	16.4±2.0[a]

Each drug was given for 10 days 3 weeks after 6-OHDA injection, 10 mg kg^{-1} day^{-1} (i.p.) for SKF38393, 1 mg kg^{-1} day^{-1} (i.p.) for the quinpirole and 100 mg kg^{-1} day^{-1} (i.p.) for (*S*) α-flouromethylhistidine (FMH). In the control group, rats received the same volume of saline. Values (fmol mg^{-1} tissue) are the mean ± s.d. from 6 to 8 rats. $^{*}P<0.05$, $^{**}P<0.01$ versus the intact side (paired *t* test); $^{a}P<0.05$ in the corresponding regions and sides of the treated groups versus control group (Dunnett's multiple range test).

Table 3 Effects of SKF38393, quinpirole, and FMH on dopamine D_2-receptor binding labelled with [^{3}H]-YM-09151-2 in rats with unilateral 6-OHDA lesions of substantia nigra

Brain regions	*Control*		*SKF38393*		*Quinpirole*		*FMH*	
	Contralateral	*Ipsilateral*	*Contralateral*	*Ipsilateral*	*Contralateral*	*Ipsilateral*	*Contralateral*	*Ipsilateral*
Substantia nigra	4.0±0.7	1.9±0.6**	3.6±0.3	1.9±0.3**	2.8±0.6[a]	1.5±0.3**	3.4±0.5	1.5±0.3**
Striatum	25.2±1.7	29.6±2.3*	23.1±1.4	26.3±2.5*	19.9±1.0[a]	23.7±1.2**[b]	23.7±2.8	28.5±3.1*
DM	26.5±1.9	32.6±1.9**	25.2±1.5	29.3±2.2**	20.7±1.1[b]	25.1±1.6**[b]	25.5±2.4	31.2±3.5**
DL	31.4±2.2	35.3±2.2*	28.2±2.2	31.6±4.6	26.2±2.4[a]	30.1±2.3*	28.4±4.4	33.2±3.7
VM	19.0±1.8	23.7±3.3*	17.6±1.0	20.0±2.1*	13.8±1.3[b]	15.9±0.9*[b]	19.9±1.7	23.9±3.0*
VL	22.2±2.6	24.3±1.5	20.0±2.4	22.1±3.2	17.9±1.5[a]	20.6±1.3*	19.9±3.0	24.0±2.7*
Accumbens	11.1±1.2	12.4±2.2	9.3±0.6[a]	10.1±0.7	7.2±0.8[b]	7.7±0.2[b]	12.2±1.2	12.8±2.4
Cortex	0.8±0.2	0.8±0.5	0.7±0.2	0.5±0.2[a]	0.8±0.2	0.6±0.2	0.6±0.3	0.7±0.2
Globus pallidus	1.2±0.4	1.1±0.3	1.0±0.2	1.6±0.4	1.0±0.3	1.0±0.4	1.7±0.5	1.1±0.5

Each drug was given for 10 days 3 weeks after 6-OHDA injection, 10 mg kg^{-1} day^{-1}(i.p.) for SKF38393, 1 mg kg^{-1} day^{-1} (i.p.) for the quinpirole and 100 mg kg^{-1} day^{-1} (i.p.) for (*S*)α-flouromethylhistidine (FMH). In the control group, rats received the same volume of saline. Values (fmol mg^{-1} tissue) are the mean±s.d. from 6 to 7 rats. $^*P<0.05$, $^{**}P<0.01$ versus the intact side (paired *t* test); $^aP<0.05$; $^bP<0.01$ versus the corresponding regions and sides of the group (Dunnett's multiple range test).

tralateral side, while the dorsal lateral geniculate nucleus receives 15%. Taken together, histamine H_3-receptors have a unique characteristic in that they are markedly increased following nearly complete loss of neuronal inputs. Our studies on chemical and physical denervation models support the hypothesis that H_3-receptors are involved in some respects in plasticity induced by neuronal damage.

Dopamine D_1- and D_2-receptor binding was also changed by the injection of 6-OHDA in the striatum and substantia nigra. D_2-receptors have been shown to be located on dopamine neurones and to act as autoreceptors in the substantia nigra (Cross & Waddington, 1981; Creese, 1982). D_2-receptor binding in the substantia nigra was markedly decreased following the degeneration of dopamine neurones caused by the administration of 6-OHDA. A proportion of D_1-receptor binding was assumed to be located on the perikarya from the substantia nigra pars compacta, while the major part was distributed in the striatonigral projections (Cross & Waddington, 1981; Savasta *et al.*, 1986; Barone *et al.*, 1987; Mansour *et al.*, 1992). The finding that D_1-receptor binding in the substantia nigra was not affected by dopaminergic degeneration might be attributed to the distribution of D_1-receptors in the substantia nigra.

Following destruction of the ascending mesostriatal dopaminergic projections with 6-OHDA, rats exhibit increased densities of D_2-receptors in the striatum, a major target of dopaminergic afferents. In addition, chronic treatment with D_2-receptor antagonists such as haloperidol increases striatal D_2-receptor density (LaHoste & Marshall, 1992; Marin & Chase, 1993). In each case, the maximal increase is about 20–40%. The effects of selective dopaminergic destruction on D_1-receptors is less clear. Although some studies have demonstrated increased D_1-receptor density and decreased D_1 mRNA following 6-OHDA treatment, others have not confirmed these results (Ariano, 1989; Gerfen *et al.*, 1990; Fornaretto *et al.*, 1993; Jongen-Rêlo *et al.*, 1994). Some studies have, in fact, shown a decreased density of D_1-receptors in the striatum following dopaminergic denervation (Porceddu *et al.*, 1987; Radja *et al.*, 1993). By contrast, chronic treatment with the selective D_1 antagonist SCH23390 has consistently been found to increase striatal D_1 density by about 25–40% (Hess *et al.*, 1986; McGonigle *et al.*, 1989).

Receptor binding studies investigating the effects of chronic treatments with dopamine agonists have also observed conflicting changes in the 6-OHDA lesioned rat model (Engber *et al.*, 1993). The up-regulation in striatal D_2 dopamine receptor density observed in 6-OHDA lesioned rats is reduced, or further enhanced following chronic intermittent levodopa administration (Gnaalingham & Robertson, 1994; Rouillard *et al.*, 1987). Chronic intermittent levodopa treatment has also been shown to reverse the D_1-receptor upregulation observed in the denervated striatum (Juncos *et al.*, 1989). However, in some studies, [^{3}H]-SCH23390 binding in the striatum and nucleus accumbens was unaffected by levodopa treatments (Rioux *et al.*, 1993). Differences in the dose, duration, frequency of administration (chronic continuous and intermittent administrations) and drugs used in the study may underlie these discrepancies.

It has been well established that contraversive turning behaviour induced by 6-OHDA is associated with supersensitivity of dopamine receptors (Ungerstedt, 1971; Creese *et al.*, 1977; Marshall & Ungerstedt, 1977; Heikkila *et al.*, 1981). In our study, obvious contraversive turning behaviour was not observed 1 week after treatment. Therefore, we checked the turning behaviour of rats 2 weeks after 6-OHDA injection before the autoradiographic study (Cadet & Zhu, 1992). Chronic treatment of the dopamine precursor levodopa induces an increase in the circling response to levodopa itself, the mixed D_1/D_2 agonist apomorphine and the D_2 agonist quinpirole. Moreover, this effect is evident to a greater degree following chronic intermittent rather than a continuous infusion regime of levodopa administration. It has also been shown that foetal nigral grafts prevent dopamine supersensitivity in the 6-OHDA lesioned rat model (Gagnon *et al.*, 1991; Savasta *et al.*, 1992; Rioux *et al.*, 1993). From our results, it is interesting that H_3 receptor binding was found in these animal models to be a marker of dopamine supersensitivity.

The functional roles and mechanisms of up-regulation of H_3-receptors on the striatonigral projection neurones are still unknown. However, the present study strongly suggests that the H_3-receptors in the striatum and substantia nigra are present on striatonigral neurones and that they are regulated by dopaminergic inputs through dopamine D_1-receptors. Overall, these results provide evidence that histamine H_3-receptors in the striatum and substantia nigra are markedly up-regulated by nigrostriatal dopaminergic denervation, and demonstrate that the increased H_3-receptors are down-regulated by a selective dopamine D_1 agonist, SKF38393. Further work is clearly required to discover the functional relationship between histamine H_3-receptors and dopamine D_1-receptors in the striatum and substantia nigra.

This study was supported by grants-in-aid from the Ministry of Education, Science and Culture of Japan (#04857021, #05557008, #05454663, #06833002 and #07558107), Uehara Memorial Foundation, and Sasagawa Foundation of Health Care.

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(Received December 18, 1995
Revised February 12, 1996
Accepted February 13, 1996)