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Modification of adenosine extracellular levels and adenosine A_{2A} receptor mRNA by dopamine denervation

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Abstract

Adenosine A_{2A} receptor antagonists have been proposed as an effective therapy in the treatment of Parkinson's disease. To explore the possibility that dopamine denervation may produce modifications in adenosine A_{2A} transmission, we measured the extracellular concentration of adenosine and adenosine A_{2A} receptor mRNA in the striatum of rats infused unilaterally with 6-hydroxydopamine in the medial forebrain bundle. Fifteen days after 6-hydroxydopamine infusion, extracellular adenosine levels, measured by in vivo microdialysis, were significantly lower (-35%) in the dopamine-denervated striatum. At the time of the decrease in adenosine levels, an increase in striatal adenosine A_{2A} receptor mRNA levels ($+20\%$), measured by in situ hybridization, was observed. Modifications in adenosine A_{2A} transmission, following nigrostriatal dopamine neuron degeneration, establish a potential neural basis for the effectiveness of adenosine A_{2A} receptor antagonists in the treatment of Parkinson's disease.

Keywords: Adenosine, extracellular level; 6-Hydroxydopamine-lesion; Microdialysis; Adenosine A_{2A} receptor mRNA; Striatum

1. Introduction

Adenosine extracellular levels and adenosine A_{2A} receptors play a critical role in the control of motor behaviors originating in the basal ganglia. The sources of extracellular adenosine are dependent both on the degradation of vesicular released ATP, through the action of ecto-5′ -nucleotidase, and on the intracellular adenosine concentration, through the action of a nucleoside transporter which transports adenosine to the extracellular space. The modalities of adenosine release in the central nervous system were recently reviewed and discussed (Cunha, 2001; Latini and Pedata, 2001).

Adenosine A_{2A} receptors have a higher density in the striatum (Jarvis and Williams, 1989; Svenningsson et al., 1997a; Rosin et al., 1998), where they are colocalized with dopamine D2 receptors on neurons of the indirect striatopallidal pathway (Schiffmann et al., 1991; Fink et al., 1992). Stimulation of adenosine A_{2A} receptors decreases the binding affinity of dopamine D2 receptors (Ferré et al., 1991) and elicits effects opposite to those elicited by dopamine D2 receptor activation at the level of second messenger systems and early gene expression (Morelli et al., 1995; Olah and Stiles, 2000). Adenosine A_{2A} receptor agonists induce sedation and catalepsy and inhibit the motor-stimulating effects of dopamine receptor agonists (Durcan and Morgan, 1989; Hauber and Munkle, 1995; Ferré, 1997; Rimondini et al., 1997). Furthermore, in rats with a unilateral 6-hydroxydopamine lesion of the dopaminergic nigrostriatal pathway, parenteral administration of adenosine A_{2A} receptor agonists reduces the contralateral turning behavior induced by direct dopamine receptor agonists (Vellucci et al., 1993; Morelli et al., 1994). In contrast, adenosine receptor antagonists such as caffeine, through an action on adenosine A_{2A} receptors, produce motor stimulant effects by enhancing locomotor activity (Griebel et al., 1991; Svenningsson et al., 1997b; Hauber et al., 1998).

Recent studies with dopamine D2 receptor knockout mice have demonstrated that adenosine A_{2A} receptor agonists and antagonists elicit behavioral and cellular responses in these mice, suggesting that endogenous

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adenosine acting on striatal adenosine A_{2A} receptors might have an independent role rather than simply causing inhibition of dopamine D2 receptor transmission (Chen et al., 2001).

Studies in *N*-methyl-1,2,3,6-tetrahydropyridine (MPTP) and 6-hydroxydopamine models of Parkinson's disease have shown that adenosine A_{2A} receptor antagonists might be good candidates for the treatment of Parkinson's disease due to their positive effect on motor deficits and their potentiation of dopamine receptor agonist-stimulated motor behavior (Pinna et al., 1996; Pollack and Fink, 1996; Fenu et al., 1997; Richardson et al., 1997). In addition to motor activation, the minimal dyskinetic potential further supports the potential use of adenosine A_{2A} receptor antagonists in Parkinson's disease (Kanda et al., 1998; Grondin et al., 1999; Pinna et al., 2001). Despite the highly promising therapeutic potential of adenosine A_{2A} receptor antagonists, the neural basis for the positive effects of adenosine A_{2A} receptor antagonists in Parkinson's disease is still poorly understood.

Modifications of dopamine transmission have long-term consequences on adenosine transmission and on the action of drugs binding to adenosine A_{2A} receptors (Fenu and Morelli, 1998; Fredholm et al., 1999; Fenu et al., 2000). Changes in adenosine transmission at the level of adenosine A_{2A} receptors, produced by dopamine neuron denervation, might therefore be the best explanation for the positive role of adenosine A_{2A} receptor antagonists in animal models of Parkinson's disease.

To address this issue, we correlated the changes in extracellular adenosine levels, measured by in vivo microdialysis, with the expression of adenosine A_{2A} receptors, measured by in situ hybridization of adenosine A_{2A} receptor mRNA, in the striatum of rats infused in the medial forebrain bundle with 6-hydroxydopamine.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (Charles River, Italy) weighing 275–300 g were used. Rats were housed in groups of four with free access to food and water and kept on a 12-h light/dark cycle. The guidelines of the European Community for animal experiments were followed.

2.2. 6-Hydroxydopamine lesion

Rats were anesthetized with chloral hydrate (400 mg/kg i.p.), placed in a David Kopf stereotaxic apparatus and injected unilaterally in the left medial forebrain bundle at coordinates AP = − 2.2, ML = + 1.5, DV = − 7.8 according to the atlas of Pellegrino et al. (1979) with 6-hydroxydopamine·HCl (8 μg/4 μl of saline containing 0.05% ascorbic acid) through a stainless steel cannula. Rats were pretreated with desipramine (10 mg/kg i.p.) in order to prevent 6-hydroxydopamine-induced neurotoxicity to noradrenergic neurons.

2.3. Evaluation of turning behavior

Ten days after the 6-hydroxydopamine infusion, rats were screened on the basis of their contralateral rotations in response to apomorphine (0.2 mg/kg s.c.). Rats not showing at least 100 contralateral rotations during a 1-h testing period were eliminated from the study. For recording of turning behavior, rats were placed in Plexiglas hemispherical bowls (50 cm in diameter) 30 min before the administration of apomorphine, and the number of contralateral rotations was counted by automated rotameters.

2.4. Microdialysis studies

2.4.1. Surgery

Rats were anesthetized with chloral hydrate (400 mg/kg i.p.) and placed in the David Kopf stereotaxic apparatus. Vertical microdialysis probes were implanted bilaterally in the rat dorsolateral striatum. The microdialysis membranes (AN 69 Hospal membrane; 220 μm ID and 310 μm OD; molecular weight cut-off > 15,000 Da) were 3 mm long. The coordinates used for implantation of the microdialysis probe were 0.7 mm anterior and 3.2 mm lateral to the bregma and 6.5 mm ventral from "dura" (Paxinos and Watson, 1998). The external portion of the probe was fixed to the skull with dental cement. After surgery, rats were individually housed in hemispherical bowls which also served as the experimental environment.

Microdialysis experiments were performed 15 days after unilateral infusion of 6-hydroxydopamine into the medial forebrain bundle. Rats were infused with 6-hydroxydopamine on day 1, screened with apomorphine on day 10, implanted with microdialysis probes in both striata on day 14 and used for the microdialysis experiments on day 15.

2.4.2. Microdialysis procedure

Perfusion was started 24 h after implantation of the microdialysis probes in freely moving rats as previously described (Tanda et al., 1997). The inlet of the microdialysis probe was connected to a microperfusion pump (CMA/100 microinjection pump, Carnegie Medicine, Sweden), while the outlet was inserted into a 200-μl test tube. Microdialysis probes were perfused continuously with Ringer's solution (147 mM NaCl, 2.2 mM $CaCl_2$, 4.0 mM KCl, pH 7.0) at a constant flow rate of 3 μl/min. After a 1.5-h stabilization period, 20-min samples were collected. The samples were frozen at − 80 °C until assay (Pazzagli et al., 1993).

2.4.3. Histological control

At the end of experiments, rats were anesthetized with chloral hydrate (400 mg/kg i.p.) and killed by decapitation. The brains were rapidly removed and placed in a vial

containing 10 ml of 9% phosphate-buffered formaldehyde solution. Coronal slices (50 μm) were cut using a microtome and examined to verify the position of the dialysis probes. Samples obtained from rats in which the probes were not correctly positioned were discarded.

2.4.4. Dopamine assay

Extracellular dopamine concentration was evaluated in dialysate samples collected at 0, 60, 120 and 180 min. Ten microliters of dialysate samples was injected without purification into a high-performance liquid chromatograph (HPLC) equipped with a reverse-phase column (LC-18 DB, 15 cm, 5 μm particle size, Supelco) and a coulometric detector (ESA, Coulochem II, Bedford, MA, USA) in order to quantitate dopamine. The first electrode of the detector was set at +150 mV (oxidation) and the second at −250 mV (reduction).

The composition of the mobile phase was 50 mM NaH_2PO_4, 5 mM Na_2HPO_4, 0.1 mM Na_2EDTA, 0.5 mM *n*-octyl sodium sulphate and 15% methanol; pH was adjusted to 5.50. The mobile phase was pumped with an LKB 2150 pump at a flow rate of 1.0 ml/min. The sensitivity of the assay for dopamine was 2 fmol per sample. The concentration of dopamine in 6-hydroxydopamine-lesioned striatum was calculated as a percentage of that in the intact striatum.

2.4.5. Adenosine assay

The adenosine content of the samples was analyzed by HPLC coupled to a spectrofluorimetric detector (LC-240, Perkin Elmer, Norwalk, CT, USA) with a fixed excitation wavelength set at 270 nm and fixed emission wavelength set at 394 nm (Melani et al., 1999). A Nucleosil C-18 column (i.d.: 4.6 mm; length: 150 mm; Waters, MA, USA) with a particle size of 3.5 μm was used. To protect the system from clogging with particulate matter, a Waters in-line filter with a 2-μm pore size was incorporated into the HPLC system upstream from the stationary phase column. The mobile phase was a 50 mmol/l acetate buffer (pH = 5) containing 5% acetonitrile (v/v) and 1 mmol/l 1-octanesulfonic acid sodium salt (Eastman Kodak, Rochester, NY) and was pumped at a flow rate of 0.8 ml/min.

Adenosine was detected as a fluorescent derivative (1,N^6-ethenoadenosine) following derivation with chloroacetaldehyde. Four microliters of Zn acetate (0.1 mmol/l) was added to 30 μl of each sample. The solution was transferred to glass vials, where 0.18 μl of chloroacetaldehyde (4.5%) was added for each microliter of solution obtained. This solution was kept at 100 °C for 20 min.

The adenosine peaks were identified and quantified by comparing retention time and peak heights with those of known standards run according to the sample procedure. Adenosine was identified by its disappearance after incubation of the sample with 1 U of adenosine deaminase at room temperature for 1 min. The minimum detectable amount of adenosine was 0.1 pmol. The data are expressed as absolute values (in μmol/l). The mean adenosine extracellular levels reported in the figures were calculated by averaging the mean ± S.E.M. of the sample values of each rat.

2.4.6. In vitro recovery experiments

To evaluate adenosine recovery through the dialysis membrane, in vitro experiments were performed. Dialysis probes were immersed at room temperature in Ringer's solution containing known concentrations of adenosine. The probes were perfused with Ringer's solution at 3 μl/min and samples were collected every 20 min. Recovery rate was 6.7 ± 0.8% ($n = 3$ probes). Adenosine values reported in this paper were not corrected for recovery.

2.5. In situ hybridization

Rats infused with 6-hydroxydopamine were killed with CO_2. The brains were rapidly removed, frozen in dry ice-cooled isopentane and stored at −20 °C. Cryostat coronal sections (12 μm) were mounted on glass slides coated with gelatin, dried on a warm plate and stored at −20 °C. Slides were then warmed to room temperature, post-fixed in 4% paraformaldehyde 0.9% NaCl solution for 10 min, dehydrated in an ascending series of alcohols, delipidated in chloroform, rehydrated in a descending series of alcohols, air-dried and stored at −20 °C.

For synthesis of radionucleotide probes, a plasmid containing a cDNA sequence complementary to adenosine A_{2A} or dopamine D2 receptors was linearized with *Apa*I or *Xho*I restriction enzymes, respectively, and the antisense probe was generated using SP6 or T3 RNA polymerase in the presence of [^{35}S]UTP. Each slide was hybridized with 100 μl of buffer containing 2×10^6 cpm of radioactively labeled probe. Hybridization was carried out at 55 °C for 12 h. The next morning, slides were washed [1 × saline sodium citrate (SSC), room temperature; 20 mg/ml RNase A for 15 min; 4 × 20 min in 0.2 × SSC at 60 °C, brief rinse in water], air-dried and apposed to film.

The expression of adenosine A_{2A} and dopamine D2 receptor mRNAs was measured in the lateral and medial striatum, 10 mm rostral to the interaural line according to the atlas of Paxinos and Watson (1998).

2.6. Drugs

6-Hydroxydopamine–HCl, desipramine and apomorphine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Drugs administered parenterally were dissolved in saline and injected in a volume of 0.3 ml i.p. per 100 g body weight or in a volume of 0.1 ml s.c. per 100 g body weight.

2.7. Data analysis and statistics

For microdialysis experiments, statistically significant differences in extracellular adenosine levels between groups were evaluated by two-way analysis of variance (ANOVA)

followed by post hoc Fisher's least significant difference (LSD) multiple comparison test. Differences in mean adenosine levels were evaluated by a paired Student's *t*-test, $P < 0.05$.

Film autoradiograms were analyzed with an image analysis program (Scion Image). Data are presented as mean density and are corrected for background density ($MD_{gray\ matter} - MD_{white\ matter}$); values from lateral and medial 6-hydroxydopamine-lesioned striatum were calculated as a percentage of the levels in the lateral and medial intact striatum. Drug effects on mRNA levels were determined by one-factor ANOVA, with $P < 0.05$.

Table 1
Striatal extracellular dopamine levels

6-OHDA infusion	Time (min)	6-OHDA striatum (% of control)
15 days	0	5.6 ± 2
	60	10.5 ± 3
	120	10.6 ± 3.7
	180	8.5 ± 3.7

Concentration of dopamine in the striatum on the 6-hydroxydopamine-infused side of the brain was calculated as percentage of values of the control striatum. Data from 15 days after 6-hydroxydopamine (6-OHDA) infusion in the left medial forebrain bundle. Extracellular dopamine concentration was evaluated in dialysate samples collected at 0, 60, 120 and 180 min ($N = 11$).

3. Results

3.1. Dopamine and adenosine extracellular levels

Fifteen days after 6-hydroxydopamine infusion into the medial forebrain bundle, extracellular adenosine levels were significantly lower (−35%) in the striatum on the 6-hydroxydopamine-infused side of the brain than in the striatum on the non-infused side. Fig. 1 shows the time course of striatal extracellular levels of adenosine expressed as absolute values (in μM). The mean adenosine concentration was 0.017 ± 0.002 μM in the control striatum and 0.011 ± 0.002 μM in the striatum on the 6-hydroxydopamine-infused side of the brain ($N = 14$).

Two-way ANOVA analysis of the time course of extracellular adenosine levels from control and 6-hydroxydopamine-lesioned striatum showed a significant effect of treatment (6-hydroxydopamine infusion) [$F(1,256) = 15.5$; $P < 0.0001$], no significant effect of time [$F(8,256) = 0.45$; $P = 0.90$] and no interaction between treatment and time [$F(8,256) = 0.16$; $P = 0.99$]. Extracellular adenosine levels were therefore significantly reduced by the lesion but were not changed during the experiment in either striata.

In order to evaluate the extent of dopamine neuron degeneration, extracellular dopamine concentration was evaluated in dialysate samples collected 15 days after 6-hydroxydopamine infusion. Table 1 shows the levels of dopamine at 0, 60, 120 and 180 min, expressed as percentage of the values obtained from the striatum on the non-infused side of the brain. Extracellular dopamine levels were reduced by 91% in the striatum on the 6-hydroxydopamine-infused side as compared to those in the striatum on the non-infused side of the brain 15 days after 6-hydroxydopamine infusion ($N = 11$) (Table 1).

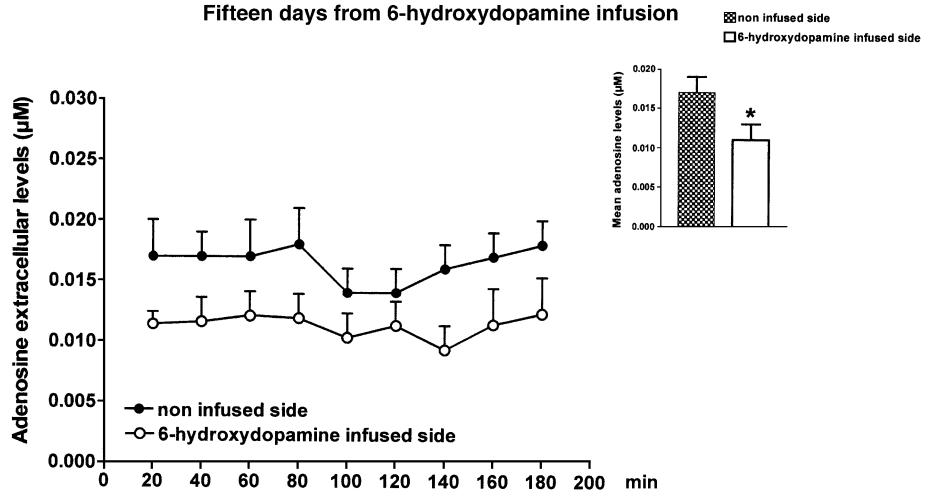

Fig. 1. Time course of extracellular adenosine levels in the striatum on the control and 6-hydroxydopamine-infused side 15 days after 6-hydroxydopamine infusion. Each point is the mean ± S.E.M. expressed as absolute values (in μM) not corrected for membrane recovery. Closed circles: control non-infused side; open circles: 6-hydroxydopamine-infused side. In the inset, the mean extracellular adenosine level is represented. Grey bars: control non-infused side; white bars: 6-hydroxydopamine-infused side. Paired Student's *t*-test: $^*P < 0.04$ for 6-hydroxydopamine-infused striatum vs. control striatum ($N = 14$).

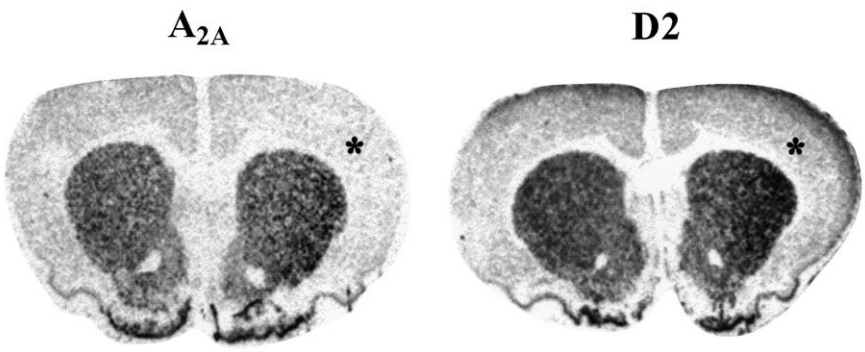

Fig. 2. In situ hybridization for adenosine A_{2A} and dopamine D2 receptor mRNA in coronal striatal sections from unilaterally 6-hydroxydopamine-lesioned rats. Asterisk indicates the 6-hydroxydopamine-infused side of the brain.

3.2. Adenosine A_{2A} and dopamine D2 receptor mRNA

Levels of mRNA for adenosine A_{2A} receptors and dopamine D2 receptors were analyzed in both lateral and medial portions of the striatum 15 days after 6-hydroxydopamine infusion in the medial forebrain bundle. Mean density values for lateral and medial 6-hydroxydopamine-lesioned striatum were calculated as percentages of the values for the corresponding lateral and medial intact striatum.

Adenosine A_{2A} receptor mRNA levels increased in the striatum on the 6-hydroxydopamine-infused side of the brain as compared to those in the striatum on the non-infused side ($N=6$). The increase reached a significant level (20%) in the lateral but not in the medial portion of the striatum (Figs. 2 and 3).

Dopamine D2 receptor mRNA levels were also significantly increased in the lateral but not in the medial portion of the striatum on the 6-hydroxydopamine-infused side of the brain as compared to those in the striatum on the non-infused side ($N=6$) (Figs. 2 and 3).

4. Discussion

The present results show that 15 days after unilateral 6-hydroxydopamine infusion in the medial forebrain bundle, when dopamine extracellular levels had decreased by 91%, striatal extracellular adenosine levels were significantly lower (-35%) in the striatum on the 6-hydroxydopamine-infused side than in the intact control striatum.

The decrease in extracellular striatal adenosine levels, produced by degeneration of dopaminergic nigrostriatal neurons, might suggest that a pool of adenosine is associated with dopaminergic neurons, although this pool is not the sole source of adenosine because after dopamine denervation, extracellular adenosine levels were significantly reduced but still measurable. The decrease in extracellular adenosine levels after 6-hydroxydopamine might be influenced by the chronic lack of dopamine transmission via dopamine D1 receptors, which control the activity of both γ-aminobutyric acid (GABA)-containing striatonigral neurons and cholinergic interneurons. This might result in a decreased cAMP formation and subse-

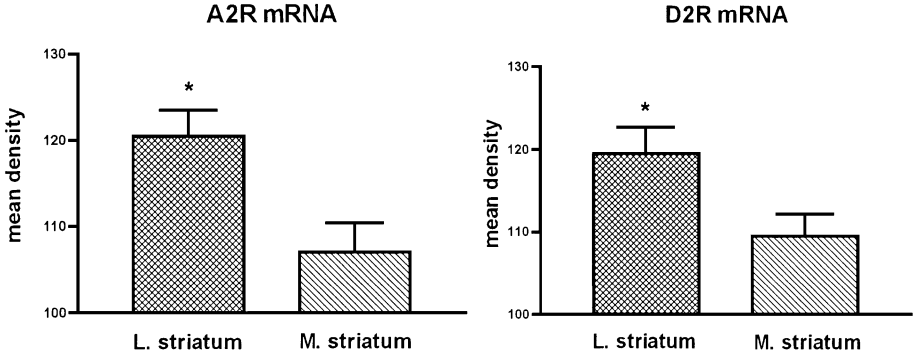

Fig. 3. Adenosine A_{2A} and dopamine D2 receptor mRNA levels in the lateral (L) and medial (M) portion of striatum from 6-hydroxydopamine-lesioned rats. Data are presented as mean density and are corrected for background ($MD_{gray\ matter} - MD_{white\ matter}$). Values from lateral and medial 6-hydroxydopamine-lesioned striatum were calculated as a percentage of the values for the lateral and medial intact striatum. One-way ANOVA: * $P<0.05$ for 6-hydroxydopamine-infused striatum vs. control striatum ($N=6$).

quent decrease in adenosine formation by the action of 5′-nucleotidase.

A previous study by Wojcik and Neff (1983), performed with striatal slices, showed that the increase in adenosine content produced by decapitation was less marked in the 6-hydroxydopamine-lesioned striatum, whereas kainic acid lesion of intrinsic striatal neurons produced a dramatic loss of adenosine content, suggesting that the main source of adenosine in the striatum is from intrinsic neurons. Further support for the important function of intrinsic striatal neurons in the formation of adenosine is given by a study of adenosine production from extracellular ATP at cholinergic synapses (James and Richardson, 1993) and by the suggestion that cholinergic neurons are a source of adenosine (Pedata et al., 1989).

Previous studies have reported that both in neonate and adult 6-hydroxydopamine-lesioned rats or in MPTP-lesioned marmosets, striatal adenosine levels are not modified by mesencephalic dopamine neuron deafferentation (Ballarin et al., 1987; Herrera-Marschitz et al., 1994; Nomoto et al., 2000). Measurement of striatal adenosine in these experiments was performed in anesthetized rats and/or on the same day of probe implantation. Implantation of the probe induces brain tissue damage, so that adenosine concentrations measured early after implantation may reflect an alteration of cellular metabolism (Latini and Pedata, 2001). In line with this finding, extracellular adenosine concentrations have been shown to be 20 times lower 24 h after probe implantation (Pazzagli et al., 1993). Furthermore, adenosine levels have been shown to be lower in anesthetized rats than in unanesthetized rats (Pazzagli et al., 1993). Under our conditions, dialysates were collected 24 h after microdialysis probe implantation from freely moving rats, a time when it is possible to detect modifications in extracellular adenosine levels not influenced by traumatic events or by anesthesia.

A study performed after acute MPTP infusion reported an increase in extracellular adenosine levels as a consequence of the hypoxic effect produced by MPP^+, the active metabolite of MPTP, at the level of the mitochondrial respiratory chain (Ballarin et al., 1989). The mechanism of action and the consequences of acute MPTP infusion therefore appear quite different from those observed in this study in which 6-hydroxydopamine was used as a toxin.

Besides modifications in striatal extracellular adenosine levels, dopamine neuron degeneration increased the level of mRNA for adenosine A_{2A} receptors (+20%). Adenosine A_{2A} receptor mRNA levels were selectively increased in the lateral portion of the lesioned striatum as compared to those in the intact striatum. Moreover, as reported in previous studies (Joyce, 1991), dopamine D2 receptor mRNA levels were significantly increased in the lateral portion of the striatum of 6-hydroxydopamine-lesioned rats. It is interesting to note that previous studies have described the lateral portion of the striatum as being the most important for the control of dopamine-mediated motor behavior (Brown and Sharp, 1995).

The restricted effect of dopamine denervation on adenosine A_{2A} mRNA levels to the lateral portion of the striatum is most probably at the origin of the failure of Kaelin-Lang et al. (2000) to detect modifications in adenosine A_{2A} receptor mRNA. Evaluation of mRNA in that study was performed in the whole striatum, with no distinction being made between the medial and lateral portions.

Previous studies, including ours (Alexander and Reddington, 1989; Martinez-Mir et al., 1991; Morelli et al., 1994; Przedborski et al., 1995), failed to report modifications in adenosine A_{2A} receptor binding after 6-hydroxydopamine lesions. The discrepancies between receptor binding studies and in situ hybridization studies might be due to the different sensitivities of the two methodologies or to the fact that binding studies detect both pre- and post-synaptic receptors. After 6-hydroxydopamine-induced lesions, the loss of adenosine A_{2A} receptors located on dopamine terminals might compensate for the increase in post-synaptic adenosine A_{2A} receptors and impair the detection of small post-synaptic changes.

Studies of post-mortem human brain tissue showed a decrease in mRNA levels for adenosine A_{2A} receptors (Hurley et al., 2000) and no changes in adenosine A_{2A} receptor binding (Martinez-Mir et al., 1991) in the striatum of patients affected by Parkinson's disease. All patients analyzed in these studies were receiving dopaminergic treatment at the time of death, and therefore, the decrease in adenosine A_{2A} mRNA levels might be produced by the dopaminergic therapy. Such changes may not be present or may be different in the brains of untreated parkinsonian patients.

On the basis of the reported results, it can be hypothesized that the loss of dopamine and the consequent decrease in extracellular adenosine levels caused by dopamine neuron degeneration might produce dopamine D2 and adenosine A_{2A} receptor supersensitivity. However, whereas dopamine D2 receptors are no longer stimulated due to the almost complete loss of dopamine, adenosine A_{2A} receptors might still be stimulated by the residual endogenous adenosine tone. In this situation, stimulation of adenosine A_{2A} receptors might prevail over dopamine D2 receptor stimulation and might contribute to the motor impairments which characterize Parkinson's disease.

Functional studies of c-*fos* expression or GABA and acetylcholine release support this interpretation by showing a stronger control of neuronal activity by adenosine A_{2A} receptors after dopamine denervation. In vivo microdialysis revealed overactivity of the striatopallidal GABAergic pathway after 6-hydroxydopamine, as shown by an increase in GABA extracellular levels in the globus pallidus (Ochi et al., 2000). Blockade of adenosine A_{2A} receptors by parenteral administration of (*E*)-1,3-diethyl-8-(3,4-dimethoxystyryl)-7-methyl-3,7-dihydro-1*H*-purine-2,6-dione (KW 6002) antagonized the increase in extracellular GABA

levels in the 6-hydroxydopamine-lesioned globus pallidus but did not affect levels in the intact globus pallidus (Ochi et al., 2000). Similarly, stimulation of adenosine A_{2A} receptors by 2-[*p*-(2-carboxyethyl)phenethylamino]-5′-*N*-ethylcarboxamido adenosine (CGS 21680) caused a greater increase in acetylcholine release and in c-*fos* expression in the 6-hydroxydopamine-lesioned striatum than in the intact striatum (Morelli et al., 1995; Kurokawa et al., 1996).

Adenosine A_{2A} receptor stimulation, by depressing locomotor activity and by increasing acetylcholine release, has a negative influence on motor performance. In the case of Parkinson's disease, an overactivity of adenosine A_{2A} receptors together with a lack of dopamine D1 and D2 receptor stimulation might play an important role in the control of neuronal functions associated with the motor disturbances which characterize Parkinson's disease. Adenosine A_{2A} receptor antagonists, by eliminating the adenosine A_{2A} receptor negative tone, might be beneficial in the treatment of Parkinson's disease.

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