Dopamine Receptor Agonists Regulate Levels of the Serotonin 5-HT_{2A} Receptor and its mRNA in a Subpopulation of Rat Striatal Neurons

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The effects of dopamine receptor agonists on the levels of the striatal serotonin 5-HT_{2A} receptor and its mRNA were investigated in rats lesioned with 6-OHDA as neonates. The mRNA encoding for the 5-HT_{2A} receptor was detected by *in situ* hybridization histochemistry and the binding to 5-HT_{2A} receptors was revealed with [¹²⁵I](2,5-dimethoxy-4-iodophenyl)2-aminopropane ([¹²⁵I]DOI). In adult control unlesioned rats, labeling with the 5-HT_{2A} cRNA probe and with [¹²⁵I]DOI was concentrated in medial sectors of the striatum. In 6-OHDA-lesioned rats, labeling with the 5-HT_{2A} cRNA probe or with [¹²⁵I]DOI was increased in the striatum, particularly in its lateral subdivisions. These increases were abolished after chronic systemic administration of the dopamine receptor agonists apomorphine or SKF-38393. The mRNA levels encoding for the 5-HT_{2A} receptor were further measured in individual striatal

neurons after double-labeling of sections with a 5-HT_{2A} and a preproenkephalin (PPE) cRNA probe. In control unlesioned rats, 5-HT_{2A} mRNA labeling was distributed in PPE-labeled as well as in PPE-unlabeled striatal neurons. In 6-OHDA-lesioned rats, increased 5-HT_{2A} mRNA labeling was found only in PPE-unlabeled neurons and it was abolished after apomorphine or SKF-38393 administration. These results demonstrate that agonists of dopamine receptors inhibit the expression of 5-HT_{2A} receptors in a subpopulation of presumed striato-nigral neurons. We propose that this regulation plays an important role in the control of motor activity by dopamine and 5-HT in the basal ganglia.

Key words: striatum; 5-HT; dopamine; 6-OHDA; 5-HT_{2A} serotonin receptor; D1 dopamine receptor

Dopamine and 5-HT play an important role in several brain functions. Altered regulation of these two neurotransmitters in the basal ganglia is associated with various behavioral dysfunctions including motor and obsessive-compulsive disorders (Sandyk et al., 1988). The striatum, one of the major components of the basal ganglia, receives a dense dopaminergic input from the substantia nigra pars compacta as well as serotonergic projections from the dorsal raphe nucleus. Serotonergic and dopaminergic systems in the striatum interact with each other, and such interaction might play a key role in their respective modes of action. For instance, it has been demonstrated that 5-HT facilitates the release of dopamine in the striatum (Benlucif et al., 1993; Gallaway et al., 1993; Yadid et al., 1994; Bonhomme et al., 1995) and regulates the firing rate of dopamine neurons in the substantia nigra (Kelland et al., 1990). Reciprocally, dopamine afferents are able to facilitate the release of 5-HT in the raphe dorsalis and, at the same time, inhibit this release in the striatum (Lee and Geyer, 1984; Ferré and Artigas, 1993; Ferré et al., 1994).

An interaction between dopaminergic and serotonergic in-

puts to the striatum is also evidenced in adult rats injected with 6-OHDA as neonates. These rats demonstrate an important decrease in the number of dopaminergic afferents and, at the same time, an increased density of 5-HT axons in the striatum, predominantly in its rostral half (Stachowiak et al., 1984; Berger et al., 1985; Snyder et al., 1986; Descarries et al., 1992). The 5-HT hyperinnervation is paralleled by an increase in striatal 5-HT content and reuptake (Luthman et al., 1987; Molina-Holgado et al., 1993, 1994). Neonatal 6-OHDA lesions also induce an increased ligand binding to striatal serotonin 5-HT_{1B}, 5-HT_{1nonAB}, and 5-HT_{2A} receptors (Radja et al., 1993) and an increased responsiveness of striatal neurons to iontophoretic application of 5-HT receptor agonists (El Mansari et al., 1994). An increase in 5-HT_{2A}, but not 5-HT_{1A} or 5-HT_{1C} (also called 5-HT_{2C}), mRNA levels has also been recently reported in the striatum of rats injected with 6-OHDA as adults (Numan et al., 1995).

Adult rats lesioned with 6-OHDA as neonates demonstrate supersensitive behavioral responses to the administration of dopamine D1 receptor agonists (Breese et al., 1985a,b). In addition, the motor hyperactivity exhibited by these rats under drug-free conditions has been shown to involve 5-HT_{2A} receptors (Luthman et al., 1991). These data indicate that both the D1 and 5-HT_{2A} receptor subtypes are preferentially involved in some of the motor abnormalities and adaptive changes exhibited by rats lesioned with 6-OHDA as neonates. In the present study, we tested the hypothesis that dopamine receptor agonists, particularly D1 agonists, are involved in the modulation of striatal 5-HT_{2A} receptor levels in adult rats lesioned with 6-OHDA as neonates. Because striatal projection neurons can be distinguished as two subpopu-

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lations that either express or do not express the preproenkephalin (PPE) mRNA, we further analyzed the fate of the mRNA encoding for the 5-HT_{2A} receptor in these two neuronal subpopulations.

MATERIALS AND METHODS

Neonatal 6-OHDA treatments. Three pregnant female Sprague–Dawley rats (Charles River, Montreal) were housed individually with water and dry food available *ad libitum*. Three days after delivery, each litter was reduced to 12 pups. Twenty-five pups were given bilateral cerebrotentorial injections of the neurotoxin 6-OHDA (Sigma, St. Louis, MO) and 6 pups (sham-operated) were injected with the vehicle (0.9% sodium chloride and 1% ascorbic acid) under anesthesia with methoxyflurane vapors. Pups were injected either with a total of 100 μ g of 6-OHDA in 10 μ l (5 μ l in each ventricle) or with 10 μ l of vehicle (sham-operated). All animals were pretreated with the noradrenaline uptake inhibitor desipramine (25 mg/kg, s.c.) 45 min before surgery, in order to protect noradrenergic neurons.

Pharmacological treatments. Sixty days after the surgery, the sham-operated and six of the 6-OHDA-lesioned rats were injected subcutaneously with vehicle (0.02% acetic acid in 0.9% NaCl). The other 6-OHDA-lesioned rats were subdivided in three groups of six or seven animals that were injected subcutaneously with the mixed dopamine D1/D2 receptor agonist apomorphine (5 mg/kg), the preferential dopamine D1 receptor agonist SKF-38393 (12.5 mg/kg), or SKF-38393 in combination with the dopamine D1 receptor antagonist SCH-23390 (0.2 mg/kg). All injections were given twice daily for 10 d. Three hours after the last injection, all rats were killed by decapitation; their brains were quickly removed and kept frozen at -70°C . Tissue sections (10 or 20 μ m thick) were cut at striatal level on a cryostat, thaw-mounted onto slides coated with gelatin, and stored at -70°C until further use.

Synthesis of the cRNA probes. Radioactive- or digoxigenin-labeled cRNA probes were produced by *in vitro* transcription from cDNA clones encoding for the rat 5-HT_{2A} receptor (Pritchett et al., 1988) or the rat PPE (Yoshikawa et al., 1984). The cDNAs inserted into a PSP64/65 plasmid vector were linearized with *EcoRI* (5-HT_{2A}) or *SacI* (PPE) restriction enzymes. Transcription of the radioactive 5-HT_{2A} cRNA probe was performed using a riboprobe kit (Promega, Madison, WI) in the presence of 2.5 μ M [³⁵S]UTP (1000 Ci/mmol, DuPont NEN, Boston, MA) and 10 μ M unlabeled UTP. Transcription of the digoxigenin-labeled PPE cRNA probe was performed in presence of 0.166 mM digoxigenin-UTP (Boehringer Mannheim, Indianapolis, IN) and 0.33 mM unlabeled UTP. In both cases, unlabeled CTP, GTP, and ATP were added in excess. The reaction was performed for 2 hr at 37°C, and then the cDNA templates were digested with the DNase I. The labeled cRNAs were purified by phenol/chloroform extraction and ethanol precipitation. The length of the cRNAs was reduced to 100–150 nucleotides by partial alkaline hydrolysis to improve accessibility of the probe (Cox et al., 1984).

In situ hybridization and radioautography. Brain sections (10 μ m thick) were quickly dried at room temperature and fixed for 5 min in a solution of 3% paraformaldehyde in phosphate buffer (1 M; pH 7.2) containing 0.02% DEPC. Sections were treated for 10 min with trichloroamine (0.1 M, pH 8.0) containing 0.25% acetic anhydride and then for 30 min with Tris-glycine (1 M, pH 7.0) before being dehydrated and air dried. Each section was covered with 3–3.5 ng of radiolabeled cRNA probe and 4 ng of the digoxigenin-labeled probe diluted in 20 μ l of hybridization solution (containing 40% formamide, 10% dextran sulfate, 4 $\times$ SSC, 10 mM dithiothreitol, 1% sheared salmon sperm DNA, 1% yeast tRNA, 1 $\times$ Denhardt's solution containing 1% RNase-free BSA). Some control sections were hybridized with a sense 5-HT_{2A} or PPE RNA probe to verify the specificity of labeling. The sections were covered with Parafilm and placed in humidified boxes, and hybridization was performed for 4 hr at 50°C. Posthybridization washes were in 50% formamide (in 2 $\times$ SSC) at 52°C for 5 min and 20 min, in RNase A (100 μ g/ml; Sigma; in 2 $\times$ SSC) for 30 min at 37°C, and in 50% formamide (in 2 $\times$ SSC) at 52°C for 5 min. Sections were further rinsed at room temperature for 30 min in 2 $\times$ SSC containing 0.05% Triton X-100 and for 3 $\times$ 5 min in Tris buffer (0.1 M, pH 7.5) containing 0.15 M NaCl, 0.3% Triton X-100 and 2% normal sheep serum. Sections were then covered with 100 μ l of an anti-digoxigenin Fab fragment conjugated with alkaline phosphatase (Boehringer Mannheim) diluted 1:500 in the same Tris buffer and left overnight at 4°C. Then, the sections were rinsed for 3 $\times$ 7 min in the antibody buffer and for 2 $\times$ 5 min in a Tris buffer (0.1 M; pH 9.5) containing 0.1 M NaCl and 0.05 M MgCl₂. The sections were then incubated in the dark for 2–5 hr in the same Tris buffer containing 0.24 mg/ml levamisole, 75 mg/ml nitroblue tetrazolium

and 50 mg/ml X-phosphate (all these chemicals were purchased from Boehringer Mannheim). The reaction was stopped by dipping the slides in Tris buffer (10 mM; pH 8.0) containing 1 mM EDTA. Sections were washed in 2 $\times$ SSC for 15 min, quickly dipped in ammonium acetate (300 mM), rinsed in 70% ethanol, and air dried. Sections were first juxtaposed to Kodak X-OMAT-AR x-ray films for 21 d and then processed for emulsion radioautography. In that case, sections were dipped in the Amersham LM-1 nuclear emulsion, air dried, and stored at 4°C in light-tight boxes in presence of desiccant. After 4–8 d of exposure, the emulsion radioautographs were developed in Kodak D-19 for 3.5 min at 14°C and mounted with Aquaperm mounting media (Fisher Scientific, Orangeburg, NY).

[¹²⁵I]DOI binding. The serotonin 5-HT_{2A} receptors were labeled with [¹²⁵I]DOI (DuPont, Billerica, MA; specific activity 2200 Ci/mmol), according to Mengod's modification (Mengod et al., 1990; Radja et al., 1993) of the protocol of McKenna et al. (1989). Briefly, the sections (20 μ m thick) were preincubated at 25°C for 30 min in 50 mM Tris-HCl buffer (pH 7.4) containing 4 mM CaCl₂, 0.1% ascorbic acid, and 0.1% bovine serum. They were then incubated for 90 min in the same buffer containing 200 pM [¹²⁵I]DOI, in the presence of 30 nM of unlabeled 5-HT to block 5-HT_{2C} sites. Nonspecific binding was determined in the presence of 4 mM cold unlabeled 5-HT. After incubation with the radioligand, the slides were washed in cold buffer (2 $\times$ 10 min) and dried under a stream of cold air. Autoradiographs were generated by juxtaposition of the slides to autoradiographic film (Hyperfilm, Amersham, Arlington Heights, IL), together with Microscales (Amersham); the exposure lasted 3 d.

[³H]mazindol binding. The density of dopamine reuptake sites in the striatum was measured by [³H]mazindol binding, as previously reported by Javitch et al. (1985). Frozen sections (10 μ m thick) were dried under a flow of air and rinsed for 5 min at 4°C in 50 mM Tris buffer with 120 mM NaCl and 5 mM KCl to wash off the endogenous ligand. They were then incubated for 40 min in 15 nM [³H]mazindol (DuPont NEN; specific activity, 22.7 Ci/mmol) in 50 mM Tris buffer containing 300 mM NaCl and 5 mM KCl. Desipramine (0.3 μ M) was added to the incubation medium to block the norepinephrine transporter. Sections were then rinsed 2 $\times$ 3 min in the incubation buffer and 10 sec in distilled water and then dried under a flow of air at room temperature. Sections were then juxtaposed to X-OMAT-AR x-ray films (Amersham) for 14 d.

[³H]citalopram binding. The density of the striatal 5-HT innervation was estimated after citalopram binding to tissue sections. Fresh frozen brain sections (10 μ m thick) were dried and preincubated at room temperature for 15 min in 50 mM Tris buffer (pH 7.4; containing 120 mM NaCl and 5 mM KCl). They were then incubated for 1 hr at room temperature in 1 nM [³H]citalopram (DuPont NEN; specific activity, 82.0 Ci/mmol) diluted in the Tris buffer. Nonspecific binding was determined by incubating control sections for 1 hr in the same solution containing 1 μ M imipramine. After the incubation, the sections were rinsed 2 $\times$ 10 min in ice-cold buffer and quickly dipped in ice-cold distilled water. They were then dried under a flow of cold air and juxtaposed for 3 weeks to tritium-sensitive films (Hyperfilms, Amersham).

Analysis of the film radioautographs. The levels of radioautographic labeling on x-ray films were quantified in the striatum by computerized densitometry with a Macintosh computer and an Ultimage image analysis software (Graftec, France). The optical density of labeling in various striatal sectors was calculated after subtracting the optical density of the film and standardization against emulsion-coated filters (Kodak). Internal ¹⁴C (for ³⁵S-labeled cRNA probe) or ¹²⁵I (for ¹²⁵I-labeled DOI) standards (Amersham) were used to insure that measurements were made in the linear portion of the film. Labeling was measured in four different sectors in order to sample the whole striatal surface. Three sections per animal were analyzed in each condition. The average level of labeling was calculated for each rat and in each striatal sector sampled. Statistically significant difference in radioautographic labeling for each striatal sector in the different experimental groups of rats was determined using a one-way ANOVA, whereas post-hoc paired comparisons were performed with the PLSD Fisher's test. Statistical significance was defined as $p < 0.05$.

Analysis of emulsion radioautographs. The cellular distribution of the 5-HT_{2A}-receptor mRNA in striatal neurons expressing or not the PPE mRNA was examined on emulsion radioautographs by light microscopy. First, all single or double-labeled neurons observed in one microscopic field were mapped on paper using a camera lucida. The mapping was performed at a magnification of 25 $\times$ and the microscopic field corresponded to an area of 0.212 mm². From these maps, the numbers of neurons labeled with the ³⁵S-radioactive 5-HT_{2A} cRNA probe alone or

with the digoxigenin-labeled PPE cRNA probe were then calculated. In order to provide an estimate of the ratio of labeled versus unlabeled neurons in each microscopic field, Nissl-stained neurons on adjacent sections were similarly mapped. The levels of mRNA encoding for the 5-HT_{2A} receptor in individual neurons expressing or not the PPE mRNA was then measured on emulsion radioautographs under dark-field (for PPE-labeled neurons) or bright-field (for PPE-unlabeled neurons) illumination at 40× magnification. The area covered by silver grains in each neuron was measured by computerized image analysis (National Institutes of Health IMAGE 1.55) and expressed as a number of pixels per neuron. The number of pixels in each neuron was determined in an area of constant dimension that was large enough to encircle the larger neuronal profiles. A sample of ~50 neurons labeled for PPE and 50 neurons unlabeled for PPE mRNA was thus analyzed for each rat. The average level of labeling from six rats in each experimental group was then calculated. Statistically significant differences in labeling for each striatal sector between the experimental groups of rats were calculated with an ANOVA. Post hoc pairwise comparisons of 5-HT_{2A} mRNA labeling between experimental groups was performed for each striatal sector with a Fisher's test with $p < 0.05$ considered significant.

RESULTS

Regional and cellular distribution of the 5-HT_{2A} receptor and its mRNA in control rats

Labeling with the 5-HT_{2A} cRNA probe or with [¹²⁵I]DOI exhibited a latero-medial gradient of distribution in the striatum of control rats (Fig. 1*A,B*). Quantitative analysis of the film radioautographs demonstrated that the labeling with the 5-HT_{2A} cRNA probe was higher (+33% on average) in the medial than in the lateral sectors of the striatum (0.048 ± 0.002 vs 0.032 ± 0.002 ; $n = 6$). Similarly, the level of [¹²⁵I]DOI binding was higher (+43% on average) in the medial than in the lateral striatal sectors (0.136 ± 0.023 vs 0.078 ± 0.012 ; $n = 6$).

Observation of the emulsion radioautographs revealed the presence of neurons intensely labeled with the dark-blue alkaline-phosphatase reaction product in the striatum. In contrast, no such labeling could be detected in the overlying cerebral cortex (not shown). In many instances, the digoxigenin-unlabeled neuronal profiles could be distinguished from the surrounding neuropil as light blue spots (see Fig. 5). In addition, the presence of silver grains accumulations on digoxigenin-unlabeled neurons allowed their unambiguous identification. Neuronal profiles showing an accumulation of three or more silver grains were considered labeled with the 5-HT_{2A} cRNA probe.

Labeling with the 5-HT_{2A} cRNA probe was visible in digoxigenin-labeled as well as in digoxigenin-unlabeled neurons. Comparison with adjacent Nissl-stained sections indicated that almost 90% of the striatal neurons in the medial and ~70% of the striatal neurons in the lateral sector were labeled with the 5-HT_{2A} cRNA probe (Table 1). The average numbers of neurons labeled with the 5-HT_{2A} cRNA probe (PPE-labeled and PPE-unlabeled) were slightly lower but not significantly different in the lateral than in the medial striatal sector (Table 1). A large majority (over 95%) of PPE-labeled neurons exhibited 5-HT_{2A} labeling in the lateral and medial striatal sectors. In both striatal sectors, a proportion of 50–60% of neurons expressing the 5-HT_{2A} mRNA was also labeled with the PPE cRNA probe. As estimated from one representative control rat, the level of labeling with the 5-HT_{2A} cRNA probe was higher in neurons of the ventromedial than in neurons of the ventrolateral striatal sector in both PPE-unlabeled (+48%; 100.2 ± 7.7 vs 67.9 ± 4.2 pixels per neuron; $n = 50$) and PPE-labeled (+35%; 107.6 ± 8.4 vs 79.7 ± 7.2 pixels per neuron; $n = 50$) neurons.

Table 1. Average numbers (per 0.212 mm² of tissue section) of neurons labeled with the 5-HT_{2A} cRNA, double-labeled with the 5-HT_{2A} and PPE cRNAs, or Nissl-stained on adjacent sections in two sectors of the striatum

	5HT _{2A} (n)		5HT _{2A} +PPE (n)		Nissl	
	Medial	Lateral	Medial	Lateral	Medial	Lateral
Control	129 ± 12	110 ± 10	66 ± 5	61 ± 5	146 ± 10	151 ± 10
Lesioned	160 ± 9	156 ± 7*	81 ± 6	83 ± 3*	152 ± 10	170 ± 7
Apomorphine	139 ± 8	105 ± 10	91 ± 4	69 ± 5	134 ± 12	133 ± 19
SKF-38393	139 ± 17	119 ± 9	80 ± 8	75 ± 7	151 ± 10	143 ± 2

Average numbers of neurons sampled in a microscopic field corresponding to an area of 0.212 mm². The total number of neurons labeled with the 5-HT_{2A} cRNA probe (5-HT_{2A}), double-labeled with the 5-HT_{2A} and the PPE cRNA probes (5-HT_{2A}+PPE), or Nissl-stained on adjacent sections was calculated in two sectors, medial and lateral, of the striatum. Data are from adult control sham-operated rats (control), rats that received 6-OHDA as neonates (lesioned), and rats that received 6-OHDA as neonates and were treated with apomorphine or SKF-38393 as adults. The values are means (±SEM) from six rats per condition. ANOVAs indicated significant differences between experimental groups for the lateral striatal sector only (see Results). *, Significantly different from the controls or from the apomorphine- and SKF-38393-treated rats with the Fisher's test.

Effect of neonatal 6-OHDA injections and administration of dopamine-receptor agonists on 5-HT_{2A} mRNA levels

Brain sections from adult, sham-operated or 6-OHDA-lesioned, rats were first processed for [³H]mazindol binding to evaluate the loss of dopamine axon terminals in the striatum after neonatal 6-OHDA injections. Intense [³H]mazindol labeling was observed in the striatum of sham-operated rats (Fig. 2*A*). In contrast, very weak labeling was observed in the striatum of rats injected with 6-OHDA as neonates and treated or not with apomorphine or SKF-38393 as adults (Figs. 2*B, C*). In accordance with a previous report (Molina-Holgado et al., 1994), the levels of [³H]citalopram binding to 5-HT reuptake sites were significantly increased in the striatum of rats injected with 6-OHDA as neonates (Fig. 2*C,D*). Chronic administration of apomorphine or SKF-38393 to these rats did not affect the levels of [³H]citalopram binding that remained significantly higher than the levels measured in sham-operated rats (Fig. 3).

The quantification of labeling with the 5-HT_{2A} cRNA probe was performed on x-ray film radioautographs at two frontal levels of the striatum; that is, A = 10 and A = 9.2, according to the stereotaxic atlas of Paxinos and Watson (1986). At the rostral-most level (A = 10.0), the ANOVAs demonstrated significant differences in 5-HT_{2A} mRNA levels between the five experimental groups of rats in the dorsomedial ($F_{(4,23)} = 7.4$; $p = 0.0006$), the ventromedial ($F_{(4,23)} = 6.6$; $p = 0.0011$), the dorsolateral ($F_{(4,23)} = 5.8$, $p = 0.0022$), and the ventrolateral ($F_{(4,23)} = 5.9$, $p = 0.0021$) striatal sectors. At the caudal-most level (A = 9.2), the ANOVAs demonstrated significant differences in 5-HT_{2A} mRNA levels between the five experimental groups in the dorsomedial ($F_{(4,25)} = 8.6$, $p = 0.0002$), the dorsolateral ($F_{(4,25)} = 8.5$, $p = 0.0002$), the ventrolateral ($F_{(4,25)} = 9.0$, $p = 0.0001$) but not the ventromedial striatal sector.

At the rostral-most level of the striatum, the average level of labeling with the 5-HT_{2A} cRNA probe was significantly increased in the four striatal sectors of 6-OHDA-lesioned rats when compared to the labeling in sham-operated rats (Fig. 4*A*). The increased labeling was more prominent in the lateral than in the medial striatal sectors (Fig. 4*A*). At the caudal-most level of the striatum, the average 5-HT_{2A} mRNA labeling was increased in the dorsolateral, the ventrolateral, and the dorsomedial sectors of

Figure 1. Negative images of x-ray films from frontal brain sections processed for *in situ* hybridization with a ³⁵S-labeled 5-HT_{2A} cRNA probe (exposure time 10 d; *A*, *C*, *E*, *G*) or incubated with [¹²⁵I]DOI (exposure time 3 d; *B*, *D*, *F*, *H*). Sections are from adult control sham-operated rats (*A*, *B*), adult rats injected with 6-OHDA as neonates (*C*, *D*), and from adult rats injected with 6-OHDA as neonates and treated chronically with apomorphine (*E*, *F*) or SKF-38393 (*G*, *H*). The labeling intensity in each condition was measured by computerized densitometry in the four striatal sectors illustrated in Figure 1*B*. The surface of analysis shown for the dorsomedial sector was identical for the three other striatal sectors.

Figure 2. Negative images of x-ray films from frontal brain sections processed for [³H]mazindol (exposure time 3 weeks; *A*, *B*) or [³H]citalopram (exposure time 4 weeks; *C*, *D*) binding. Sections are from adult control sham-operated rats (*A*, *C*) or adult rats injected with 6-OHDA as neonates (*B*, *D*).

6-OHDA-lesioned rats (Fig. 4*B*). At the two frontal levels examined, the labeling with the 5-HT_{2A} cRNA probe in 6-OHDA-lesioned rats appeared homogeneously distributed over the whole striatal surface (Fig. 1*C*). Chronic apomorphine or SKF-38393 administration to adult rats lesioned with 6-OHDA as neonates abolished the increases in 5-HT_{2A} mRNA levels in all, except the ventromedial, striatal sectors (Figs. 1*C,E,G*, 4*A,B*). As a consequence, the striatum of rats lesioned with 6-OHDA as neonates and treated with apomorphine or SKF-38393 exhibited a pronounced latero-medial gradient of labeling with the 5-HT_{2A} cRNA probe that resembled the gradient observed in sham-operated rats (Fig. 1*E,G*). The effects of SKF-38393 on 5-HT_{2A} mRNA levels were blocked by concomitant administration of the dopamine D1 receptor antagonist SCH-23390 (Figs. 4*A,B*).

Effects of neonatal 6-OHDA injections and administration of dopamine receptor agonists on [¹²⁵I]DOI binding levels

[¹²⁵I]DOI binding levels were measured on x-ray film radioautographs at only one frontal level of the striatum ($A = 9.2$). The ANOVAs performed for each striatal sector revealed highly significant differences in [¹²⁵I]DOI binding levels between experimental groups in the dorsomedial ($F_{(4,21)} = 5.2$, $p = 0.0047$), the dorsolateral ($F_{(4,21)} = 10.8$, $p < 0.0001$), and the ventrolateral ($F_{(4,21)} = 9.2$, $p = 0.002$) but not the ventromedial ($F_{(4,21)} = 2.8$, $p = 0.0507$) striatal sector.

When compared to sham-operated rats, [¹²⁵I]DOI binding levels in 6-OHDA-lesioned rats were significantly increased in the

dorsolateral and the ventrolateral striatal sectors only (Figs. 1*D* 4*C*). In the medial striatal sectors, there were small increases in labeling that did not reach statistical significance (Fig. 4*C*). As a result of the preferential increase in the lateral striatal sectors, [¹²⁵I]DOI labeling in 6-OHDA-lesioned rats appeared homogeneously distributed over the whole striatal surface (Fig. 1*D*). Chronic administration of apomorphine or SKF-38393 to 6-OHDA-lesioned rats abolished the increases in [¹²⁵I]DOI binding levels in the dorsolateral and ventrolateral striatal sectors but did not produce any statistically significant effect in the dorsomedial or ventromedial striatal sectors (Figs. 1*D,F,H*, 4*C*). The selective effect of apomorphine and SKF-38393 in the lateral striatal sectors resulted in a pronounced latero-medial gradient of distribution of [¹²⁵I]DOI labeling that resembled the distribution observed in sham-operated rats (Fig. 1*F,H*). The effects of SKF-38393 on striatal [¹²⁵I]DOI levels were antagonized by the concomitant administration of SCH-23390 (Fig. 4*C*).

Cellular distribution of the mRNA encoding for the 5-HT_{2A} receptor

Analysis of the emulsion radioautographs indicated that, as in control rats, the 5-HT_{2A} mRNA labeling in 6-OHDA-lesioned rats was distributed in PPE-labeled as well as in PPE-unlabeled neurons (Table 1). In addition, more than 95% of PPE-labeled neurons also expressed the 5-HT_{2A} mRNA. In each experimental group, the numbers of neurons exclusively labeled with the radioactive 5-HT_{2A} cRNA probe or double-labeled with the 5-HT_{2A} and the PPE cRNA probes were not significantly different in the

Figure 3. Level of [³H]mazindol or [³H]citalopram binding in the striatum of adult control sham-operated rats (control), adult rats lesioned with 6-OHDA as neonates (lesioned), and adult rats injected with 6-OHDA as neonates and chronically injected with apomorphine, SKF-38393, or a combination of SKF-38393 and SCH-23390. Labeling was measured by computerized densitometry on x-ray film radioautographs. The values represent the average labeling from six rats in each experimental group and are expressed as a percentage of the controls. ANOVAs for [³H]mazindol or [³H]citalopram binding indicated statistical significant differences between experimental groups ($F_{(4,24)} = 4.5$, $p < 0.0001$ and $F_{(4,24)} = 3.1$, $p < 0.05$, respectively). * $p < 0.05$, ** $p < 0.005$ when compared to controls with the Fisher's test.

lateral and medial striatal sectors (Table 1). In addition, the numbers of single- or double-labeled neurons in the medial striatum were not significantly different between experimental groups (Table 1). In the lateral striatal sector, however, the ANOVAs indicated a significant difference between experimental groups in the number of neurons expressing the 5-HT_{2A} mRNA ($F_{(4,23)} = 3.5$; $p = 0.0231$) or expressing both the 5-HT_{2A} and the PPE mRNAs ($F_{(4,23)} = 4.6$; $p = 0.0073$). Therefore, the numbers of neurons (labeled or not with the PPE cRNA probe) expressing the 5-HT_{2A} mRNA were slightly higher in 6-OHDA-lesioned rats when compared to the sham-operated rats or when compared to the 6-OHDA-lesioned rats that were treated with apomorphine or SKF-38393 (Table 1). In contrast, the numbers of Nissl-stained neuronal profiles in all these groups were not significantly different (Table 1). This indicated that some striatal neurons in the lateral sector of control and 6-OHDA-lesioned rats treated with apomorphine or SKF-38393 did not express the 5-HT_{2A} mRNA or were below the threshold of detection.

Quantification of 5-HT_{2A} mRNA levels was then performed on emulsion radioautographs in individual neurons labeled or unlabeled with PPE in a ventrolateral striatal sector (Fig. 5). The ANOVAs indicated significant differences between experimental groups in the number of pixels per neuron in PPE-unlabeled ($F_{(4,20)} = 6.4$, $p = 0.0018$) but not PPE-labeled ($F_{(4,20)} = 0.406$, $p = 0.8020$) striatal neurons (Fig. 6). Pairwise comparisons with sham-operated rats showed that the 5-HT_{2A} mRNA labeling in 6-OHDA-lesioned rats was significantly increased in PPE-unlabeled neurons (Figs. 5A,B, 6). This increase was abolished after apomorphine or SKF-38393 administration (Figs. 5B-D, 6). The effect of SKF-38393 on 5-HT_{2A} mRNA labeling in PPE-unlabeled neurons was blocked by concomitant administration of SCH-23390 (Fig. 6). The histograms of frequency distribution of the 5-HT_{2A} mRNA labeling in PPE-labeled and PPE-unlabeled neurons shown in Figure 7 illustrate the increase of 5-HT_{2A} mRNA labeling in the population of PPE-unlabeled

Figure 4. Levels of 5-HT_{2A} mRNA (A, B) or [¹²⁵I]DOI binding (C) in the striatum of adult control sham-operated rats (control), adult rats lesioned with 6-OHDA as neonates (lesioned), and adult rats injected with 6-OHDA as neonates and chronically injected with apomorphine, SKF-38393, or a combination of SKF-38393 and SCH-23390. The values represent the average intensity of labeling measured on x-ray films by computerized densitometry and expressed as a percentage of the controls at frontal levels A = 10 or A = 9.2 according to the stereotaxic atlas of Paxinos and Watson (1986). The data (mean $\pm$ SEM) were obtained from six rats in each group. Labeling was measured in four different striatal sectors (DM, dorsomedial; VM, ventromedial; DL, dorsolateral; and VL, ventrolateral). Statistical differences in labeling in each striatal sector were determined after a one-way ANOVA. Pairwise comparisons between different experimental conditions were made according to the Fisher's test. *, $p < 0.01$, **, $p < 0.005$ when compared to the controls; #, $p < 0.01$, or ##, $p < 0.005$ when compared to the 6-OHDA-lesioned; ¶, $p < 0.01$, or ¶¶, $p < 0.005$ when compared to the SKF-38393-treated rats.

Figure 5. Bright-field photomicrographs of brain sections processed for *in situ* hybridization histochemistry with a ³⁵S-labeled 5-HT_{2A} cRNA probe and a digoxigenin-labeled PPE cRNA probe in a ventrolateral striatal sector. Labeling is from an adult control sham-operated rat (*A*), an adult rat lesioned with 6-OHDA as neonate (*B*), and an adult rat lesioned with 6-OHDA as neonate and chronically injected with apomorphine (*C*) or SKF-38393 (*D*). Neurons labeled with the 5-HT_{2A} cRNA probe are indicated by the arrows. Note the increased labeling on the PPE-unlabeled neuron of the 6-OHDA-lesioned rat (*B*). Scale bar, 10 μ m.

neurons in 6-OHDA-lesioned rats and its reversal after apomorphine or SKF-38393 administration.

DISCUSSION

Our results indicate that neonatal 6-OHDA lesions induce concomitant increases in the levels of serotonin 5-HT_{2A} receptor and mRNA in the adult rat striatum. Such increases are abolished in the lateral sectors of the striatum after chronic and systemic administration of apomorphine or SKF-38393. The changes in mRNA levels encoding for the 5-HT_{2A} receptor are restricted to a subpopulation of striatal neurons that do not express the PPE mRNA.

Distribution of the striatal 5-HT_{2A} receptor and its mRNA

The distribution of labeling with the 5-HT_{2A} cRNA probe in the striatum of control rats was similar to the distribution observed with [¹²⁵I]DOI. In both cases, labeling was heterogeneous and was more intense in the medial sectors of the striatum. This similar distribution of labeling is a strong indication that the cRNA probe and [¹²⁵I]DOI specifically labeled the 5-HT_{2A} mRNA and receptor, respectively. This is consistent with previous reports showing that DOI in presence of 30 nM 5-HT labels the 5-HT_{2A} but not the closely related 5-HT_{2C} (formerly 5-HT_{1C}) receptor site (Mengod et al., 1990). The comparable distribution of labeling with the cRNA probe and with [¹²⁵I]DOI also suggests that most striatal 5-HT_{2A} receptors are distributed in cell bodies. This conclusion is

consistent with previous reports (Fishette et al., 1988; Mengod et al., 1990; Pompeiano et al., 1994). A proportion of striatal 5-HT_{2A} receptors would also be localized on dopaminergic nerve terminals (Muramatsu et al., 1988). However, this fraction of receptors was probably not detected in 6-OHDA-lesioned rats, and the changes in striatal [¹²⁵I]DOI binding levels measured in these rats most likely reflect changes in the number of postsynaptic receptors.

After neonatal 6-OHDA lesions, increased levels of the 5-HT_{2A} receptor and mRNA were particularly prominent in the lateral striatal sectors. As a consequence, the heterogeneous distribution of labeling observed in control rats became rather homogeneous in 6-OHDA-lesioned rats. Chronic administration of apomorphine or SKF-38393 resulted again in a pronounced latero-medial gradient of distribution of labeling. Cellular analysis indicated that this gradient was primarily attributable to higher 5-HT_{2A} mRNA levels in neurons of the medial striatal sectors. Altogether, these results suggest that the heterogeneous distribution of the 5-HT_{2A} receptor in the rat striatum is under the control of dopamine. In particular, dopamine appears to exert an inhibitory control on the expression of the 5-HT_{2A} receptor and/or mRNA in neurons of the lateral striatum.

At the caudal-most level examined, the correspondence between the levels of 5-HT_{2A} mRNA and [¹²⁵I]DOI binding was not observed in the dorsomedial sector of the striatum. In this sector, increased 5-HT_{2A} mRNA levels in 6-OHDA-lesioned rats were paralleled by a

Figure 6. Levels of 5-HT_{2A} mRNA labeling in single PPE-labeled and PPE-unlabeled neurons in a ventrolateral sector of the striatum. Radioautographic labeling was measured by computerized image analysis (see Materials and Methods for details). The values are means $\pm$ SEM of the average number of pixels per neuron and are expressed as a percentage of the controls. Data are from adult control sham-operated rats (control), rats that received 6-OHDA as neonates (lesioned), and rats that received 6-OHDA as neonates and were treated with apomorphine, SKF-38393, or a combination of SKF-38393 and SCH-23390 as adults. A sample of 50 neurons per rat from six rats per experimental condition was analyzed. Pairwise comparisons between experimental groups were made with a Fisher's test. *, $p < 0.01$ when compared to the controls; #, $p < 0.01$ when compared to the lesioned rats; and ∇ , $p < 0.01$ when compared to the SKF-38393-treated rats.

small but nonsignificant increase in [¹²⁵I]DOI binding levels. In addition, administration of apomorphine or SKF-38393 abolished the increased levels of the 5-HT_{2A} mRNA, but it had no consistent effect on [¹²⁵I]DOI binding levels. This suggests a certain degree of mismatch between the regulation of the mRNA and the receptor itself in this dorsomedial striatal sector.

Regulation of striatal 5-HT receptors by dopamine receptor agonists

Administration of apomorphine or SKF-38393 had a comparable inhibitory effect on the levels of the striatal 5-HT_{2A} receptor and its mRNA. Furthermore, the effect of SKF-38393 was blocked by the preferential dopamine D1 receptor antagonist SCH-23390. These results strongly suggest that the effects of apomorphine and SKF-38393 are mediated by D1 receptors. In normal rats, systemic administration of apomorphine has been shown to induce an increase in the intracellular levels of 5-HT in the raphe dorsalis and a decrease in the extracellular concentration of 5-HT in the striatum (Lee and Geyer, 1992; Ferré et al., 1994). The regulation of 5-HT levels by apomorphine is mediated by dopamine D2, but not D1, receptors in the raphe dorsalis (Ferré and Artigas, 1993). In addition, when directly infused into the striatum, apomorphine or SKF-38393 do not alter the extracellular concentration of serotonin (Ferré et al., 1994). In light of these previous and our own results, it seems unlikely that the effects of apomorphine or SKF-38393 on the levels of the 5-HT_{2A} receptor and its mRNA involve an action on striatal 5-HT neurons. This interpretation is also supported by the fact that apomorphine or SKF-38393 failed to alter the increases in citalopram binding levels measured in 6-OHDA-lesioned rats.

Changes in 5-HT_{2A} mRNA levels in 6-OHDA-lesioned rats were exclusively observed in the subpopulation of striatal neurons

that do not express the PPE mRNA. It has been previously shown that the majority of striato-pallidal neurons contain the mRNA encoding for enkephalin whereas the majority of striato-nigral neurons express the mRNAs encoding for dynorphin and substance P, but not enkephalin (Gerfen et al., 1990; for review, see also Gerfen, 1992). Thus, our results suggest that the 5-HT_{2A} mRNA is expressed in both striato-pallidal and striato-nigral neurons but its regulation by dopamine receptors occurs only in striato-nigral neurons. Striato-nigral neurons have been shown to preferentially express the dopamine D1 receptor (Gerfen et al., 1990) whereas striato-pallidal neurons express the D2 receptor (Gerfen et al., 1990; Le Moine et al., 1990). It can thus be speculated that D1 receptors are coupled to intracellular pathways that directly participate in the regulation of the 5-HT_{2A} receptor and/or mRNA.

Functional consequences of 5-HT_{2A} receptor regulation

The increased number of 5-HT_{2A} receptors after neonatal 6-OHDA injections may result in hypersensitive responses of striatal neurons to serotonin. This interpretation is supported by previous findings of increased responsiveness of striatal neurons to the inhibitory action of 5-HT or DOI (El Mansari et al., 1994). Another study has shown, however, that 5-HT in such rats elicit excitations rather than inhibitions of striatal neurons (Luthman et al., 1993). Eventual changes in the responsiveness of striatal neurons to 5-HT_{2A} receptor agonists after neonatal 6-OHDA would be associated with an increase in evoked release of striatal 5-HT (Jackson and Abercrombie, 1992) without concomitant changes in the extracellular levels or basal release of 5-HT (Jackson and Abercrombie, 1992; Luthmann et al., 1993; Molina-Holgado et al., 1993, 1994). After chronic administration of dopamine receptor agonists to rats lesioned with 6-OHDA as neonates, it can be expected that the hypersensitivity of striatal neurons to 5-HT receptor agonists will be reversed or attenuated as a consequence of decreased expression of the 5-HT_{2A} receptor.

Previous reports have shown that systemic administration of DOI to adult rats can induce an increase in striatal substance P mRNA and peptide levels (Walker et al., 1991). In addition, lesions of 5-HT neurons with 5,7-dihydroxytryptamine result in a decrease in dynorphin levels without concomitant changes in the levels of striatal PPE mRNA (Morris et al., 1992). On the other hand, a facilitatory role of D1 receptor agonists on the levels of striatal dynorphin and substance P mRNAs has been documented previously (Gerfen et al., 1990). Altogether, these studies indicate that dopamine through D1 receptors, and 5-HT through 5-HT_{2A} receptors, exert a facilitatory control on the expression of peptides in striato-nigral neurons. It is therefore possible that the control of dopamine D1 receptors on the expression of serotonin 5-HT_{2A} receptors has important consequences on the regulation of neurotransmitters in striato-nigral neurons.

Adult rats injected with 6-OHDA as neonates do not exhibit the severe behavioral abnormalities observed when similar extensive lesions are performed on adults (Breese et al., 1984; Bruno et al., 1987; Weihmuller and Bruno, 1989; Zigmond et al., 1990; Johnson and Bruno, 1992). However, these rats exhibit some learning deficits as well as a motor hyperactivity and a behavioral hypersensitivity to the administration of dopamine D1 agonists (Erinoff et al., 1979; Heffner et al., 1982; Breese et al., 1984, 1985a,b; Schallert et al., 1989; Gong et al., 1992, 1993). The motor hyperactivity can be reversed by the systemic administration of ketanserin or mianserin and therefore appears to be mediated by

Figure 7. Histograms of frequency distributions of 5-HT_{2A} mRNA labeling in PPE-labeled and PPE-unlabeled neurons of the lateral striatum. Data are from adult control sham-operated rats (*Control*), rats that received 6-OHDA as neonates (*Lesioned*), and rats that received 6-OHDA as neonates and were treated with apomorphine, SKF-38393 or a combination of SKF-38393 and SCH-23390 as adults. Quantification of silver grains over individual striatal neurons was performed by computerized image analysis (see Materials and Methods for details). The area covered by silver grains is expressed in number of pixels per neuron. A sample of 50 neurons per rat from six rats in each experimental condition was analyzed.

5-HT_{2A} receptors (Luthman et al., 1991). Altered expression of 5-HT_{2A} receptors in rats lesioned with 6-OHDA as neonates might thus play a critical role in the genesis and maintenance of this motor hyperactivity.

Conclusions

The major finding of the present study is that stimulation of dopamine D1 receptors inhibits the expression of 5-HT_{2A} receptors in presumed striato-nigral neurons of the lateral striatum. In the rat striatum, the lateral regions are involved in sensorimotor functions (Dunnett and Iversen, 1981). The control of serotonin 5-HT_{2A} receptors by D1 receptors in the lateral striatum might thus represent an important mechanism involved in the regulation of sensorimotor and motor striatal functions. In keeping with evidence showing that 5-HT increases the release of dopamine in the striatum (Benloucif et al., 1993; Galloway et al., 1993; Yadid et al., 1994; Bonhomme et al., 1995), the negative control of dopamine receptors on the expression of 5-HT_{2A} receptors can be

viewed as a homeostatic mechanism aimed at balancing the effects of dopamine and 5-HT on motor activity.

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