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Modulation of histamine H_3 receptors in the brain of 6-hydroxydopamine-lesioned rats

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Abstract

Parkinson's disease is a major neurological disorder that primarily affects the nigral dopaminergic cells. Nigral histamine innervation is altered in human postmortem Parkinson's disease brains. However, it is not known if the altered innervation is a consequence of dopamine deficiency. The aim of the present study was to investigate possible changes in the H_3 receptor system in a well-characterized model of Parkinson's disease – the 6-hydroxydopamine (6-OHDA) lesioned rats. Histamine immunohistochemistry showed a minor increase of the fibre density index but we did not find any robust increase of histaminergic innervation in the ipsilateral substantia nigra on the lesioned side. *In situ* hybridization showed equal histidine decarboxylase mRNA expression on both sides in the posterior hypothalamus. H_3 receptors were labelled with N-alpha-[3H]-methyl histamine dihydrochloride ([^{3}H] NAMH). Upregulation of binding to H_3 receptors was found in the substantia nigra and ventral aspects of striatum on the ipsilateral side. An increase of GTP-γ-[^{35}S] binding after H_3 agonist activation was found in the striatum and substantia nigra on the lesioned side. *In situ* hybridization of H_3 receptor mRNA demonstrated region-specific mRNA expression and an increase of H_3 receptor mRNA in ipsilateral striatum. Thus, the histaminergic system is involved in the pathological process after 6-OHDA lesion of the rat brain at least through H_3 receptor. On the later stages of the neurotoxic damage, less H_3 receptors became functionally active. Increased H_3 receptor mRNA expression and binding may, for example, modulate GABAergic neuronal activity in dopamine-depleted striatum.

Introduction

Animal models of Parkinson's disease are needed for better understanding the pathogenesis and for drug screening. Neurotoxins, such as 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) or 6-hydroxydopamine (6-OHDA), produce parkinsonism in several species, including fish, rodent, primates and humans (Burns *et al*., 1983; Langston *et al*., 1983; Perese *et al*., 1989; Pollard *et al*., 1992) and provide useful experimental models. 6-OHDA cannot pass through the brain blood barrier, so that intraventicular or intracerebral injections are needed to produce lesions. 6-OHDA is taken up and accumulates in catecholaminergic nerve terminals, hence the damage occurs quite specifically in these neurotransmitter pathways. The cytotoxic effect of 6-OHDA is considered to be related to the formation of oxygen-based free radicals and oxidation products (Reader & Dewar, 1999) in catecholaminergic terminals. However, during the development of experimental parkinsonism, different other neurotransmitter pathways are also modulated, e.g. the serotoninergic, GABAergic and cholinergic ones (Lloyd & Hornykiewicz, 1973; Scatton *et al*., 1983; Reader & Dewar, 1999).

The histaminergic cell bodies and fibres in the rat brain have been mapped with antibodies against histamine (Panula *et al*., 1984; Panula *et al*., 1989). Histamine-containing cell bodies are located exclusively in the tuberomammillary nucleus of the posterior hypothalamus, and their projections extend to all regions of the brain. The involvement of the histaminergic system in control of movement has been suggested, since intracerebroventricular administration of histamine produces biphasic changes in the locomotor activity (Nistico *et al*., 1980; Tuomisto & Eriksson, 1980), and the histamine-induced hypoactivity is inhibited by administration of the histamine H_3 receptor antagonist thioperamide (Chiavegatto *et al*., 1998). Moreover, the central histaminergic system has been suggested to have an inhibitory role in methamphetamine-induced stereotyped behaviour (Ito *et al*., 1997).

Parkinson's disease is one of the major brain-degenerative disorders, characterized by bradykinesia, rigidity and resting tremor. The pathophysiological core of Parkinson's disease is a specific degeneration of dopamine-containing nerve cells in the substantia nigra and ventral tegmental area with their projections to striatum, hypothalamus and thalamus. A growing body of evidence indicates possible involvement of the histaminergic system in the pathogenesis of Parkinson's disease or animal models of this disease. H_3 receptor binding is upregulated in the substantia nigra and striatum of 6-OHDA lesioned rats (Ryu *et al*., 1994, 1996), but is not changed in the striatum of the Parkinson's disease patients (Goodchild *et al*., 1999). Central histamine was unaffected by MPTP treatment in the neocortex and hypothalamus in mice (Cumming *et al*., 1989), whereas nigral and

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striatal levels were not examined. Recently we observed increased histaminergic innervation in the substantia nigra of Parkinson's disease patients (Anichtchik *et al*., 2000). The aim of this study was therefore to examine the potential plastic changes of the histaminergic system in rats after 6-OHDA lesion of the medial forebrain bundle lesion with special emphasis on the H_3 receptor system.

Materials and methods

6-OHDA lesion experiments

Unilateral lesions of the medial forebrain bundle were made in Wistar male rats (National Animal Center, University of Kuopio, Finland) weighing 180–230 g. Thirty minutes before the 6-OHDA infusion the rats were injected with desipramine, a noradrenaline uptake blocker (20 mg/kg i.p.) to restrict the damage to the dopaminergic system only. The animals were anaesthetized with 350 mg/kg chloral hydrate (i.p.), placed in a Kopf stereotactic frame (David Kopf, Tujunga, USA) and the skull was exposed. A stainless steel guide cannula was lowered through a drilled burr hole 2.0 mm above the target. An infusion needle was lowered 2.0 mm below the lower tip of the guide cannula. The coordinates measured from bregma for the final infusion site in the medial forebrain bundle near substantia nigra were AP 4.4, L 1.2, DV 8.3 (Paxinos & Watson, 1986). The volume of freshly made infused 6-OHDA (12 µg in 4 µL of NaCl containing 0.2% ascorbic acid) was 4 µL, given over 8 min using CMA syringes (1 mL) connected to PE 10 type plastic tubing and run by a motor-driven slow-motion syringe pump (CMA/102, CMA Microdialysis, Solna, Sweden). The needle was retained in its position for 4 min after the infusion of 6-OHDA. Following recovery from anaesthesia (about 1 week), the animals were housed singly and after the recovery period in groups of three to five rats per cage. Water and food were available *ad libitum*. Appropriate permits were obtained for all studies. Experiments were in accordance with the National Institutes guidelines and were approved by the Animals Ethics Committee of the University of Kuopio.

Testing of turning behaviour

The rats wore a vest having two holes for the front legs and were placed in individual hemispherical plastic bowls (diameter 35 cm). The vest was connected to the automatic 8-channel rotameter (Coulbourn Instruments, Inc., Allentown, PA, USA). The rotameter registered right and left full turns separately. To induce apomorphine-induced rotation, apomorphine (0.1 mg/kg, 1 mL/kg) was given subcutaneously about 14 days after the toxin infusion. As a criteria for successful lesion the lower limit of 100 contralateral turns per hour was used.

Immunohistochemistry

Rats were anaesthetized with chloral hydrate and perfused transcardially with saline followed by 4% 1-ethyl-3, 3(dimethyl-aminopropyl) carbodiimide (EDAC, Sigma, MO, USA) dissolved in 0.05 M phosphate buffer (PB) pH 7.6. Brains were removed, postfixed in 4% EDAC for 4 days and then in 2% PFA in 0.05 M PB pH 7.6 overnight, transferred to 20% sucrose in 0.05 M PB until they sunk. Then brains were frozen in liquid nitrogen, 20 µm thick coronal sections were cut in a cryostat at –20 °C, thaw-mounted on gelatin-coated slides and stored at –20 °C until examined. All incubations were performed at room temperature, except where noted. Slides were dried at the room temperature for 30 min, then washed in PB saline (PBS) for 15 min. Antibodies were routinely diluted in PBS, in which 0.25% Triton X-100 was added (PBS-T). Sections were pretreated with 1% H_2O_2 in methanol for 30 min to reduce the activity of endogenous peroxidase.

In order to visualize the histaminergic system in the brain of rats bearing unilateral 6-OHDA lesion, we used the immunohistochemical protocol described previously (Anichtchik *et al*., 2000) with minor modifications. Briefly, slides with sections were incubated in the antiserum against histamine raised in the rabbit over two nights at +4 °C. The immunoreactivity was detected using biotin-streptavidin method (Vectastain Elite Kit, Vector Labs, CA, USA). This was finished by the coupled oxidation reaction in a solution containing 0.025% diaminobenzidine (Sigma), 0.01% H_2O_2 and 0.3% $NiSO_4$ in 0.05 M Tris-HCl buffer, pH 7.6 for 2–3 min until a bluish-black product of the reaction appeared. Parallel sections were processed for antityrosine hydroxylase immunohistochemistry with polyclonal antibodies against tyrosine hydroxylase (2 µg/mL) raised in sheep, affinity purified and characterized as described (Haycock, 1989). The reaction was stopped in distilled water, then slides were dried and coverslipped with Permount (Fisher Chemical, NJ, USA). Control sections were incubated as previously described (Panula *et al*., 1989) with preimmune serum or antiserum HA19C preabsorbed with a histamine–protein conjugate. As an additional control, absence of the primary antibody in the first incubation was also used. No immunoreaction was seen in these samples.

H_3 binding experiments

H_3 receptors were labelled with N-alpha-[methyl-^{3}H]-methylhistamine dihydrochloride ([^{3}H] NAMH) (NEN Life Science Products, Inc., MA, USA) (81.500 Ci/mmol). Rats, used for binding experiments, were decapitated, brains were rapidly removed and fresh frozen in a liquid nitrogen. 15 µm thick coronal sections were cut in a cryostat at –20 °C, thaw-mounted on poly (L)-lysine-coated slides and stored at –70 °C until used. Before binding procedures, slides with sections were left to thaw and dry at room temperature. The binding buffer contained 150 mM Na_2/K phosphate buffer pH 7.4, 2 mM $MgCl_2$, 100 µM dithiothreitol and 4 nM [^{3}H]-NAMH. Sections were incubated in the buffer for 45 min at room temperature. The specificity of receptor binding was assessed by addition of 5 µM of specific histamine H_3 receptor antagonist clobenpropit (kindly provided by Professor H. Timmerman) to the incubation buffer. After incubation, sections were washed several times with ice-cold binding buffer without the specific ligand, dipped in ice-cold distilled water and rapidly dried under a stream of cold air. After drying, sections were exposed to tritium-sensitive film (Hyperfilm, Amersham International, UK) for 4 weeks. The experiment groups consisted of four (5 weeks) plus four (8 weeks) rats, which were used for the statistical analysis. The experiment was repeated once with similar groups.

GTP-γ-[^{35}S] binding experiment

The details of the protocol have been described previously (Sim *et al*., 1995; Laitinen & Jokinen, 1998). In the present study, the following procedures were used. Sections of fresh frozen brains were left to thaw and dry at room temperature. All incubations were performed at the room temperature. In the preincubation step, slides were placed in a humidified chamber and incubated with assay buffer, containing 50 mM Tris-HCl, 5 mM $MgCl_2$, 1 mM EDTA and 100 mM NaCl, pH 7.4 for 10 min. Then all sections were loaded with 2 mM GDP in assay buffer for 15 min. Agonist-stimulated activity was determined by incubation of slides in 40 pM of [^{35}S]guanosine-5′-*O*-(3-thio)triphosphate (GTP-γ-[^{35}S]) (NEN Life Science Products Inc.) with an appropriate concentration of the nonspecific H_3 agonist histamine or specific H_3 agonist immepip (kindly provided by Professor H. Timmerman) and 2 mM GDP (Sigma) in the assay buffer for 2 h. Basal binding was assessed by incubation of sections with GDP in the

absence of agonist and nonspecific binding was determined by incubation with 10 μM GTP-γ-S. Specificity of agonist-evoked GTP-γ-[^{35}S] binding was determined by addition of an appropriate concentration of the specific H_3 antagonist clobenpropit in the GDP loading and in GTP-γ-[^{35}S] binding steps. In order to reduce nonspecific GTP-γ-[^{35}S] binding to adenosine A_1 receptors, 8-cyclopentyl-1, 3-dipropylxantine (RBI, Sigma-Aldrich, Natrick, USA) was used in all incubations at 10 μM concentration. The incubation buffer was removed by aspiration; sections were shortly washed twice in ice-cold 50 mM Tris-HCl, pH 7.4, rinsed in ice-cold distilled water and dried overnight. After drying, sections were exposed to Kodak BioMax MR film (Kodak, New Haven, CT, USA) for 2 days.

In situ *hybridization experiments*

The protocol for oligonucleotide *in situ* hybridization used in our laboratory was described previously (Castren & Panula, 1990; Karlstedt *et al*., 1999). We used this protocol with minor modifications. Shortly, two oligonucleotide probes were targeted to bases 583–632 (CGA GCG TTC AGA GAG GAC TCA TCA GCA TTG GGC TCA TGC GCT TTC ATT TC) and to bases 1603–1652 (ATC TTC CGG ACC TGT ACT GGG TCA TCT CCT CCC TCA TTG ACA GAC TCC AG), respectively, of the rat HDC mRNA sequence (Joseph *et al*., 1990). To detect the expression of H_3 receptor mRNA, we used 45-mer-oligonucleotide (GGC TTG GAG CCC CTC TTG AGT GAG CGC GGC CTC TCA GTG CCC CTC), targeted to bases 1231–1275 of the human H_3 receptor mRNA sequence (Lovenberg *et al*., 1999). This oligonucleotide has a three base pair mismatch with the rat H_3 receptor sequence (Lovenberg *et al*., 1999), with 95.4% of the identity. A 50-mer oligonucleotide probe (GAA GCA TGG TAA CCA TCA CAT ACA GCA TGA TGA AGC TGC AAG GCA ACT GG) complementary to *Staphylococcus aureus* chloramphenicol acetyltransferase was used as a negative control. Probes were labelled at the 3′ end using terminal deoxynucleotide transferase (Promega Corporation, WI, USA) and [^{35}S] α-dATP (1000 Ci/mol, New England Nuclear, MA, USA). For *in situ* hybridization, slides were left to thaw and dry at room temperature and were exposed to UV light for 5 min (Schambra *et al*., 1994). Sections were hybridized with hybridizing buffer according to (Karlstedt *et al*., 1999) and ~10^7 cpm of [^{35}S]-labelled HDC, H_3 or control probe per millilitre of hybridization buffer for 24 h at 50 °C. After hybridization slides were washed with 1 × SSC (0.6 M sodium chloride, 0.06 M sodium citrate) at 56 °C and then dehydrated in graded ethanols 30 s in each. After drying, sections were exposed to Kodak BioMax film for 5–10 days and then dipped in Kodak NTB2 emulsion (Kodak, CT, USA) and exposed for 40 days at +4 °C.

Image analysis and statistical procedures

To reveal the morphology and the degree of the degeneration, sections were stained with cresyl violet and examined by light microscopy. Brain areas were identified using the atlas of the rat brain (Paxinos & Watson, 1986). In order to quantify the distribution of immunoreactive fibres, a counting grid in the ocular of the microscope was used, which covered an area 45 × 45 μm. We determined the number of fibres crossing the horizontal and vertical lines of the grid using a × 25 objective. The sum of crosses of the fibres and horizontal or vertical gridlines was considered as the index of fibre density.

Quantification of the autoradiographic *in situ* hybridization and binding films was done by digitizing the film images with a computer-based MCID image analysis system (Imaging Research, Ont., Canada) and by measuring different brain areas in grey-scale pixel values. The relative optical density was converted to a linear grey scale value based on an appropriately derived ^{14}C- or ^{3}H-standard curve. Statistical analysis was performed using repeated measures one-way ANOVA test with Bonferoni post-test by GraphPad Prism (GraphPad Software, CA, USA) only after subtraction of the nonspecific and basal values in the GTP-γ-[^{35}S] binding assay and blocked values in the [^{3}H]-NAM binding assay. Three or four rats were used in every experiment, and every experiment was repeated at least once.

Results

Immunohistochemistry

The success of the 6-OHDA lesion was assessed by immunostaining with anti-tyrosine hydroxylase antibodies. The injection of 6-OHDA to the medial forebrain bundle led to virtually complete disappearance of the tyrosine hydroxylase immunoreactivity from the ipsilateral side of the brain at the levels of the striatum and substantia nigra. This staining was also significantly lower at the level of hypothalamus, as compared with the contralateral side (Fig. 1A and B).

Histaminergic cell bodies were located exclusively in the posterior hypothalamus, corresponding with the tuberomammillary nuclear complex. Varicotic and nonvaricotic nerve fibres and terminal-like structures were localized in all brain regions with different density. When compared with the contralateral side, histaminergic fibre density index was not changed in the striatum and hypothalamus ipsilaterally, whereas a small increase in the substantia nigra (Fig. 1C and D, and Table 1) and the ventral tegmental area was observed. In the posterior cortical nucleus of amygdala, the density of histaminergic fibres was decreased on the lesioned side of the brain (Fig. 1G and F, and Table 1).

Binding experiments

Histamine H_3 receptor binding, determined by [^{3}H]-NAMH autoradiography, was detected in different areas of the brain with different density, being strongest in the substantia nigra, caudate putamen, and less intense in the amygdala, hypothalamus, hippocampus and cortex. It was difficult to the define the exact location of the compact part of substantia nigra on an autoradiography film; therefore the whole substantia nigra was measured. In the striatum, the binding had a clear dorso-ventral gradient with the highest [^{3}H]-NAMH binding in the fundus striati and pallidum and lowest binding in the dorsal aspects of caudate putamen (Fig. 2C). Nucleus accumbens had a relatively high level of [^{3}H]-NAMH binding.

At 5 weeks after the lesion, the increase of H_3 receptor binding (209%) was detected ipsilaterally in substantia nigra when compared with the corresponding site of the contralateral side of the brain (considered 100%). At 8 weeks after the lesion, the relative increase was less pronounced (170%) (Fig. 5A and B). However, at this time the [^{3}H]-NAMH binding on the contralateral side was also increased in comparison with 5 weeks after the lesion. In ventral aspects of the striatum (nucleus accumbens and ventral caudate putamen) [^{3}H]-NAMH binding was also slightly increased (115%). A decrease of [^{3}H]-NAMH binding in the globus pallidus was detected on the ipsilateral side, when compared with the contralateral side (Fig. 2C). [^{3}H]-NAMH binding in the hippocampus and hypothalamus did not differ between the sides of the brain at any time-points after the lesion. Eight weeks after the lesion, H_3 receptor binding density was increased in the posterior cortical nucleus of the amygdala on the control side.

To evaluate the activation of G-protein after stimulation of H_3 receptor, GTP-γ-[^{35}S] binding assay was used. Different concentra-

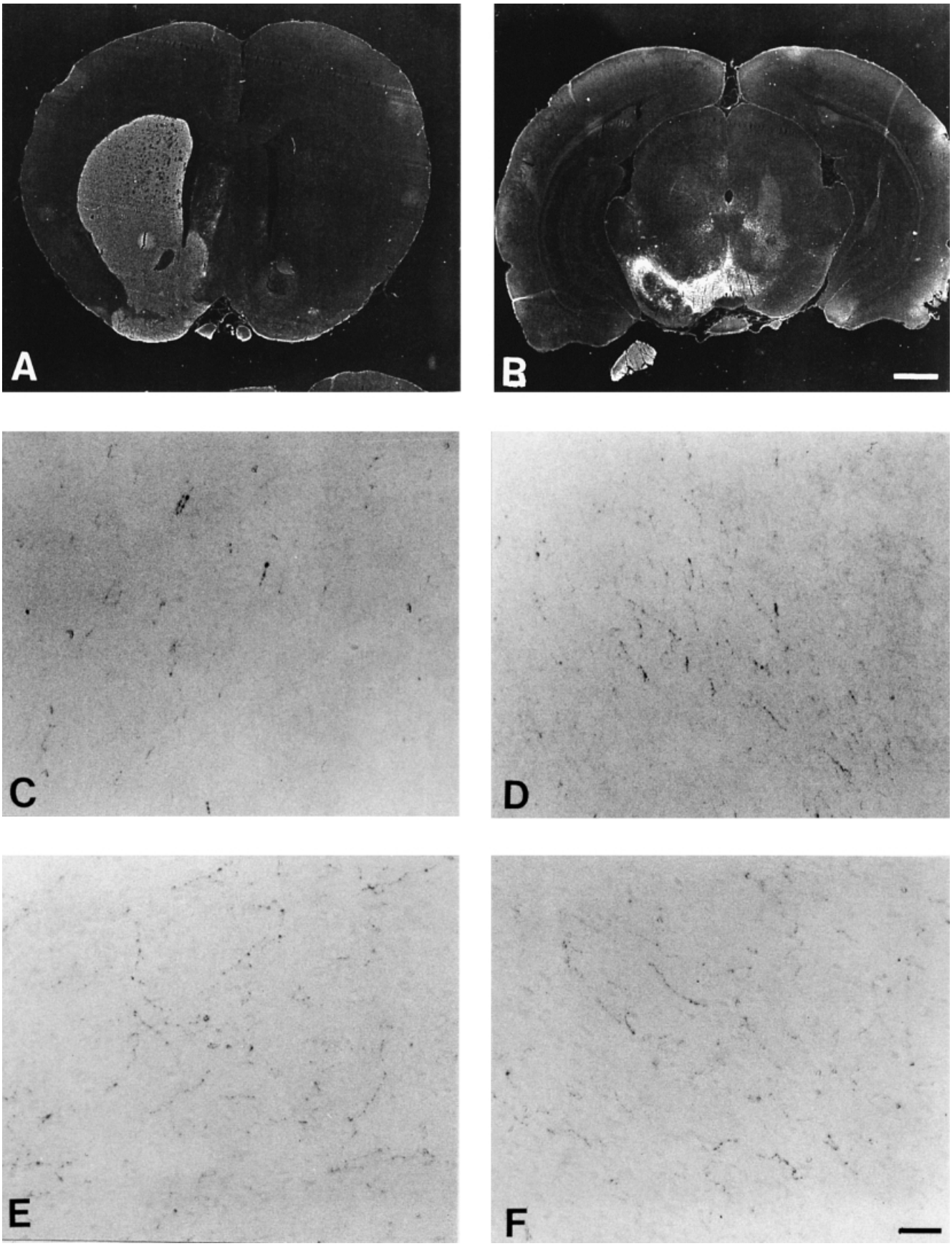

FIG. 1. Immunohistochemical detection of tyrosine hydroxylase in striatum (A), substantia nigra (B) and histamine in the substantia nigra (C and D), and amygdala (E and F) of 6-OHDA lesioned rats. In A and B the lesion is on the right side. The tyrosine hydroxylase immunoreactivity appears white against dark background. In panels C–F the control side is shown in C and E, lesioned side in D and F. Scale bars, 15 mm (A–B) and 50 μm (C–F).

TABLE 1. Histaminergic fibre density index in the brain of 6-OHDA lesioned rats

Time Brain area	Intact side (mean ± SEM)	Lesioned side (mean ± SEM)
5 weeks after lesion		
Substantia nigra	4.17 ± 0.33	4.69 ± 0.36
Amygdala	8.4 ± 0.52	9.4 ± 0.5**
8 weeks after lesion		
Substantia nigra	7.78 ± 0.33	8.81 ± 0.4*
Amygdala	13.1 ± 0.64	10.8 ± 0.63**

*$P < 0.05$, **$P < 0.01$, compared with the contralateral side.

tions of histamine produced a clear dose-dependent increase of the binding in all brain areas (data not shown), and at the dose of 1 μM, GTP-γ-[^{35}S], binding over the basal level was detected in the striatum, substantia nigra, hypothalamus, amygdala and hippocampus. This increase of the binding was mimicked by administration of selective the H_3 receptor agonist immepip. The binding of GTP-γ-[^{35}S] was abolished by 0.5 μM of the selective H_3 antagonist clobenpropit to almost basal levels (Fig. 3). After subtraction of the nonspecific values, we detected a significant difference between sides in G-protein activation after immepip application (Fig. 5C and D). In ventral aspects of the striatum, GTP-γ-[^{35}S] binding was increased to 142% in 5 weeks after the lesion (119% on the normal side) and to 149% after 8 weeks (140% on the normal side) after the lesion, as

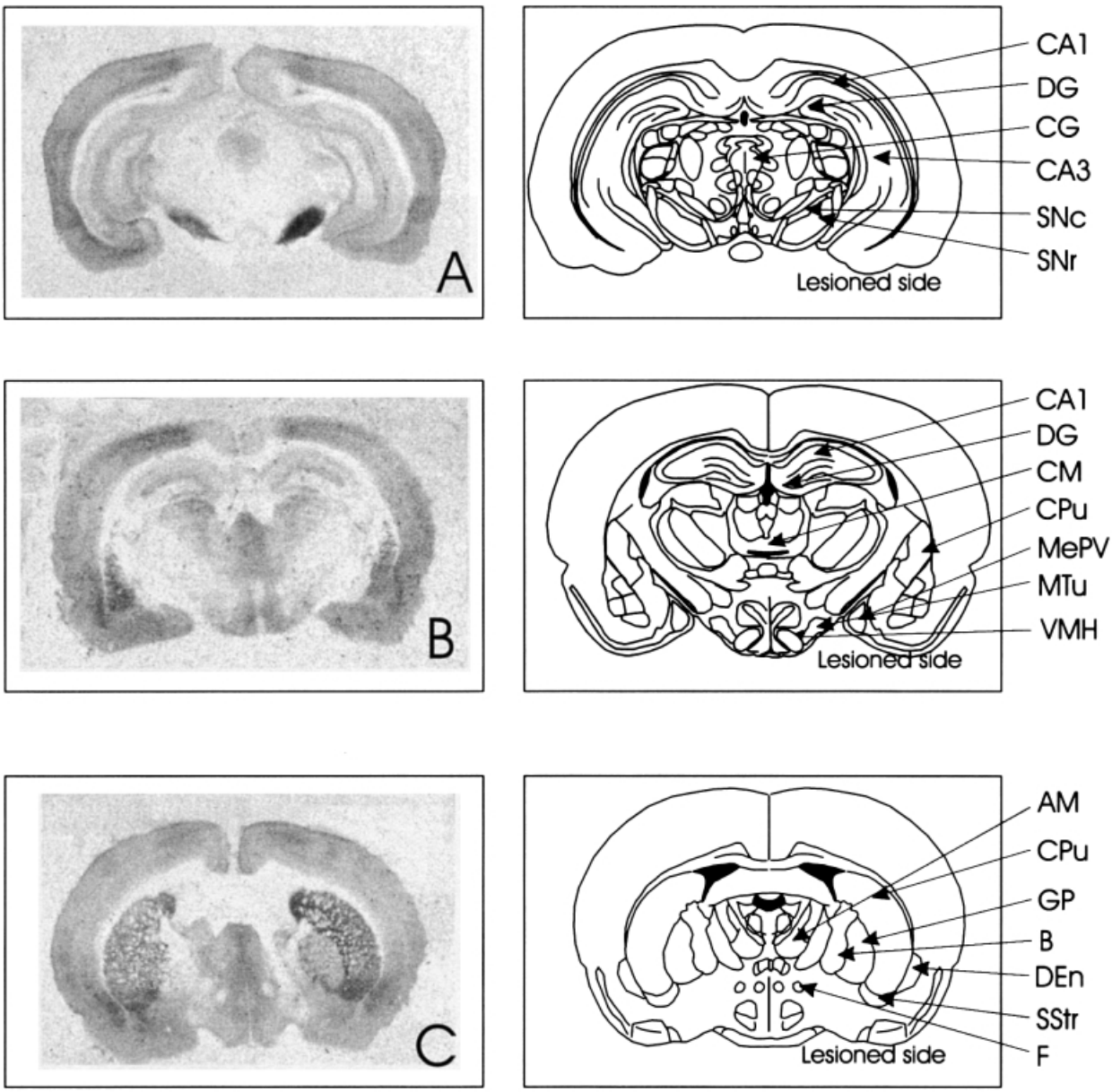

FIG. 2. Autoradiography of [^{3}H]-NAMH binding to H_3 receptors in the brain of 6-OHDA rats. (A) The level of substantia nigra. (B) The level of the hypothalamus. (C) The level of the stratum. Schematic drawing of the same region shows the most important anatomical structures. Abbreviations: AM, anteromedial thalamic nucleus; B, basal nucleus of Meynert; CA1, field CA1 of Ammon's horn; CA3, field CA3 of Ammon's horn; CM, central medial thalamic nucleus; CPu, caudate putamen; DEn, dorsal endopiriform nucleus; DG, dentate gyrus; CG, central grey; F, fornix; MePV, medial amygdaloid nucleus, posteroventral part; MTu, medial tuberal nucleus; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; SStr, substriatal area; VMH, ventromedial hypothalamic nucleus.

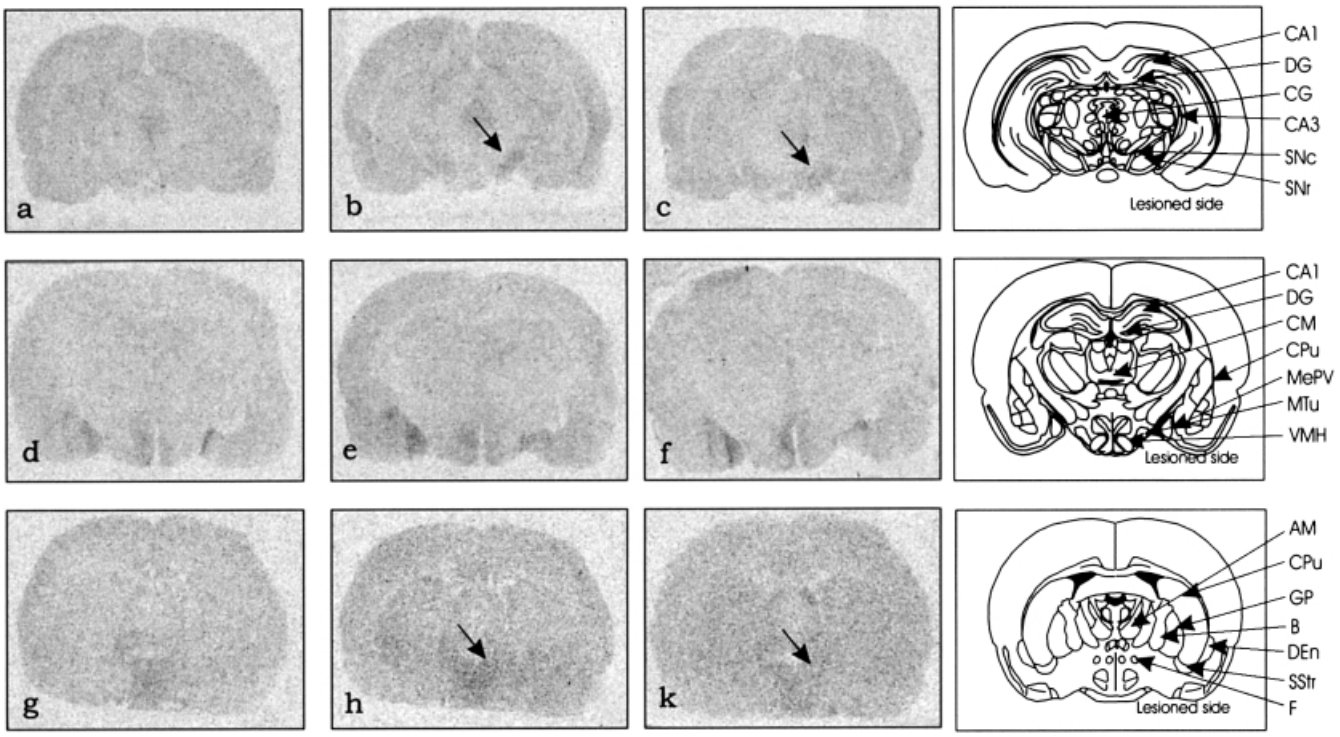

FIG. 3. Autoradiography of GTP-γ-[^{35}S] binding to the activated H_3 receptors at the level of substantia nigra (a, b and c), hypothalamus (d, e and f) and striatum (g, h and k). Basal binding shown in (a, d and g), after H_3 agonist stimulation immepip in (b, e and h) and blocked with receptor antagonist clobenpropit in (c, f and k). Arrowed areas of the H_3 receptor activation in the substantia nigra and ventral aspects of the striatum. Lesioned side is indicated on the scheme. Abbreviations: CA1, field CA1 of Ammon's horn; CA3, field CA3 of Ammon's horn; CPu, caudate putamen; Ctx, cortex; DG, dentate gyrus; LM, lateral mammillary nucleus; MG, medial geniculate nucleus; ML, medial mammillary nucleus, lateral part; MM, medial mammillary nucleus, medial part; PMCo, posteromedial cortical amygdaloid nucleus; SN, substantia nigra; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; Tu, olfactory tubercule.

compared with the basal values (set as 100%). In the substantia nigra, increase on the lesioned side was 162% after 5 weeks (137% on the normal side), and 150% in 8 weeks after the lesion (120% on the normal side). However, when the GTP-γ-[^{35}S] binding on the lesioned side was practically the same at the two time-points, the control side values decreased with a longer time after the lesion (Fig. 5C–D).

In situ *hybridization experiments*

HDC *in situ* hybridization with the oligonucleotide probe revealed HDC mRNA expression only in the posterior hypothalamus of 6-OHDA rats (Fig. 4A). We failed to detect any other sites of HDC mRNA expression in these brains. The distribution and intensity of the hybridization signal was similar when the sides were compared, and no change in expression was detected at different time-points after the lesion (Table 2).

H_3 receptor *in situ* hybridization revealed abundant expression of this receptor mRNA in the central nervous system of the rat (Fig. 4C–F). The most prominent signal was detected in the caudatus-putamen, thalamus, hypothalamus and hippocampus. The cerebral cortex expressed a high level of the mRNA, being most prominent in the middle layers (III–IV) of the parietal cortex. The expression of H_3 receptor mRNA was elevated in the caudate putamen of the lesioned side at all time-points, being statistically significant at 8 weeks after the lesion (Fig. 5E and F). The expression of H_3 receptor mRNA in the substantia nigra was low, and did not differ between the sides at any time-points (Figs 4C, and 5E and F). A moderate *in situ* signal was detected in the posterior hypothalamus, where histaminergic neurons are located.

Discussion

We investigated the histaminergic innervation of the rat brains bearing unilateral 6-OHDA lesions in the medial forebrain bundle, which is a widely accepted model to induce the unilateral catecholamine deficiency. To restrict the damage to the dopaminergic system, rats were injected with desipramine to protect noradrenergic neurons. The contralateral side of the brain served as a undamaged control. In our study, unilateral depletion of dopamine did not affect the general patterns of the histaminergic innervation. We did not detect significant alterations of the histaminergic innervation in the striatum and hypothalamus after the lesion. Only a minor increase of histaminergic fibres was detected in lesioned substantia nigra 8 weeks after the lesion. Also, the histaminergic projections from the tuberomammillary nucleus are bilateral with unilateral predominance (Inagaki *et al.*, 1990), which provides opportunities for compensation of any putative alterations of innervation. At the same time, expression of HDC mRNA was identical on the two sides in the hypothalamus of 6-OHDA lesioned rats. In this study we found a decrease of the fibre density index in the ipsilateral dorsal area of amygdala, the same area where there was also a decrease in H_3 receptor binding. The significance of such a decrease needs further investigation. However, it is possible to suggest that the decreased portion of the H_3 receptor in the ipsilateral amygdala corresponds with H_3 autoreceptors, which are known to reside on histaminergic terminals (Arrang *et al.*, 1983). Altogether, the changes in the histaminergic innervation in the brains of 6-OHDA-treated rats resemble those in the human Parkinson's disease substantia nigra (Anichtchik *et al.*, 2000) but were less robust. We can therefore suggest that the dramatic increase of the histaminergic innervation in

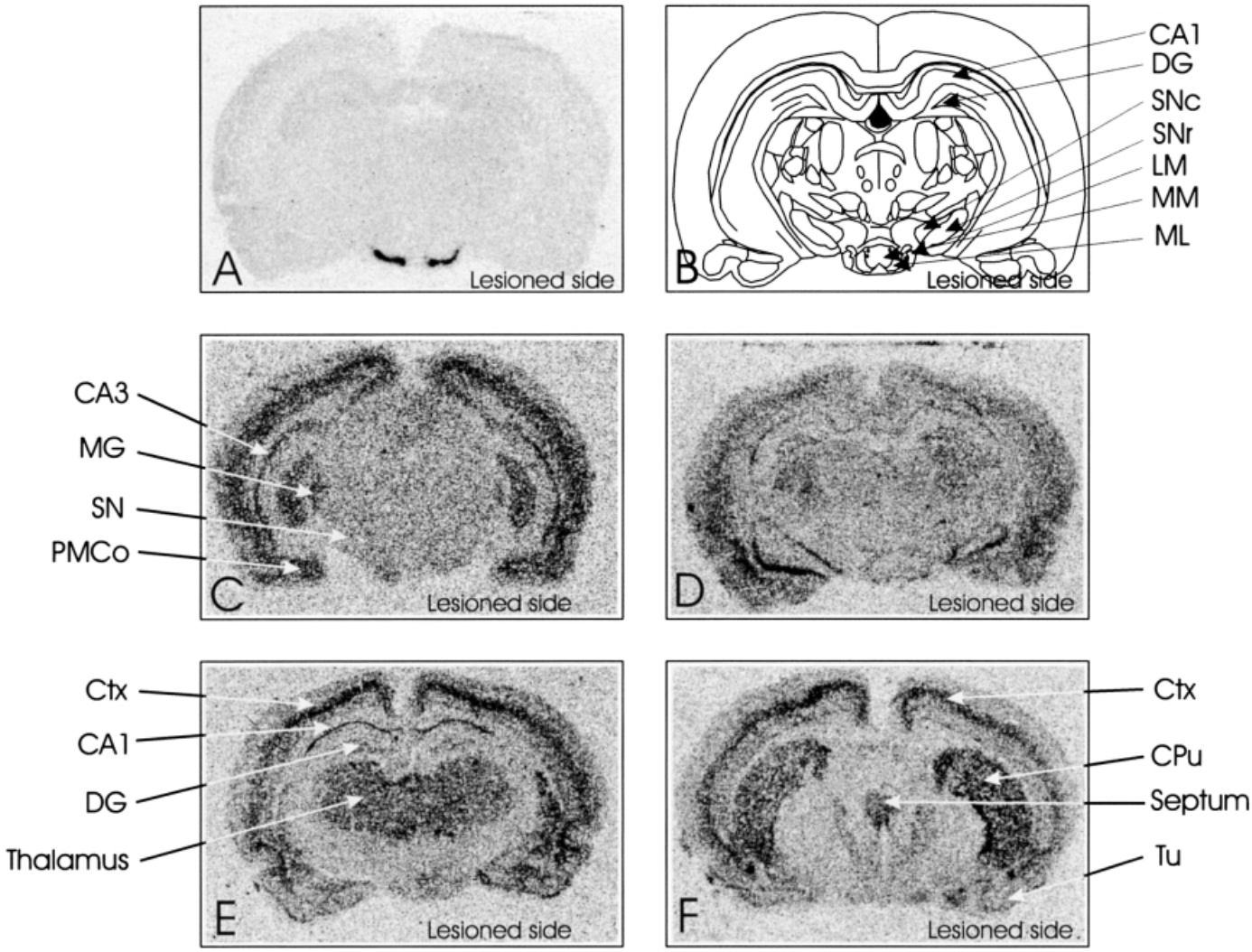

FIG. 4. *In situ* hybridization autoradiography with HDC oligonucleotide probe (A) and H_3 receptor oligonucleotide probe. A schematic drawing of anatomical areas for HDC *in situ* hybridization signal is shown on (B). Abbreviations: CA1, field CA1 of Ammon's horn; CA3, field CA3 of Ammon's horn; CPu, caudate putamen; Ctx, cortex; DG, dentate gyrus; LM, lateral mammillary nucleus; MG, medial geniculate nucleus; ML, medial mammillary nucleus, lateral part; MM, medial mammillary nucleus, medial part; PMCo, posteromedial cortical amygdaloid nucleus; SN, substantia nigra; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; Tu, olfactory tubercule.

TABLE 2. HDC mRNA expression in the posterior hypothalamus of 6-OHDA treated rats

	ROD on lesioned side (mean ± SEM)	ROD on normal side (mean ± SEM)
5 weeks	0.114 ± 0.029	0.113 ± 0.033
8 weeks	0.121 ± 0.059	0.121 ± 0.037

ROD = relative optical density in the posterior hypothalamus.

the Parkinson's disease substantia nigra in humans is not only due to the dopamine deficiency, but reflects a more complex pathological event.

Brain histamine is involved in the control of the locomotion (Onodera *et al*., 1994), and H_3 receptors may be involved in the control of motor activity. For example, the H_3 antagonist thioperamide blocks histamine-induced hypoactivity and the administration of a H_3 agonist α-methylhistamine has an opposite effect (Chiavegatto *et al*., 1998). Thioperamide alone only slightly affects locomotion in a light/dark test measuring anxiety in mice (Imaizumi & Onodera, 1993), but the involvement of H_1 receptors could not be ruled out (Imaizumi *et al*., 1996). In our study, the H_3 receptor binding was increased on the ipsilateral side in the substantia nigra and ventral aspects of the striatum at all time-points, most pronounced at 8 weeks after lesion. These findings are in agreement with previous reports on marked upregulation of the H_3 receptor binding in the substantia nigra and striatum in 6-OHDA lesioned rats, as revealed by [^{3}H]-NAMH binding (Ryu *et al*., 1994, 1996). However, in our study we detected a higher level of upregulation of H_3 receptor binding at 5 weeks than Ryu *et al*. (1994, 1996). It might be due to a methodological difference: we used medial forebrain bundle lesions, not direct substantia nigra lesions. Our approach allows evaluation of the morphology of dopamine-depleted substantia nigra, and excludes the possibility that changes in the receptor density in the nigra are a direct reaction to the lesion.

We demonstrated for the first time the distribution of the H_3 receptor mRNA expression in 6-OHDA lesioned rats, as revealed by oligonucleotide *in situ* hybridization. We found a strong and uneven distribution of the *in situ* signal. Our data on the control material is in agreement with a previous report on H_3 receptor mRNA expression

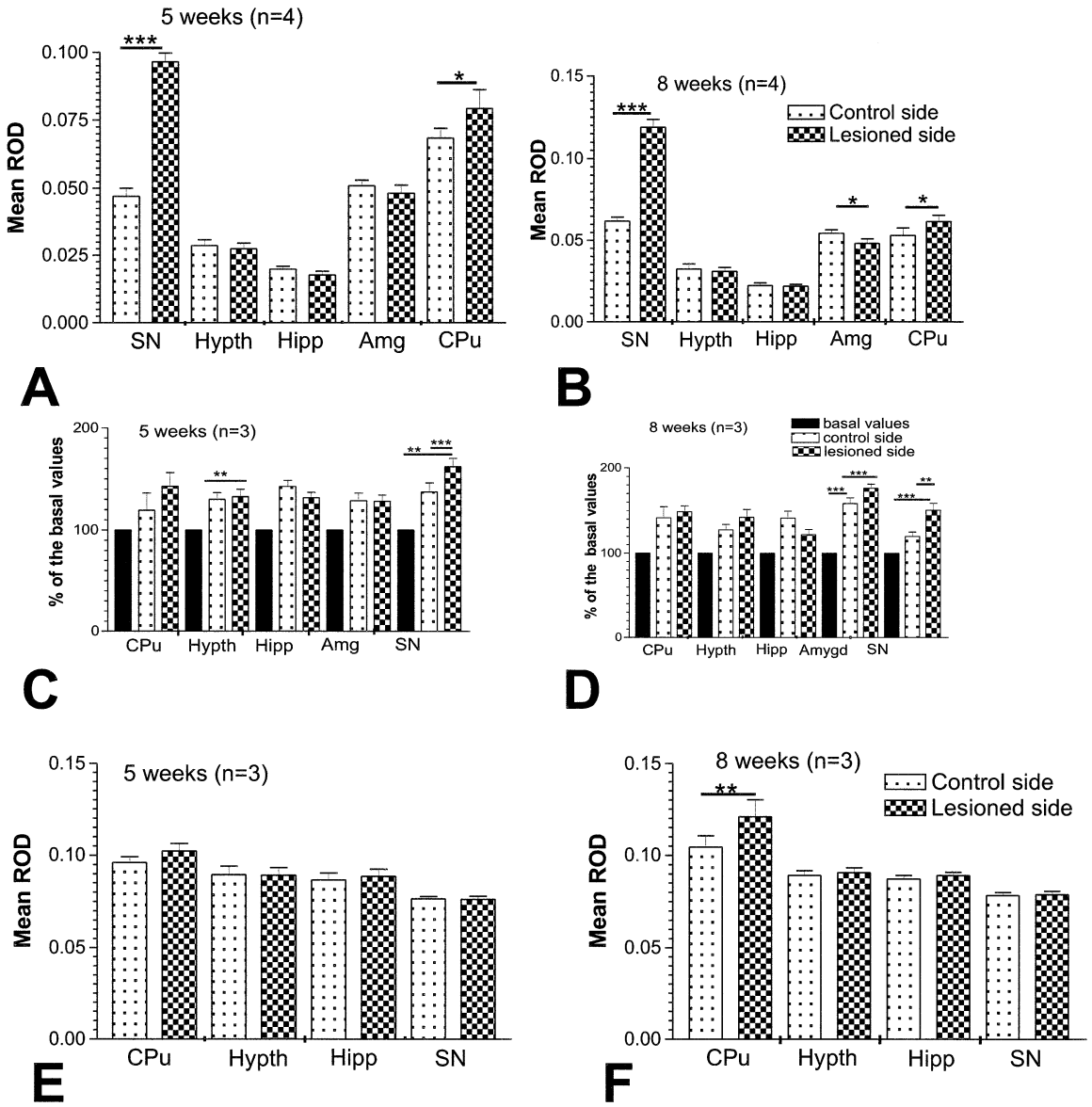

FIG. 5. The histamine H_3 receptor binding (A and B), GTP-γ-[^{35}S] binding (C and D) and histamine H_3 receptor *in situ* hybridization in the brain of 6-OHDA lesioned rats at 5 weeks (A, C and E) and 8 weeks (B, D and F) after the lesion. Statistical analysis was performed using repeated measures one-way ANOVA with Bonferoni post-test. The number of animals is shown in parenthesis. Abbreviations: SN, substantia nigra; Hypth, hypothalamus; Hipp, hippocampus; Amg, amygdala; Cpu, caudatus-putamen. *$P < 0.05$ as compared with control, **$P < 0.01$ as compared with control, ***$P < 0.001$ as compared with control.

(Lovenberg *et al.*, 1999), using cRNA *in situ* hybridization. In the substantia nigra, we did not detect any significant increase of the H_3 receptor mRNA expression or difference between sides. Generally the signal in this region was very low, suggesting that the H_3 receptor binding in this area occurs on projections of neurons with cell bodies outside the substantia nigra. In agreement with this, our finding shows high binding levels and functional activity of H_3 receptors in this region. An increase of the histamine H_3 receptor mRNA expression in the ipsilateral striatum of the 6-OHDA lesioned rats was not unexpected, since we found the increased receptor binding signal in the same region. Expression of several other genes is also increased in the striatum of 6-OHDA-treated animals. The mRNA expression of 5-hydroxytryptamine-2 receptor (Numan *et al.*, 1995), dopamine D_2 receptor and enkephalin increased ipsilaterally (Gerfen *et al.*, 1990; Lisovoski *et al.*, 1992), when dopamine D_1 receptor and substance P mRNA expression decreased in the same region (Gerfen *et al.*, 1990). Also, glial fibrillary acidic protein mRNA, the marker of astrocytes, was increased in the ipsilateral striatum of 6-OHDA rats (Rataboul *et al.*, 1989).

The increased number of H_3 receptors was correlated with the increase of GTP-γ-[^{35}S] binding in the substantia nigra of the lesioned side of the brain. This increase reflects the activation of the G_i/G_o proteins upon stimulation of the receptor with agonist (Sim *et al.*, 1995). Moreover, this technique was proven to be suitable for studying H_3 receptor activation which is G_i/G_o protein coupled (Clark & Hill, 1996; Laitinen & Jokinen, 1998; Lovenberg *et al.*, 1999). In our study, the concordance of the effects after histamine and immepip activation and blockade of the response by clobenpropit further proves that the GTP-γ-[^{35}S] binding occurred in the sites of H_3-dependent G-protein activation. The different magnitude of the H_3 receptor binding and GTP-γ-[^{35}S] binding suggest that, in spite of increased receptor numbers, not all of them are active, especially

during the later stages of the neurotoxic damage. The density of H_3 receptors in substantia nigra decreased ipsilaterally when compared with the contralateral side from 5 to 8 weeks after the lesion from 207 to 170%, being 100% on the contralateral side. An unexpected finding, the decrease of H_3 receptor binding in ipsilateral posterior amygdala, indicates the involvement of the amygdala in the pathological process in 6-OHDA toxic damage.

The destruction of the medial forebrain bundle abolishes the ascending dopaminergic pathway and at the same time tyrosine hydroxylase-positive dopaminergic cells disappear from the substantia nigra. Upregulation of the H_3 receptor number and activity of receptor in the substantia nigra and striatum suggests that this receptor is under a dopaminergic influence. Intrastriatal injection of kainic acid or quinolinic acid produced a significant reduction in striatal H_3 receptor binding sites (Cumming *et al*., 1991; Pollard *et al*., 1993). One of the possible sites for the H_3 receptor localization within the nigrostriatal system is GABAergic cell bodies in the caudate putamen and their terminals. In fact, more than 80% of striatal efferents are estimated to have GABA as a neurotransmitter (Kita & Kitai, 1988). Different populations of GABA neurons possess different types of dopamine receptors: striatopallidal neurons (with substance P and GABA as neurotransmitters) have D_2 receptors, while striatonigral neurons (with enkephalin and GABA as neurotransmitters) have D_1 receptors. As mentioned before, 6-OHDA lesions produce opposite effects on the dopamine receptor mRNA expression and binding in the striatum of rats: the ipsilateral increase of D_2 mRNA expression and decrease of D_1 mRNA expression was reported (Gerfen *et al*., 1990; Lisovoski *et al*., 1992). We detected here the upregulation of H_3 mRNA expression after dopamine depletion. It is interesting to note that both H_3 and D_2 receptors react in a similar way on the dopamine depletion, and both these receptors are G_i/G_o coupled receptors. It may provide an additional link to the interaction between histaminergic and dopaminergic systems.

Histamine apparently controls via H_3 receptors D_1-dependent release of GABA in rat substantia nigra pars reticulata *in vitro* (Garcia *et al*., 1997) and in hypothalamus *in vivo* (Yamamoto *et al*., 1997). We have reported here an increase of the H_3 receptors in the striatum and substantia nigra on the lesioned side. Since we did not detect H_3 receptor mRNA expression in the substantia nigra (or its levels were below detection), we suggest that the receptor mRNA is transcribed and the receptor protein produced in the cell bodies of the striatum, most likely in the GABAergic projection neurons or cholinergic interneurons. The H_3-dependent release of acetylcholine in the cholinergic interneurons of the striatum has been reported recently (Prast *et al*., 1999).

GABAergic neurons of the SNr are under tonic inhibition by input from GABAergic neurons of striatum (Lindefors *et al*., 1990). Striatal GABAergic neurons posses D_1 receptors, get activated by the nigral dopaminergic projections, and their activity is inhibited via D_2 receptors (Reid *et al*., 1990). H_3 heteroreceptors located on GABAergic cell bodies and terminals would contribute to the tonic control of the GABAergic neurons of SNr and striatum. In the situation of dopamine deficiency, GABAergic neurons of SNr may escape from the tonic inhibition of striatonigral projections, hence producing 'hyperinhibition' of the thalamus. Increase of the H_3 receptors in a region of GABA overactivity would lead to at least partial restoration of the tonic control of GABA neurons.

In conclusion, we have detected changes in the histamine innervation, histamine H_3 receptor density and mRNA expression in the brain of unilaterally 6-hydroxydopamine lesioned rats.

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Abbreviations

[^{3}H] NAMH, N-alpha-[methyl-3H]-methylhistamine dihydrochloride; 6-OHDA, 6-hydroxydopamine; AM, anteromedial thalamic nucleus; B, basal nucleus of Meynert; CA1, field CA1 of Ammon's horn; CA3, field CA3 of Ammon's horn; CG, central grey; CM, central medial thalamic nucleus; CPu, caudate putamen; Ctx, cortex; DEn, dorsal endopiriform nucleus; DG, dentate gyrus; EDAC–1-ethyl-3, 3(dimethyl-aminopropyl) carbodiimide; F, fornix; GDP, guanosine 5′-diphosphate; GP, globus pallidus; GTP-γ-[^{35}S], [^{35}S] guanosine, 5′-*O*-(3-thio)triphosphate; GTP-γ-S, guanosine, 5′-*O*-(3-thio)triphosphate; HDC, histidine decarboxylase; LM, lateral mammillary nucleus; MePV, medial amygdaloid nucleus, posteroventral part; MG, medial geniculate nucleus; ML, medial mammillary nucleus, lateral part; MM, medial mammillary nucleus, medial part; MPTP, 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine; MTu, medial tuberal nucleus; PB, phosphate buffer; PBS, phosphate buffer saline; PBS-T, phosphate buffer saline with Triton; Parkinson's disease, Parkinson's disease; PMCo, posteromedial cortical amygdaloid nucleus; SN, substantia nigra; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; SStr, substriatal area; Tu, olfactory tubercule; VMH, ventromedial hypothalamic nucleus.

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