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Adenosine A_{2A} receptor antagonism increases striatal glutamate outflow in dopamine-denervated rats

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Abstract

The objective of the work was to study, by in vivo microdialysis, the effect of the adenosine A_{2A} receptor antagonist 7-(2-phenylethyl)-5-amino-2-(2-furyl)-pyrazolo-[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (SCH 58261) on glutamate outflow in the striata of unilateral 6-hydroxydopamine-infused rats. Two vertical microdialysis probes were implanted bilaterally in both the denervated striatum and in the intact striatum. Glutamate concentrations in the dialysate were determined by high-performance liquid chromatography (HPLC). Infusion of the adenosine A_{2A} receptor antagonist SCH 58261 (50 nM), through the microdialysis fiber, significantly increased glutamate outflow from the denervated striatum while it decreased glutamate outflow from the intact striatum. The opposite effects of SCH 58261 on glutamate outflow in the intact and 6-hydroxydopamine-lesioned striatum might be attributed to blockade of striatal adenosine A_{2A} receptors located on either striatal indirect output pathways or glutamatergic terminals. These results may be relevant to our understanding of the mechanism of action of adenosine A_{2A} receptor antagonists in Parkinson's disease.

Keywords: Adenosine A_{2A} receptor; Parkinson's disease; Striatum; Microdialysis; Glutamate

1. Introduction

Parkinson's disease is due to severe degeneration of nigrostriatal dopaminergic neurons, which leads to a disorder of extrapyramidal motor function. Current therapy for Parkinson's disease is based on dopamine replacement, but this is followed by long-term complications such as loss of drug efficacy and dyskinesia (Marsden, 1982). Recent evidence suggests that antagonism of striatal adenosine A_{2A} receptors may represent an alternative therapeutic approach to Parkinson's disease. Selective adenosine A_{2A} receptor antagonists have been reported to reduce motor (Kanda et al., 1998) and cognitive deficits (Gevaerd et al., 2001) in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) rodent and primate models of Parkinson's disease and to potentiate L-3,4-dihydroxyphenylalanine (L-DOPA) and selective dopamine agonist-induced turning behavior in unilaterally 6-hydroxydopamine-lesioned rats (Fenu et al., 1997). Furthermore, recent epidemiological studies have described an association between the consumption of the adenosine A_1/A_{2A} receptor antagonist caffeine and a reduced risk of developing Parkinson's disease in humans (Ascherio et al., 2001).

Adenosine A_{2A} receptor mRNA is highly expressed in the striatum, nucleus accumbens, olfactory tubercle and the lateral segment of the globus pallidus (Jarvis and Williams, 1989). In the striatum, adenosine A_{2A} receptors are expressed by γ-aminobutyric acid (GABA)-enkephalin striatopallidal neurons (Schiffmann et al., 1991), where they are co-localized with dopamine D2 receptors (Fink et al., 1992), and also in 25% of the large cholinergic interneurons (Dixon et al., 1996). Stimulation of adenosine A_{2A} receptors is known to decrease the binding affinity of dopamine D2 receptors (Ferre et al., 1991). It was recently demonstrated that compounds acting on adenosine A_{2A} receptors elicit behavioral and cellular responses in dopamine D2 receptor knockout mice, suggesting that adenosine acting on adenosine A_{2A} receptors may exert a role, independent of dopamine D2 receptors (Chen et al., 2001). The striatum receives dense glutamatergic innervation from the cerebral

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cortex, which has a tonic stimulatory role on intrinsic and efferent pathways associated with dopamine (Hauber, 1998). Accordingly, adenosine A_{2A} receptors regulate glutamate outflow from the striatum (Corsi et al., 2000). Glutamate therefore contributes to the coordinated balance of the functions of the major striatal output pathways, which control movements.

We have demonstrated, by using in vivo microdialysis, that the basal extracellular concentration of striatal glutamate is reduced in aged rats compared to young rats (Corsi et al., 1999, 2000) and that the selective adenosine A_{2A} receptor antagonist 7-(2-phenylethyl)-5-amino-2-(2-furyl)-pyrazolo-[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (SCH 58261) restores glutamate outflow (Corsi et al., 2000). Since Parkinson's disease is an age-related pathology, the purpose of the present investigation was to study the effect of the selective adenosine A_{2A} antagonist SCH 58261 on in vivo glutamate outflow from the rat striatum, in the model of Parkinson's disease induced by 6-hydroxydopamine lesion of the dopaminergic nigrostriatal pathway.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (Charles River Farm, Italy) weighing 275–300 g were used. They 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. Drugs

SCH 58261 was synthesized by Department of Chemistry, Schering Plough Research Institute, Kenilworth, NJ. 6-Hydroxydopamine–HCl, desipramine and apomorphine were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.3. 6-Hydroxydopamine lesion

Rats were anesthetized with chloral hydrate (400 mg/kg i.p.) and 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. dissolved in saline) in order to prevent 6-hydroxydopamine-induced neurotoxicity to noradrenergic neurons (Waddington, 1980).

2.4. Evaluation of turning behavior

Ten days after the lesion, rats were screened for contralateral rotations in response to apomorphine (0.2 mg/kg s.c. dissolved in saline). Rats not showing at least 100 contralateral rotations during a 1-h testing period were eliminated from the study. To record turning behavior, rats were placed in plexiglass hemispheric bowls (50 cm of diameter) 30 min before the administration of apomorphine, and the number of contralateral rotations was counted by automated rotameters.

2.5. Microdialysis studies

Rats were anesthetized with chloral hydrate (400 mg/kg i.p.) and placed in the David Kopf stereotaxic frame. Two vertical microdialysis probes were implanted bilaterally in the rat dorsolateral striatum: in the left "lesioned" striatum and in the right "intact" striatum, respectively. 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, 1982). The external portion of the probe was fixed to the skull with dental cement. After surgery, rats were individually housed in hemispheric bowls, which also served as the experimental environment.

Microdialysis experiments were performed 24 h or 15 days after unilateral infusion of 6-hydroxydopamine in the medial forebrain bundle. In experiments performed 24 h after 6-hydroxydopamine infusion, rats were infused with 6-hydroxydopamine and implanted with microdialysis probes in both striata in the same surgery session. In experiments performed 15 days after 6-hydroxydopamine infusion, 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 in the microdialysis experiment on day 15.

Perfusion was started 24 h after implantation of the microdialysis probe, in freely moving rats. 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 (NaCl 147 mM, $CaCl_2$ 2.2 mM, KCl 4.0 mM, pH 7.0) at a constant flow rate of 3 μl/min. After a 1.5-h settling period, 20-min samples were collected. The samples were frozen at – 80 °C until assay. SCH 58261 dissolved in dimethyl sulphoxide (DMSO) (0.025%) was administered through the microdialysis probe after the fifth sample.

2.6. Histological control

At the end of the experiment, the rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and killed by decapitation. The brain was 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 probe. Samples obtained from rats in which the probe was not correctly positioned were not assayed.

2.7. Assay of glutamate in the perfusate

Glutamate analysis was carried out with a high-performance liquid chromatography (HPLC) method with fluorimetric detection as previously described (Bianchi et al., 1999). Briefly, the amino acids were derivatized with mercaptoethanol and *o*-phthalaldehyde. The *o*-phthalaldehyde derivatives were then separated on a 5-μm reverse-phase Nucleosil C18 column (250 × 4 mm; Macherey-Nagel, Duren, Germany) kept at room temperature, using a mobile phase consisting of methanol and potassium acetate (0.1 M, pH adjusted to 5.52 with glacial acetic acid) at a flow rate of 1 ml/min in a three-linear step gradient (from 25% to 90% methanol). The levels of glutamate in the perfusate samples were measured as pmol of amino acid/μl of perfusate (μM) and expressed as absolute levels. The mean glutamate outflow was calculated using the average amino acid outflow of nine samples from both right "intact" and left "lesioned" striata. In the experiments where the effect of SCH 58261 was studied, glutamate outflow is expressed as percent variation of the mean of the first five determinations. The mean glutamate outflow was calculated by taking the average glutamate outflow of each rat before ($n = 5$ samples) and during the administration of the drug ($n = 6$ samples).

2.8. "In vitro" recovery experiments

In order to evaluate the recovery through the dialysis membrane, in vitro experiments were performed at room temperature. Dialysis probes were immersed, at room temperature, in Ringer's solution containing known concentrations of glutamate. The probes were perfused with Ringer's solution at 3 μl/min and samples were collected every 20 min. Glutamate recovery was 10 ± 2% ($n = 3$). Glutamate values reported in this paper are not corrected for recovery.

2.9. Statistical analysis

Statistically significant differences between extracellular glutamate levels were evaluated by Student's *t*-test and the two-way analysis of variance (ANOVA) followed by post hoc Fisher's least-significant difference (LSD) Multiple-Comparison test.

3. Results

Striatal extracellular glutamate levels, measured by in vivo microdialysis, were not significantly modified either 24 h or 15 days after 6-hydroxydopamine infusion, compared with those on the control non-infused side.

Fig. 1A shows the time course of glutamate outflow, 24 h after 6-hydroxydopamine infusion, from the intact striatum and from the lesioned (6-hydroxydopamine-infused) striatum ($n = 7$ rats), expressed as absolute values (μM). Two-way ANOVA, calculated for two factors, time course and treatment with 6-hydroxydopamine, showed that glutamate outflow was not significantly modified by 6-hydroxydopamine infusion. The mean glutamate outflow was 0.77 ± 0.20 μM in the intact striatum and 0.85 ± 0.11 μM in the lesioned striatum. Fig. 1B shows the time course of glutamate outflow, 15 days after 6-hydroxydopamine infusion, from the intact striatum and from the lesioned striatum ($n = 14$ rats), expressed as absolute values (μM). Two-way ANOVA, calculated for the two factors, time course and treatment with 6-hydroxydopamine, showed that glutamate outflow was not significantly modified by the 6-hydroxydopamine lesion. The mean glutamate outflow was 0.92 ± 0.14 μM in the lesioned striatum and 0.92 ± 0.21 μM in the intact striatum.

The effect of the selective adenosine A_{2A} receptor antagonist SCH 58261 (50 nM) on glutamate outflow was evaluated 15 days after 6-hydroxydopamine infusion. Previous studies have shown that at this time, extensive striatal dopamine depletion of at least 90% is reached in the striatum

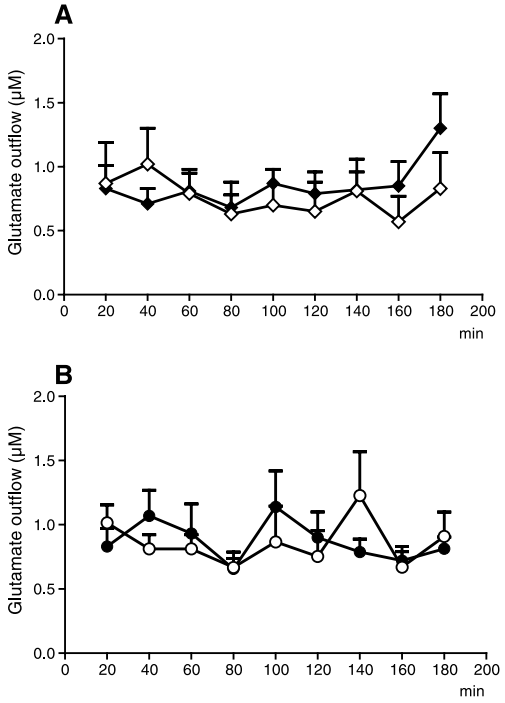

Fig. 1. (A) Time course of striatal glutamate outflow 24 h after 6-hydroxydopamine lesion. Each point is the mean ± S.E.M. for $n = 7$ rats. (◇) Intact striatum; (◆) striatum corresponding to the 6-hydroxydopamine-infused side. Full statistical analysis by two-way ANOVA followed by post hoc Fisher's LSD test is described in the text. (B) Time course of striatal glutamate outflow 15 days after 6-hydroxydopamine lesion. Each point is the mean ± S.E.M. for $n = 14$ rats. (○) Intact striatum; (●) striatum corresponding to the 6-hydroxydopamine-infused side. Full statistical analysis by two-way ANOVA is described in the text.

corresponding to the side of 6-hydroxydopamine infusion (Pinna et al., 2002). Fig. 2A shows the effect of SCH 58261 on glutamate outflow from both the lesioned striatum and the intact striatum ($n = 15$ rats). The two-way ANOVA calculated for the two factors, time course and treatment with 6-hydroxydopamine, showed that both factors were statistically significant ($F(10,316) = 2.86$, $P < 0.002$; $F(1,316) = 27.33$, $P < 0.0000001$, respectively). Moreover, the statistical analysis showed that there was a significant interaction between time course and treatment with 6-hydroxydopamine ($F(10,316) = 3.53$, $P < 0.0002$), which underlines the different response to SCH 58261 in relation to 6-hydroxydopamine treatment. The mean glutamate outflow, represented as histograms, before and during the SCH 58261 treatment in intact striatum and in lesioned striatum, is reported in Fig. 2B. SCH 58261 induced a statistically significant decrease (− 13%) of glutamate outflow in the intact striatum and a statistically significant increase (+143%) of glutamate outflow in the lesioned striatum.

We also evaluated if glutamate outflow was modified in the intact striatum of lesioned rats by the 6-hydroxydopamine lesion. The mean glutamate outflow in the intact striatum of the 6-hydroxydopamine-infused rats, expressed as absolute level and calculated as the average of the outflow at 24 h and 15 days after 6-hydroxydopamine infusion, was 0.86 ± 0.15 μM ($n = 21$ rats). This value was not significantly different from that in the striatum of control rats (0.75 ± 0.11 μM; $n = 17$ rats), thus indicating that the 6-hydroxydopamine lesion did not modify glutamate outflow in the intact striatum of unilaterally 6-hydroxydopamine-infused rats (data not shown).

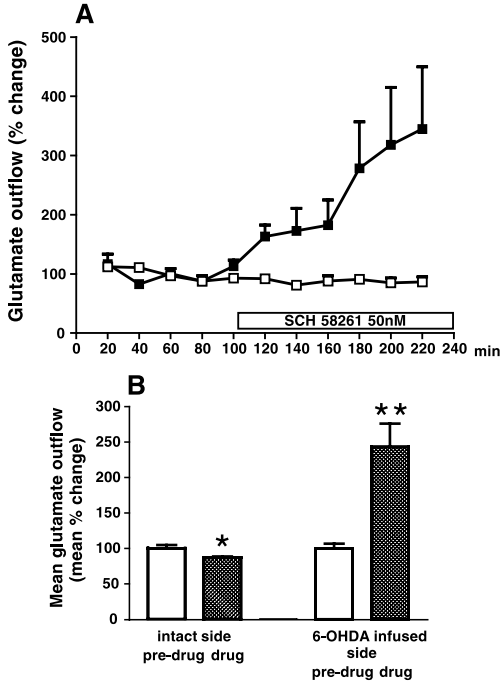

Fig. 2. (A) Effect of SCH 58261 (50 nM) on the time course of glutamate outflow in the intact striatum (□) and in the striatum corresponding to the 6-hydroxydopamine-infused side (■). Each point is the mean ± S.E.M. for $n = 15$ rats. Values are expressed as percent variations in the mean of the first five determinations. Full statistical analysis by two-way ANOVA is described in the text. (B) The histograms show the effect of 50 nM SCH 58261 on the mean percent glutamate outflow, calculated as described in the Materials and methods, in the intact striatum and in the striatum corresponding to the 6-hydroxydopamine-infused side. White bar: pre-drug samples; hatching bar: SCH 58261 treated samples. Student's *t*-test: $^{*}P < 0.024$; $^{**}P < 0.004$ versus pre-drug values.

4. Discussion

Adenosine A_{2A} receptor antagonists are known to reverse motor deficits in a variety of Parkinson's disease models. In order to evaluate the role of glutamate in these effects, we evaluated whether striatal glutamate outflow in the 6-hydroxydopamine model of Parkinson's disease was modified by local infusion of the adenosine A_{2A} receptors antagonist SCH 58261.

Our data show that in unilaterally 6-hydroxydopamine-infused rats, extracellular glutamate levels were not modified in the denervated striatum in comparison to those in the intact striatum at both 24 h and 15 days after lesion. Previous studies of Abarca and Bustos (1999) did not find any change in glutamate outflow 7 days after 6-hydroxydopamine infusion. In contrast, Meshul et al. (1999) found a glutamate increase and decrease 1 and 3 months after 6-hydroxydopamine injection, respectively, whereas Jonkers et al. (2002) reported increased glutamate outflow 18 to 20 days after 6-hydroxydopamine injection. Taken together, these results suggest that glutamate outflow is differentially regulated, depending on the time elapsed from the 6-hydroxydopamine lesion.

Antagonism of the adenosine A_{2A} receptor by the selective antagonist SCH 58261, 15 days after unilateral 6-hydroxydopamine infusion in the medial forebrain bundle, increased striatal glutamate outflow in the striatum corresponding to the 6-hydroxydopamine-infused side, whereas glutamate outflow was decreased by SCH 58261 in the intact striatum. The results obtained for the intact striatum are in line with the previous observations of our group, showing that adenosine A_{2A} receptor antagonism decreases glutamate outflow in young normal rats (Corsi et al., 2000). The decrease in glutamate outflow could be attributed to antagonism of adenosine A_{2A} receptors located on corticostriatal glutamatergic terminals, which stimulate the release of excitatory amino acids (Dixon et al., 1996).

The finding that SCH 58261 stimulated glutamate outflow from the striatum corresponding to the 6-hydroxydopamine-infused side is similar to the finding that the selective adenosine A_{2A} receptor antagonist SCH 58261 increases glutamate outflow in aged rats (Corsi et al., 2000). This latter effect was attributed to antagonism of adenosine A_{2A} receptors located on the striatopallidal GABA-enkephalin neurons. The effect of antagonism of the adenosine A_{2A} receptors located on striatopallidal GABA-enkephalin neurons may be

pronounced in comparison to the effect of antagonism of adenosine A_{2A} receptors located on glutamatergic terminals when the balance of the interaction between adenosine A_{2A} receptors and dopamine D2 receptors changes in favor of adenosine activity. This imbalance has been described both in aged rats (Popoli et al., 1998) and in 6-hydroxydopamine-lesioned rats (Pinna et al., 2002). Adenosine acting on adenosine A_{2A} receptors, besides decreasing dopamine D2 receptor affinity for dopamine (Ferre et al., 1991), may directly activate the indirect striatopallidal output pathway by activating second messenger systems (Olah and Stiles, 2000; Fenu et al., 1997; Svenningsson et al., 2000). Therefore, since dopamine levels are negligible in 6-hydroxydopamine-denervated rats (Pinna et al., 2002), our data support the suggestion of Chen et al. (2001) that adenosine acting on adenosine A_{2A} receptors, besides regulating dopamine D2 receptor-mediated transmission, may exert independent effects opposite to those elicited by dopamine D2 receptor activation. According to basal ganglia circuitry, blockade of adenosine A_{2A} receptors located in the striatopallidal pathway would result in stimulation of thalamic glutamatergic projections to the cortex and therefore to the striatum.

We hypothesized that in those situations in which the balance of interaction between adenosine A_{2A} and dopamine D2 receptors favors adenosine A_{2A} receptors, the indirect striatal output pathway might be activated and the pronounced effect of adenosine A_{2A} receptor antagonism would produce a positive regulation of striatal glutamate outflow which may contribute to the regulation of motor disability related to aging or neurodegenerative diseases such as Parkinson's disease. Recent data showed that in the 6-hydroxydopamine denervation model of Parkinson's disease, L-DOPA (Jonkers et al., 2002) and the *N*-methyl-D-aspartate (NMDA) receptor antagonist MK-801 ((+)-5-methyl-10,11-dihydroxy-5*H*-dibenzo (a,d) cyclohepten-5,10-imine) (Abarca and Bustos, 1999) increase striatal glutamate outflow. An increase in the neurotransmitter glutamate could be viewed as an effort to maintain adequate neuronal transmission in the basal ganglia in response to extensive tissue dopamine depletion.

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References

Abarca, J., Bustos, G., 1999. Differential regulation of glutamate, aspartate and gamma-amino-butyrate release by *N*-methyl-D-aspartate receptors in rat striatum after partial and extensive lesions to the nigro-striatal dopamine pathway. Neurochem. Int. 35, 19–33.

Ascherio, A., Zhang, S.M., Hernan, M.A., Kawachi, I., Colditz, G.A., Speizer, F.E., Willett, W.C., 2001. Prospective study of caffeine consumption and risk of Parkinson's disease in men and women. Ann. Neurol. 50, 56–63.

Bianchi, L., Della, C.L., Tipton, K.F., 1999. Simultaneous determination of basal and evoked output levels of aspartate, glutamate, taurine and 4-aminobutyric acid during microdialysis and from superfused brain slices. J. Chromatogr., B, Biomed. Sci. Appl. 723, 47–59.

Chen, J.F., Moratalla, R., Impagnatiello, F., Grandy, D.K., Cuellar, B., Rubinstein, M., Beilstein, M.A., Hackett, E., Fink, J.S., Low, M.J., Ongini, E., Schwarzschild, M.A., 2001. The role of the D2 dopamine receptor (D2R) in A_{2A} adenosine receptor (A_{2A}R)-mediated behavioral and cellular responses as revealed by A_{2A} and D2 receptor knockout mice. Proc. Natl. Acad. Sci. U. S. A. 98, 1970–1975.

Corsi, C., Melani, A., Bianchi, L., Pepeu, G., Pedata, F., 1999. Striatal A_{2A} adenosine receptors differentially regulate spontaneous and K^+-evoked glutamate release in vivo in young and aged rats. NeuroReport 10, 687–691.

Corsi, C., Melani, A., Bianchi, L., Pedata, F., 2000. Striatal A_{2A} adenosine receptor antagonism differentially modifies striatal glutamate outflow in vivo in young and aged rats. NeuroReport 11, 2591–2595.

Dixon, A.K., Gubitz, A.K., Sirinathsinghji, D.J., Richardson, P.J., Freeman, T.C., 1996. Tissue distribution of adenosine receptor mRNAs in the rat. Br. J. Pharmacol. 118, 1461–1468.

Fenu, S., Pinna, A., Ongini, E., Morelli, M., 1997. Adenosine A_{2A} receptor antagonism potentiates L-DOPA-induced turning behaviour and c-*fos* expression in 6-hydroxydopamine-lesioned rats. Eur. J. Pharmacol. 321, 143–147.

Ferre, S., von Euler, G., Johansson, B., Fredholm, B.B., Fuxe, K., 1991. Stimulation of high-affinity adenosine A_2 receptors decreases the affinity of dopamine D2 receptors in rat striatal membranes. Proc. Natl. Acad. Sci. U. S. A. 88, 7238–7241.

Fink, J.S., Weaver, D.R., Rivkees, S.A., Peterfreund, R.A., Pollack, A.E., Adler, E.M., Reppert, S.M., 1992. Molecular cloning of the rat A_2 adenosine receptor: selective co-expression with D2 dopamine receptors in rat striatum. Brain Res. Mol. Brain Res. 14, 186–195.

Gevaerd, M.S., Takahashi, R.N., Silveira, R., Da Cunha, C., 2001. Caffeine reverses the memory disruption induced by intra-nigral MPTP-injection in rats. Brain Res. Bull. 55, 101–106.

Hauber, W., 1998. Involvement of basal ganglia transmitter systems in movement initiation. Prog. Neurobiol. 56, 507–540.

Jarvis, M.F., Williams, M., 1989. Direct autoradiographic localization of adenosine A_2 receptors in the rat brain using the A_2-selective agonist, [3H]CGS 21680. Eur. J. Pharmacol. 168, 243–246.

Jonkers, N., Sarre, S., Ebinger, G., Michotte, Y., 2002. MK801 suppresses the L-DOPA-induced increase of glutamate in striatum of hemi-Parkinson rats. Brain Res. 926, 149–155.

Kanda, T., Jackson, M.J., Smith, L.A., Pearce, R.K., Nakamura, J., Kase, H., Kuwana, Y., Jenner, P., 1998. Adenosine A_{2A} antagonist: a novel antiparkinsonian agent that does not provoke dyskinesia in parkinsonian monkeys. Ann. Neurol. 43, 507–513.

Marsden, C.D., 1982. Basal ganglia disease. Lancet 2, 1141–1147.

Meshul, C.K., Emre, N., Nakamura, C.M., Allen, C., Donohue, M.K., Buckman, J.F., 1999. Time-dependent changes in striatal glutamate synapses following a 6-hydroxydopamine lesion. Neuroscience 88, 1–16.

Olah, M.E., Stiles, G.L., 2000. The role of receptor structure in determining adenosine receptor activity. Pharmacol. Ther. 85, 55–75.

Paxinos, G., Watson, G., 1982. The Rat Brain in Stereo-Taxic Coordinates. Academic Press, New York.

Pellegrino, L.J., Pellegrino, A.S., Cushman, A.J., 1979. A Stereotaxic Atlas of the Rat Brain. Plenum, New York.

Pinna, A., Corsi, C., Carta, A.R., Valentini, V., Pedata, F., Morelli, M., 2002. Modifications in adenosine extracellular levels and adenosine A_{2A} receptor mRNA by dopamine denervation. Eur. J. Pharmacol. 446, 75–82.

Popoli, P., Betto, P., Rimondini, R., Reggio, R., Pezzola, A., Ricciarello, G., Fuxe, K., Ferre, S., 1998. Age-related alteration of the adenosine/dopamine balance in the rat striatum. Brain Res. 795, 297–300.

Schiffmann, S.N., Jacobs, O., Vanderhaeghen, J.J., 1991. Striatal restricted adenosine A_2 receptor (RDC8) is expressed by enkephalin but not by substance P neurons: an in situ hybridization histochemistry study. J. Neurochem. 57, 1062–1067.

Svenningsson, P., Lindskog, M., Ledent, C., Parmentier, M., Greengard, P., Fredholm, B.B., Fisone, G., 2000. Regulation of the phosphorylation of the dopamine- and cAMP-regulated phosphoprotein of 32 kDa in vivo by dopamine D1, dopamine D2, and adenosine A_{2A} receptors. Proc. Natl. Acad. Sci. U. S. A. 97, 1856–1860.

Waddington, J.L., 1980. Effects of nomifensine and desipramine on the sequelae of intracerebrally-injected 6-OHDA and 5,6-DHT. Pharmacol. Biochem. Behav. 13, 915–917.