A Common Signaling Pathway for Striatal NMDA and Adenosine A_{2a} Receptors: Implications for the Treatment of Parkinson's Disease

Joanne E. Nash[1] and Jonathan M. Brotchie[1,2]

[1]Manchester Movement Disorder Laboratory, Division of Neuroscience, School of Biological Sciences, University of Manchester, M13 9PT, United Kingdom, and [2]Motac Neuroscience Ltd., Incubator Building, Manchester, M13 9XX, United Kingdom

The striatum is the major input region of the basal ganglia, playing a pivotal role in the selection, initiation, and coordination of movement both physiologically and in pathophysiological situations such as Parkinson's disease. In the present study, we characterize interactions between NMDA receptors, adenosine receptors, and cAMP signaling within the striatum. Both NMDA (100 μM) and the adenosine A_{2a} receptor agonist CPCA (3 μM) increased cAMP levels (218.9 $\pm$ 19.9% and 395.7 $\pm$ 67.2%, respectively; cf. basal). The NMDA-induced increase in cAMP was completely blocked when slices were preincubated with either the NMDA receptor antagonist 7-chlorokynurenate or the adenosine A_2 receptor antagonist DMPX (100 μM), suggesting that striatal NMDA receptors increase cAMP indirectly via stimulation of adenosine A_{2a} receptors. Thus, NMDA receptors and adenosine A_{2a} receptors might share a common signaling pathway within the striatum. In striatal slices prepared from the 6-hydroxydopamine-lesioned rat model of Parkinson's disease, NMDA receptor-mediated increases in cAMP were greater on the lesioned side compared with the unlesioned side (349.6 $\pm$ 40.2% compared with 200.9 $\pm$ 21.9% of basal levels, respectively). This finding substantiates previous evidence implicating overactivity of striatal NMDA receptors in parkinsonism and suggests that a common NMDA receptor–adenosine A_{2a} receptor–cAMP signaling cascade might be an important mechanism responsible for mediating parkinsonian symptoms.

Key words: striatum; NMDA receptors; adenosine A_{2a} receptors; cAMP; 6-OHDA; Parkinson's disease

For >30 years, dopamine replacement has formed the basis of the majority of symptomatic treatments for Parkinson's disease (Cotzias et al., 1969; Rascol et al., 1979). Because long-term use of dopamine-replacing agents is associated with severely disabling side effects, most notably dyskinesia (Quinn et al., 1987; Papa et al., 1994) or lack of maintained efficacy (Rascol, 2000), there has been increasing interest in potential non-dopaminergic treatments as monotherapies (Klockgether et al., 1994; Brotchie, 1997) or as adjuncts with L-DOPA (Wullner et al., 1992; Brotchie, 1997).

Previously, we and others have shown that overactivity of the indirect striatal output pathway connecting the striatum with the lateral segment of the globus pallidus is a key component of the neural mechanisms responsible for generating parkinsonian symptoms (Crossman et al., 1985; Pan et al., 1985; Mitchell et al., 1986; Miller and DeLong, 1987; Crossman, 1989; Mitchell and Crossman, 1989; Griffiths et al., 1990; Robertson et al., 1990; Robertson et al., 1991; Maneuf et al., 1994). The exact mechanisms driving overactivity of the indirect pathway are unclear. However, abnormal NMDA receptor transmission may be involved, because striatal NMDA receptor binding is enhanced in animal models of Parkinson's disease (Weihmuller et al., 1992; Ulas et al., 1994), and intrastriatal injection of NMDA induces parkinsonian symptoms (Klockgether and Turski, 1993). In addition, intrastriatal injection of certain NMDA receptor antagonists can reverse parkinsonism, whereas systemic administration of the NMDA receptor antagonists CP101–606, Ro 25-6981, MDL 100,453, and ifenprodil have anti-parkinsonian actions in the MPTP-lesioned primate model of Parkinson's disease (Loschmann, 1997; Nash et al., 1997; Blanchet et al., 1999; Steece-Collier et al., 2000).

Adenosine A_{2a} receptor antagonists also have anti-parkinsonian actions when administered systemically to MPTP-lesioned primates (Kanda et al., 1998). Because within the striatum adenosine A_{2a} expression is confined to the indirect striatal output pathway, it is probable that their anti-parkinsonian actions are mediated by reducing activity of the indirect striatal output pathway (Barraco et al., 1993; Sebastiao and Ribeiro, 1996). Thus, increased activation of adenosine A_{2a} receptors may underlie, in part at least, the generation of parkinsonian symptoms. Such a possibility is suggested by the finding that intrastriatal injection of adenosine A_{2a} receptor agonists induces parkinsonism (Hauber and Munkle, 1995).

Similarities between the anti-parkinsonian actions of adenosine A_{2a} and NMDA receptor antagonists are apparent. Although both classes of compounds alleviate parkinsonism to a level comparable with those observed with dopamine replacement (Nash et al., 1997, 2000; Kanda et al., 1998), both have properties that distinguish them from "traditional" dopamine replacement therapies. For example, when administered at supraoptimal doses, neither adenosine A_{2a} receptor antagonists nor NMDA receptor antagonists elicit hyperlocomotion (Nash et al., 1997, 2000; Kanda et al., 1998), whereas dopamine-replacing agents elicit hyperactivity at supraoptimal doses in MPTP-lesioned marmosets (Pearce et al., 1995). It has also been suggested that both adenosine A_{2a} receptor antagonists and NMDA receptor antagonists can alleviate parkinsonism without eliciting dyskinesia in animals that show dyskinesia when treated with dopamine replacement therapy (Bedard et al., 1998; Blanchet et al., 1999). The studies presented here investigate a signaling mechanism common to striatal NMDA and adenosine A_{2a} receptors in normal rat striatum and in striata from the 6-hydroxydopamine (OHDA)-lesioned rat model of Parkinson's disease.

MATERIALS AND METHODS

6-OHDA lesions and sham operations. Male Sprague Dawley rats (260–280 gm, Charles River) were treated with pargyline (5 mg/kg, Sigma, St. Louis,

Received June 8, 2000; revised July 18, 2000; accepted Aug. 2, 2000.

We thank the Medical Research Council (UK) for supporting the studies reported and Paula Ravenscroft for technical assistance with HPLC.

Correspondence should be addressed to Dr. Jonathan M. Brotchie, Manchester Movement Disorder Laboratory, Room 1.124, Division of Neuroscience, School of Biological Sciences, University of Manchester, M13 9PT, UK. E-mail: j.brotchie@man.ac.uk.

Dr. Nash's current address: Department of Neurobiology, University of Alabama at Birmingham, 1719 6th Avenue South, Birmingham, AL 35294-0021.

 0270-6474/00/207782-08$15.00/0

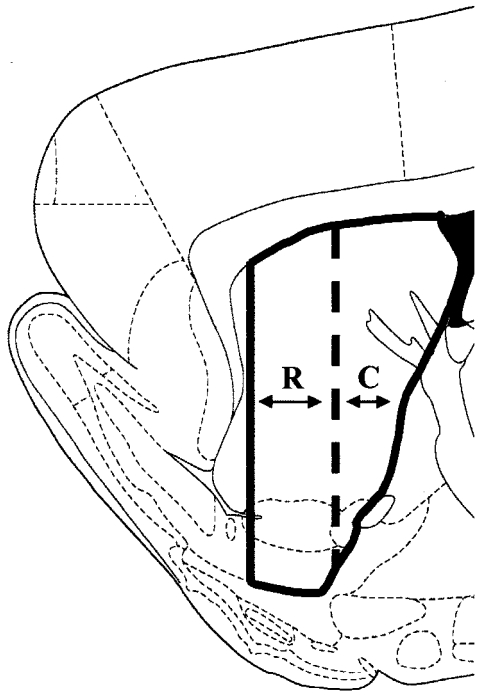

Figure 1. Schematic representation of region of striatum blocked to obtain slices for cAMP measurement. The area contained within the *solid black lines* indicates the area of striatum used for all experiments. The *dotted line* indicates the limits of rostral and caudal striatum as defined in experiments in which slices from the rostral and caudal striatum were separated.

MO) and desipramine (25 mg/kg, Sigma) 30 min before being anesthetized with sodium pentobarbitone (Sagatal, 60 mg/kg, i.p., Rhone Merieux). 6-OHDA-HCl (2.5 μl, 5 mg/ml in 0.1% ascorbic acid) (Sigma) or vehicle was infused into the right medial forebrain bundle, using routine stereotaxic procedures [coordinates: +2 mm right; −2.8 mm anterior/posterior; −9 mm dorsal/ventral to skull from Bregma, according to Paxinos and Watson (1998)]. Infusions were made manually with a 26 ga Hamilton syringe over 5 min; the needle was left in place for an additional minute before being removed. After recovery, the animals were housed in groups of six under temperature-controlled conditions (19–21°C), with 12 hr alternating light/dark cycles (lights on 8 A.M. - 8 P.M.). Food and water were available *ad libitum*. Experiments were performed a minimum of 21 d after surgery.

Slice preparation. Male Sprague Dawley rats (naïve, 200–250 gm; sham-operated/6-OHDA-lesioned, 300–500 gm, Charles River) were killed by cervical dislocation. After removal of the brain, a block of tissue (width rostrocaudally, ~7.5 mm) containing the striatum (Fig. 1) was obtained by coronal razor blade cuts. This block was fixed with cyanoacrylate glue to the stage of a Vibroslice (Campden Instruments), and coronal slices of striatum were cut at 400 μM. The cortex was removed, and striata from each hemisphere were separated. During these procedures, the tissue was maintained at 4°C in Krebs-Heinseleit solution. For each experiment, slices from six animals were distributed into 50 ml conical flasks containing 20 ml Krebs-Heinseleit solution, so that each flask contained five to eight slices from six different animals. The flasks were warmed, gassed, and maintained at 37°C in a shaking water bath for 90 min to allow the slices to recover from cutting before experimental manipulations. In experiments, which used slices prepared from 6-OHDA-lesioned or sham-operated animals to allow comparison between slices prepared from the striatum contralateral and ipsilateral to the operation, slices from the two hemispheres were distributed into separate flasks. Each experiment was repeated six times.

5′-(*N*-cyclopropyl)carboxamidoadenosine (CPCA) (0.1–100 μM), Sigma) and 3,7-dimethyl-1-propargylxanthine (DMPX) (100 μM, RBI, Natick, MA) were dissolved in Krebs-Heinseleit solution containing (in mM): NaCl 118, KCl 4.8, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11, and $CaCl_2$ 2, gassed with 5% CO_2 and 95% O_2, pH 7.4, to the desired concentration. The phospodiesterase inhibitor, Ro 20-1724 (100 μM, RBI) was dissolved in 100% ethanol to a concentration of 25 mg/ml and diluted in Krebs'-Heinseleit solution to the required concentration. NMDA (10–300 μM, Sigma) and 7-chlorokynurenate (1.3–100 μM, Sigma) were dissolved in NaOH and diluted as required in Krebs'-Heinseleit solution to a final concentration of 0.1% NaOH.

Ro 20-1734 (100 μM) was added along with antagonists or appropriate vehicle, by addition of small (>50 μl) volumes of solutions to the appropriate flask. Twenty minutes after addition of Ro 20-1734, slices were removed from the flasks and plunged into boiling sodium azide acetate buffer (10 mM) (Amersham, Arlington Heights, IL) containing 4 mM EDTA for 10 min and homogenized by sonication with a probe sonicator (Bandelin Sonoplus model HD 70). CPCA and NMDA were added 15 and 5 min, respectively, before striatal slices were removed from the flask and placed in acetate buffer.

Protein and cAMP determination. A 5 μl aliquot of the homogenate was removed and diluted with 50 μl of distilled water for the protein assay. Protein content was determined by the Bradford method (Bio-Rad kit) (Bradford, 1976) and Lambda Bio spectrometer (Perkin-Elmer, Norwalk, CT). Bovine serum albumin (10–200 μg/ml) was used to generate a standard curve. The remaining homogenate from each slice was centrifuged at 10,000 × g_{av} for 10 min in a 1.5 ml microcentrifuge tube (Eppendorf), and the supernatant was assayed for cAMP by scintillation proximity assay (kit reference: RPA 538, Amersham) following the manufacturer's nonacetylation method. A standard curve for cAMP concentrations ranged from 0.1 to 12.8 pmol per tube.

Lesion assessment. Subsequent to preparation of striatal slices, a single striatal slice was taken from each hemisphere of sham-operated and 6-OHDA-lesioned animals and stored at −20°C. Dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) levels in slices from each hemisphere were quantified using HPLC with electrochemical detection [method modified from Marsden and Joseph (1989)]. Each striatal slice was homogenized in 200 μl 0.1 M perchloric acid containing 2 mM glutathione, then centrifuged at 27,000 × g_{av} for 20 min at 4°C (centrifuge: Howe 3K30, Sigma). A 100 μl sample of the supernatant was filtered through 0.2 μM Acrodiscs (Whatman, Beckman) and injected directly (autosampler: 507e, System Gold, Beckman) onto a Hypersil 5 μM ODS-25F RP-HPLC column [flow rate of 1 ml/min (mobile phase: 12% methanol, 0.15 M NaH_2PO_4, 0.1 mM EDTA, 0.5 mM octanesulfonic acid in double-distilled deionized water); all reagents were HPLC grade (Sigma); pump: Programable Solvent Module 126, Beckman]. Current at a potential of +0.55 V was applied to carbon-based electrodes arranged in parallel (BAS Dual Amperometric LC-4B detection system). Peaks for dopamine and DOPAC were identified by retention time using System Gold Nouveau software (Beckman). Dopamine and DOPAC levels were quantified from pre-run standard curves and expressed as microgram per milligram of wet tissue. After determination of dopamine and DOPAC levels for each experiment (each including six animals), where individual striatal slices showed <85% depletion in the side ipsilateral to the 6-OHDA-induced lesion, experiments were excluded from the study.

Data analysis. Data were expressed as percentage mean picomole cAMP per milligram of protein ± SEM of basal and were normalized to the respective basal values in each experiment. In all experiments, for each condition, the mean ± SEM shown in the Figures represents mean value of cAMP levels from six experiments for that condition (each experiment using six animals; cAMP levels measured in each of five to eight striatal slices). Statistical comparisons were made using ANOVA or Student's paired *t* test where appropriate. Where ANOVA yielded significance, *post hoc* analysis was performed using Dunnett's multiple comparison tests or Tukey Kramer's multiple comparisons test.

RESULTS

To determine the incubation period required for NMDA to induce a maximal stimulation of cAMP levels within the striatum, striatal slices were exposed to NMDA (100 μM) for 2–20 min. Incubation of slices with NMDA (100 μM) at different times had a significant effect on cAMP levels compared with basal [$F_{(5,30)} = 8.5$; $p < 0.01$, ANOVA ($n = 6$)]. The maximal effect of NMDA on cAMP levels was observed after incubation for 20 min (320.0 ± 50.0% of basal), although increases were significant compared with basal postincubation with slices for 5–20 min ($p < 0.01$ at 5, 10, 20 min; $p < 0.05$ at 18 min, p*ost hoc* Dunnett's multiple comparisons test) (Fig. 2*A*).

Incubation of striatal slices with NMDA caused a concentration-dependent increase in cAMP levels [$F_{(4,25)} = 8.9$, $p < 0.01$, ANOVA ($n = 6$)]. The maximal effect of NMDA was observed at 100 μM (326.2 ± 50%), although increases were significant, compared with basal, at concentrations of 30–300 μM ($p < 0.01$ at 30–100 μM, $p < 0.05$ at 300 μM, *post hoc* Dunnett's multiple comparisons test) (Fig. 2*B*).

Previous incubation of slices with 7-chlorokynurentate (1.3–100 μM; NMDA antagonist active at the glycine site) produced a concentration-dependent inhibition of NMDA-induced increases in cAMP [$F_{(6,21)} = 4.15$ $p < 0.001$, ANOVA ($n = 6$)] (Fig. 2*C*). There was a significant difference between striatal cAMP levels in slices incubated with 7-chlorokynurenate (3.7 μM) and NMDA compared with NMDA alone ($p < 0.05$, *post hoc* Tukey Kramer's multiple comparisons test).

Incubation of striatal slices with the A_{2a} receptor agonist CPCA caused a concentration-dependent increase in cAMP

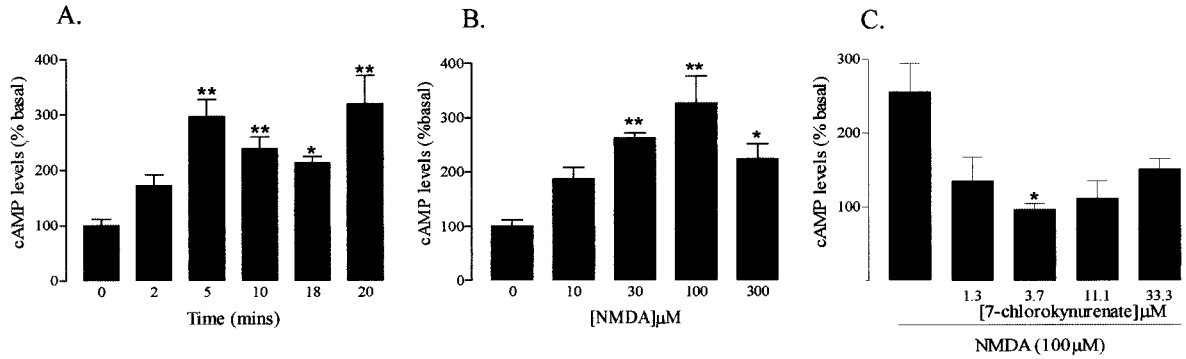

Figure 2. Effect of NMDA on striatal cAMP. *A*, cAMP levels were measured in striatal slices subsequent to exposure to NMDA (100 μM) at various time points between 2 and 20 min. *B*, cAMP levels were measured in striatal slices subsequent to exposure to NMDA (0–300 μM) (5 min). *C*, cAMP levels were measured in striatal slices subsequent to exposure to NMDA (100 μM) (5 min) that was preincubated with the glycine site NMDA receptor antagonist 7-chlorokynurenate (1.3–100 μM). Data are presented as mean percentage of basal ± SEM of six separate experiments, each with ~50–60 striatal slices taken from six individual animals. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; cf. basal, *post hoc* Dunnett's multiple comparisons test.

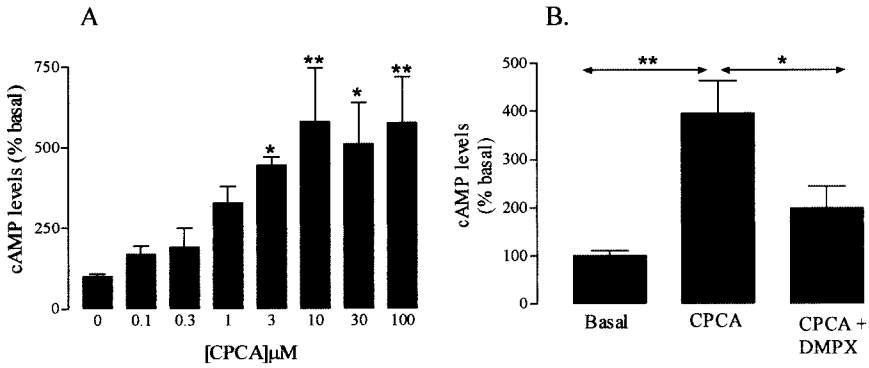

Figure 3. Effect of adenosine A_{2a} receptor stimulation on cAMP levels. *A*, cAMP levels were measured in striatal slices subsequent to exposure to the adenosine A_{2a} receptor agonist CPCA (0.1–100 μM) (15 min). *B*, cAMP levels were measured in striatal slices subsequent to exposure to the adenosine A_{2a} receptor agonist CPCA (3 μM) or CPCA after preincubation with the adenosine A2a receptor antagonist DMPX (100 μM). Data are presented as mean percentage of basal ± SEM of six separate experiments, each with 50–60 striatal slices taken from six individual animals. $^{*}p < 0.05$, $^{**}p < 0.01$ (*A*); *post hoc* Dunnett's multiple comparisons test (*B*); *post hoc* Tukey Kramer's multiple comparisons test.

levels [$F_{(8,45)} = 3.9$ $p < 0.01$, ANOVA followed by *post hoc* Dunnett's multiple comparisons test ($n = 6$)]. The maximal effect of CPCA was observed at 10 μM (579 ± 167%), although increases were significant, compared with basal, at concentrations of 3–100 μM ($p < 0.01$ at 10 and 100 μM, $p < 0.05$ at 3 and 30 μM) (Fig. 3*A*).

In a separate study, the adenosine A_{2a} receptor agonist CPCA (3 μM) significantly increased cAMP levels to 395.7 ± 67.2% of basal, which was comparable with the effect described above [$F_{(2,15} = 10.45$; $p < 0.01$, ANOVA ($n = 6$)]. This increase was inhibited when slices were preincubated with the adenosine A_2 receptor antagonist DMPX (100 μM) ($p < 0.05$ *post hoc* Tukey Kramer's multiple comparisons test) (Fig. 3*B*).

Previous incubation of slices with DMPX (100 μM) blocked the NMDA receptor-mediated increase in cAMP [$F_{(2,15)} = 20.46$; $p < 0.001$, ANOVA ($n = 6$)]. There was a significant difference between striatal cAMP levels in slices incubated with DMPX and NMDA, compared with NMDA alone ($p < 0.001$, *post hoc* Tukey Kramer's multiple comparisons test) (Fig. 4).

Incubation of striatal slices with a CPCA (100 μM) and NMDA (100 μM) induced a dramatic increase in cAMP levels (534 ± 73% of basal, $p < 0.001$), which was comparable with incubation of slices with CPCA (100 μM) alone (538 ± 43% of basal, $p < 0.001$) [$F_{(2,15)} = 137.6$, $p < 0.001$, ANOVA, *post hoc* Tukey multiple comparisons ($n = 6$)] (data not shown).

In striatal sections prepared from the unlesioned side of 6-OHDA-lesioned rats, mean dopamine and DOPAC levels were 31.6 ± 2.7 and 117 ± 17.4 ng/mg protein, respectively. Mean dopamine and DOPAC levels in striatal sections prepared from the lesioned side were 1.8 ± 0.4 and 16.7 ± 7.0 ng/mg protein, respectively. Thus, in striatal sections prepared from the lesioned striatum of 6-OHDA-lesioned animals, there was a >98% depletion of dopamine compared with the striatum on the unlesioned side. Comparisons of the mean DOPAC/dopamine ratio from lesioned

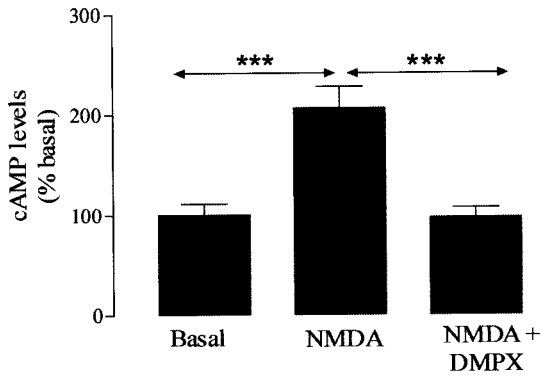

Figure 4. Effect of blockade of adenosine A_{2a} receptors on NMDA receptor-mediated increases in cAMP levels. cAMP levels were measured in striatal slices subsequent to exposure to NMDA (100 μM) or NMDA (100 μM) after preincubation with DMPX (100 μM). NMDA caused a 206.5 ± 21.89% increase in cAMP levels, which was completely blocked by DMPX. Data are presented as mean percentage of basal ± SEM of six separate experiments, each with 50–60 striatal slices taken from six individual animals. $^{***}p < 0.001$ *post hoc* Tukey Kramer's multiple comparisons test.

versus unlesioned striatum of individual animals showed that there was a significant increase in the DOPAC/dopamine ratio in the striatal sections prepared from the lesioned side (17.5 ± 3.1), compared with the unlesioned side 3.7 ± 0.8 ($p < 0.001$, Student's paired *t* test).

In this study, striatal slices prepared from 6-OHDA-lesioned and sham-operated rats were incubated with either NMDA or NMDA and DMPX. Three-way ANOVA using operation, side of injection, and drug treatment as factors showed significant effects of operation

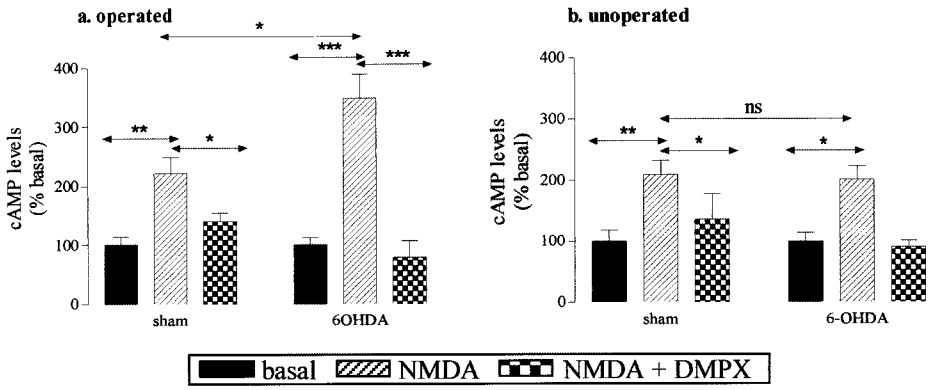

Figure 5. Effect of NMDA receptor stimulation on striatal cAMP levels in 6-OHDA rats: blockade by DMPX. Operated (*a*) and unoperated (*b*) side of 6-OHDA-lesioned and sham-operated animals. cAMP levels were measured in striatal slices subsequent to incubation with NMDA (100 μM) or NMDA and adenosine A_{2a} receptor antagonist DMPX (100 μM) and compared with basal. In slices prepared from the striatum on the operated side of sham-operated and 6-OHDA-lesioned rats, NMDA caused a 221.0 ± 28.0 and 349.6 ± 40.0% increase in cAMP levels, respectively. In striatal slices prepared from the unoperated side of sham-operated and 6-OHDA-lesioned rats, NMDA caused a 209.0 ± 23.1 and 200.9 ± 21.9% increase in cAMP levels, respectively. There was a significant difference between the increase in cAMP levels induced by NMDA in the unlesioned side compared with the lesioned side in 6-OHDA rats ($p < 0.01$, Student's *t* test). There was no significant difference between sides in the increase in cAMP levels induced by NMDA in sham-operated animals. Data are presented as mean percentage of basal ± SEM of six separate experiments, each with 50–60 striatal slices taken from six individual animals. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; *ns* = no significance *post hoc* Tukey Kramer's multiple comparisons test.

and drug, side of injection, but no significant interaction between the three groups [F_1(operation) = 3.52, $p < 0.0001$; F_1(side) = 1.49, $p < 0.05$; F_2(drug-treatment) = 14.87, $p < 0.0001$, F_1(interaction × operation × side) = 0.024, $p > 0.05$; F_2(interaction operation × drug-treatment) = 0.94, $p > 0.05$; F_2(interaction side × drug-treatment) = 0.86, $p > 0.05$; F_2(interaction operation × side × drug-treatment) = 0.357, $p > 0.05$) ($n = 18$)]. Therefore, *post hoc* comparisons were made between operation and drug treatment and within a given state for drug treatment.

In striatal slices prepared from 6-OHDA-lesioned rats, basal cAMP levels in slices taken from the lesioned (dopamine-depleted) side were not significantly different from those taken from the operated side of sham-operated rats (6-OHDA-lesioned: 15.18 ± 2.54 pmol cAMP/mg protein; cf. sham-operated: 23.31 ± 4.14 pmol cAMP/mg protein, $p < 0.05$, Student's *t* test). In striatal slices prepared from the unlesioned side of 6-OHDA-lesioned rats, basal cAMP levels were not significantly different from those prepared from the unoperated side of sham-operated rats (unlesioned: 20.36 ± 3.52 pmol cAMP/mg protein; cf. sham-unoperated: 19.06 ± 2.05 pmol cAMP/mg protein).

There was a significant increase in striatal cAMP levels after incubation of striatal slices prepared from the operated side of sham-operated and 6-OHDA-lesioned rats with NMDA (100 μM), compared with basal (221.0 ± 28.0 and 349.6 ± 40.0%, respectively) ($p < 0.01$ and $p < 0.001$, respectively, Tukey multiple comparisons test). This NMDA-induced increase in cAMP levels was greater in striatal slices prepared from the lesioned side of 6-OHDA-lesioned rats compared with the operated side of sham-operated animals ($p < 0.05$). (Fig. 5*a*). The NMDA-induced increase in cAMP was completely blocked after preincubation with DMPX (100 μM) in striatal slices from the operated side of both the sham-operated and 6-OHDA-lesioned animals ($p < 0.05$ and $p < 0.001$, respectively).

After incubation of slices prepared from the striatum on the unoperated side of sham-operated and 6-OHDA-lesioned animals, there was a significant effect of NMDA (100 μM) on striatal cAMP levels compared with basal (209.0 ± 23.1 and 200.9 ± 21.9%, respectively) ($p < 0.05$, Tukey multiple comparisons test). However, there was no significant difference in the effect of NMDA on cAMP levels in striatal slices prepared from the unoperated side of 6-OHDA-lesioned animals, compared with the unoperated side of sham-operated animals (Fig. 5*b*).

CPCA caused a significant increase in cAMP levels in striatal slices prepared from both the lesioned and unlesioned side of 6-OHDA-lesioned animals. Two-way ANOVA using treatment and side of injection as factors showed a significant effect of drug but not side of injection or any interaction between the two [F(drug) = 36.4, $p < 0.0001$, F(side of injection) = 0.12, $p > 0.05$, F(interaction between the two) F(interaction drug × side of injection) = 0.12, $p > 0.05$]. Incubation of striatal slices from the dopamine-depleted side with CPCA (3 μM) caused a significant increase in cAMP levels compared with basal (815.0 ± 205.6%) ($p < 0.01$, *post hoc* Dunnett's multiple comparisons test). Incubation of slices prepared from the lesioned striatum with CPCA (3 μM) caused a similar rise in cAMP levels compared with basal (736.8 ± 89.3%) ($p < 0.01$, *post hoc* Dunnett's multiple comparisons test) (Fig. 6).

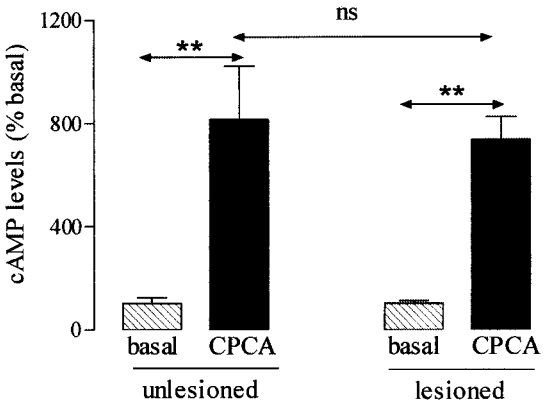

Figure 6. Effect of adenosine A_{2a} receptor stimulation on striatal cAMP levels in 6-OHDA rats. cAMP levels were measured in striatal slices prepared from the lesioned or unlesioned side of 6-OHDA rats subsequent to exposure to the adenosine A_{2a} receptor agonist CPCA (3 μM) or CPCA (3 μM) and the adenosine A_{2a} receptor antagonist DMPX (100 μM). In striatal slices prepared from the lesioned and unlesioned side of 6-OHDA-lesioned rats, CPCA caused a 815.0 ± 205.6 and 736.8 ± 89.3% increase in cAMP levels, respectively. Data are presented as mean percentage of basal ± SEM of six separate experiments, each with 50–60 striatal slices taken from six individual animals. $^{**}p < 0.01$; cf. basal, *post hoc* Tukey Kramer's multiple comparisons test; *ns*, not significantly different, *post hoc* Student's *t* test.

In striatal slices prepared from the 6-OHDA-lesioned rat, there was a significant effect of rostrocaudal level on the effect of NMDA (100 μM) on cAMP levels ($F_{(3,34)} = 4.77$, $p < 0.01$, ANOVA). In slices prepared from the rostral striatum on the lesioned side of 6-OHDA-lesioned animals, NMDA-induced increases in cAMP levels were significantly higher than basal (726.0 ± 329.8%) ($p < 0.001$). In contrast, there was no significant effect of NMDA on striatal cAMP levels prepared from the caudal striatum of the lesioned side of 6-OHDA-lesioned rats (261.5 ± 47.8%) (Fig. 7).

DISCUSSION

In this study, we show that NMDA increases striatal cAMP levels via a mechanism involving adenosine A_{2a} receptors. This signaling cascade is enhanced in the 6-OHDA-lesioned rat model of Parkinson's disease.

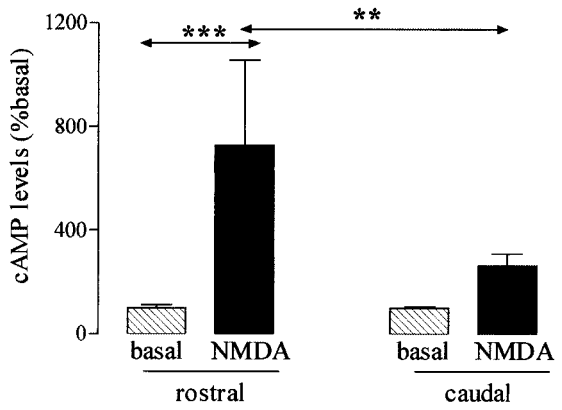

Figure 7. Topographical effect of NMDA on striatal slices prepared from the lesioned side of 6-OHDA-lesioned animals. cAMP levels were measured in striatal slices subsequent to exposure to NMDA (100 μM) and compared with basal. NMDA caused a 726.0 ± 329.8% increase in cAMP compared with basal in slices prepared from the rostral striatum of 6-OHDA-lesioned animals, and a 261.5 ± 47.8% increase compared with basal in slices prepared from the caudal striatum. Data are presented as mean percentage of basal ± SEM of six separate experiments, each with 50–60 striatal slices taken from six individual animals. $^{**}p < 0.01$, $^{***}p < 0.001$, *post hoc* Tukey Kramer's multiple comparisons test.

Incubation of striatal slices with NMDA for 5–20 min significantly increased cAMP levels. Electrophysiological studies have shown that similar slice preparations are functionally viable for >20 hr (Yu et al., 1993). In this study, incubating striatal slices for 5 min with NMDA increased cAMP levels to a level comparable with those observed with longer incubation times (up to 20 min). Thus, in subsequent experiments, striatal slices were incubated with NMDA for 5 min to minimize the risk of excitotoxicity, which can occur after incubation of brain slices with NMDA (100 μM) for periods of >30 min (Garthwaite and Garthwaite, 1986, 1989). Indeed, incubation of striatal slices with NMDA for 5 min has previously been shown to induce nontoxic, physiological changes in striatal function (Cai et al., 1991; Henselmans and Stoof, 1991).

Incubation of striatal slices with the glycine site NMDA receptor antagonist 7-chlorokynurenate produced a concentration-dependent inhibition of the effect of NMDA. This illustrates that the increase in cAMP levels observed in the presence of NMDA was an NMDA receptor-mediated event. The potency of 7-chlorokynurenate is comparable with that cited in other investigations using this antagonist to block NMDA receptor activation (Perrier and Benavides, 1995).

There was a concentration-dependent increase in the effect of the adenosine A_{2a} receptor agonist CPCA on cAMP levels in striatal slices. Because high concentrations of CPCA (10–100 μM) produced variable results, subsequent experiments used CPCA (3 μM) because this produced consistent increases in striatal cAMP levels. In addition, other studies have shown that similar concentrations of CPCA consistently activate A_{2a} adenosine receptors in brain slice preparations (Hogan et al., 1998). The CPCA-induced increase in striatal cAMP levels was inhibited when striatal slices were preincubated with the adenosine A_2 receptor antagonist DMPX, showing that the CPCA-induced increase in cAMP was mediated via adenosine A_{2a} receptors. The concentration at which DMPX inhibited the CPCA-induced increase in cAMP is comparable with that used previously to block adenosine A_{2a} receptor activation *in vitro* (Hogan et al., 1998).

NMDA receptor-mediated increases in cAMP are mediated via adenosine A_{2a} receptors

The NMDA-induced increase in striatal cAMP was completely blocked in the presence of DMPX. Although DMPX can antagonize adenosine A_{2a} and A_{2b} receptors, it is unlikely that DMPX is blocking the NMDA-induced increase in cAMP levels via blockade of adenosine A_{2b} receptors, because functional A_{2b} receptors are not expressed in rat striatum (Hide et al., 1992). Thus, the simplest explanation of the data is that NMDA receptor activation may lead to increased cAMP via indirect activation of adenosine A_{2a} receptors. If there really were a single NMDA–A2a–cAMP cascade, NMDA should not increase the effect of maximal adenosine A_{2a} receptor stimulation. Thus, the finding that incubation of a supramaximal concentration of CPCA with NMDA induces a similar increase to a supramaximal concentration of CPCA alone substantiates this explanation.

cAMP is formed from ATP subsequent to activation of the membrane-bound enzyme, adenylyl cyclase. Activation of adenylyl cyclase types I–IV, VII, and VIII (Mons et al., 1995) is calcium dependent, whereas adenylyl cyclase types V, VI, and IX are activated by G_s (Mons and Cooper, 1994; Antoni et al., 1998). Because Ca^{2+}-activated adenylyl cyclase has not been detected within the striatum, the NMDA-induced increase in cAMP is unlikely to be caused by NMDA receptor-mediated elevation of intracellular calcium activating adenylyl cyclase. Within the striatum, adenylyl cyclase type V is the most abundant adenylyl cyclase, and this is activated via G_s, subsequent to adenosine A_{2a} receptor stimulation (Mons et al., 1995, Chern et al., 1996). Because the NMDA-induced increase is completely blocked in the presence of DMPX, the increase in cAMP observed after NMDA receptor activation is probably caused by indirect activation of adenosine A_{2a} receptors. We thus propose that NMDA and adenosine A_{2a}

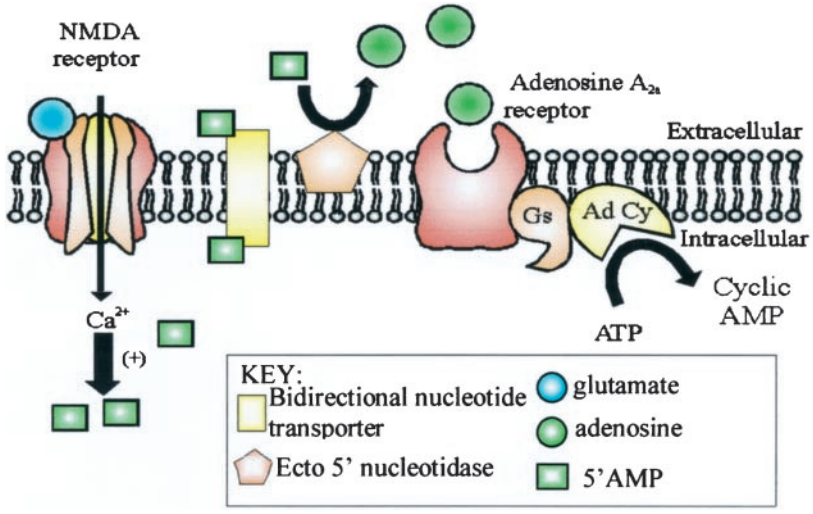

Figure 8. Proposed mechanism for NMDA receptor-mediated increases in cAMP in the striatum. Activation of NMDA receptors results in increased intracellular Ca^{2+} levels. Ca^{2+} stimulates the release of 5′AMP, which is transported across the membrane via a bidirectional nucleotide transporter (Delaney and Geiger, 1998). 5′AMP is broken down to adenosine via membrane-bound ecto 5′ nucleotidase. Adenosine then stimulates adenosine A_{2a} receptors, which are positively coupled to G_s. Once the adenosine A_{2a} receptor is stimulated, G_s activates adenylyl cyclase type V, which in turn triggers the breakdown of ATP to cAMP.

receptors share a common second messenger signaling cascade within the striatum, whereby stimulation of either NMDA receptors or adenosine A_{2a} receptors results in increased cAMP levels via activation of the same adenylyl cyclase (Fig. 8). Although not determined in the present study, such a signaling pathway may also be important in other regions of the brain, for example in the hippocampus, where both adenosine A_{2a} and NMDA receptors have been implicated in long-term potentiation (Collingridge et al., 1983; Kessey et al., 1997).

NMDA receptor–adenosine A_{2a} receptor signaling is enhanced in the 6-OHDA-lesioned animal model of Parkinson's disease

To determine the effect of NMDA on cAMP levels in the parkinsonian striatum, striatal slices were prepared from rats lesioned unilaterally with 6-OHDA. Although this is a unilateral model of Parkinson's disease, the pathological changes after 6-OHDA-induced lesions of the medial forebrain bundle correlate closely with alterations observed in idiopathic Parkinson's disease (Hornykiewicz, 1975). After infusion of 6-OHDA, although some compensatory changes may occur as a consequence of chronic dopamine depletion, these changes are thought to be similar to those occurring in patients with idiopathic Parkinson's disease.

In striatal slices prepared from 6-OHDA-lesioned rats, basal cAMP levels in slices taken from the lesioned side were comparable with those taken from the operated side of sham-operated animals. The NMDA-induced increase in cAMP observed in slices prepared from either side of sham-operated animals or the unlesioned side of 6-OHDA-lesioned animals was comparable with those observed in naïve animals (approximately twofold). NMDA caused almost a fourfold increase in cAMP levels in striatal slices prepared from the dopamine-depleted side of 6-OHDA rats. In the dopamine-depleted striatum, the enhanced effect of NMDA on cAMP levels was completely blocked by DMPX. Thus, in the dopamine-depleted striatum, the enhanced effect of NMDA on cAMP levels must be a consequence of increased signaling of the NMDA receptor–adenosine A_{2a} receptor–cAMP signaling cascade, rather than additional NMDA receptor interactions with other neurotransmitter systems. The mechanism underlying enhanced NMDA receptor stimulation in the parkinsonian striatum *in vivo* is unknown, although enhanced activation of AMPA receptors may be involved, because AMPA receptor activation has been shown to increase levels of NMDA receptor activation (Bliss and Collingridge, 1993). Furthermore, AMPA receptor antagonists elicit anti-parkinsonian actions in animal models of Parkinson's disease (Loschmann et al., 1991).

Because the effect of NMDA on cAMP levels was enhanced in the dopamine-depleted striatum, the finding that basal cAMP levels were unchanged in the lesioned striatum is perhaps surprising, because one might expect endogenous excitatory amino acids to have an effect similar to that of NMDA. One possible explanation is that transmission by endogenous excitatory amino acids within the striatum is quiescent after the recovery period of slices after preincubation. Alternatively, endogenous excitatory amino acids within the striatum may have been broken down or taken up during the recovery period. Thus, increases in basal cAMP might be seen if levels were assessed immediately postmortem or *in vivo*.

When striatal slices prepared from the 6-OHDA-lesioned striatum were incubated with the A_{2a} receptor agonist CPCA, cAMP levels were increased to the same extent as observed in the dopamine-innervated (unlesioned) striatum. Therefore, enhanced NMDA receptor–adenosine A_{2a} receptor–cAMP signaling in the dopamine-depleted striatum must be a consequence of changes occurring at the level of the NMDA receptor rather than adenosine A_{2a} receptor stimulation.

The concept that dopamine depletion results in changes in the properties of striatal NMDA receptors is in line with other data obtained in rat models of Parkinson's disease. Receptor–radioligand binding studies using washed striatal membranes in the presence of [^{3}H] MK-801 have shown that NMDA receptors in the parkinsonian striatum have increased "activatability" in the presence of glutamate and glycine (Nash et al., 1997, 1999). Increased phosphorylation of NMDA receptors may account for striatal NMDA receptors becoming more activatable (Menegoz et al., 1995; Oh et al., 1998, 1999).

NMDA receptor–adenosine A_{2a} receptor signaling is enhanced specifically in the rostral striatum in the 6-OHDA-lesioned animal model of Parkinson's disease

There was a significant effect of rostrocaudal level on the ability of NMDA to increase cAMP levels in striatal slices prepared from the 6-OHDA-lesioned striatum. NMDA only induced significant increases in striatal cAMP levels in striatal slices prepared from the rostral striatum on the lesioned side of 6-OHDA-lesioned animals. This topographical specificity is in line with previous findings in which blockade of NMDA receptors specifically within the rostral striatum mediated anti-parkinsonian actions, whereas blockade of NMDA receptors in more caudal regions had no effect on locomotion (Klockgether and Turski, 1993; Nash et al., 1997). Indeed, anatomical tracing and pharmacological and immunohistochemical studies have shown that both receptor density and neurotransmitter content, as well as afferent and efferent connections, vary between the rostral and caudal striatum (Beckstead and Cruz, 1986; Widmann and Sperk, 1986; Albin et al., 1992; Kincaid and Wilson, 1996).

Enhanced NMDA receptor–adenosine A_{2a} receptor–cAMP signaling on the indirect striatal output pathway is responsible for the induction of parkinsonian symptoms

Overactivity of the indirect striatal output pathway is thought to be the key mechanism responsible for the induction of symptoms of Parkinson's disease (Crossman et al., 1985; Pan et al., 1985; Mitchell et al., 1986; Miller and DeLong, 1987; Crossman, 1989; Mitchell and Crossman, 1989; Griffiths et al., 1990; Robertson et al., 1990, 1991; Maneuf et al., 1994). Although NMDA receptors are expressed on both the direct and indirect pathway, as well as on glia (Thompson et al., 2000), the selective location of adenosine A_{2a} receptors on the indirect pathway (Barraco et al., 1993; Sebastiao and Ribeiro, 1996) makes it probable that NMDA receptor–adenosine A_{2a} receptor–cAMP signaling occurs specifically on the indirect pathway. Furthermore, NMDA receptor antagonists are thought to mediate their anti-parkinsonian actions specifically via the indirect pathway, suggesting that NMDA receptors on this pathway are overactive in Parkinson's disease (Schmidt et al., 1990, 1992).

In conclusion, we have demonstrated that within the striatum, NMDA receptors modulate cAMP levels via adenosine A_{2a} receptors within the striatum. Furthermore, this NMDA receptor–adenosine A_{2a} receptor–cAMP signaling cascade is enhanced in the dopamine-depleted striatum. Both this enhancement and the anti-parkinsonian action of NMDA receptor antagonists are restricted to the rostral striatum, suggesting that blockade of this overactive signaling cascade may account for the anti-parkinsonian actions of NMDA receptor antagonists and adenosine A_{2a} receptor antagonists in MPTP-lesioned primates. At present, it is not clear how NMDA–adenosine A_{2a} receptor signaling is enhanced, although adenosine A_{2a} receptor function appears normal.

REFERENCES

Albin RL, Makowiec RL, Hollingsworth ZR, Dure LS, Penney JB, Young AB (1992) Excitatory amino acid binding sites in the basal ganglia of the rat: a quantitative autoradiographic study. Neuroscience 46:35–48.

Antoni FA, Palkovits M, Simpson J, Smith SM, Leitch AL, Rosie R, Fink G, Paterson JM (1998) Ca^{2+}/calcineurin-inhibited adenylyl cyclase, highly abundant in forebrain regions, is important for learning and memory. J Neurosci 18:9650–9661.

Barraco RA, Martens KA, Parizon M, Normile HJ (1993) Adenosine A2a receptors in the nucleus accumbens mediate locomotor depression. Brain Res Bull 31:397–404.

Beckstead RM, Cruz CJ (1986) Striatal axons to the globus pallidus, entopeduncular nucleus and substantia nigra come mainly from separate cell populations. Neuroscience 19:147–158.

Bedard PJ, Grondin R, Hadj Tahar A, Kase H, Mori A (1998) The A2a adenosine receptor antagonist KW-6002 has antiparkinsonian activity in MPTP treated cynomolgous monkeys. Mov Disord 13:00.

Blanchet PJ, Konitsiotis S, Whittemore ER, Zhou ZL, Woodward RM, Chase TN (1999) Differing effects of *N*-methyl-D-aspartate receptor subtype selective antagonists on dyskinesias in levodopa-treated 1-methyl-4-phenyl-tetrahydropyridine monkeys. J Pharmacol Exp Ther 290:1034–1040.

Bliss TVP, Collingridge GL (1993) A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361:31–39.

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254.

Brotchie JM (1997) Novel approaches to the symptomatic treatment of parkinsonian syndromes: alternatives and adjuncts to dopamine-replacement. Curr Opin Neurol 10:340–345.

Cai NS, Kiss B, Erdo SLJ (1991) Heterogeneity of *N*-methyl-D-aspartate receptors regulating the release of dopamine and acetylcholine from striatal slices. Neurochemistry 57:2148–2151.

Chern Y, Lee EH, Lai HL, Wang HL, Lee YC, Ching YH (1996) Circadian rhythm in the Ca^{2+}-inhibitable adenylyl activity of rat striatum. FEBS Lett 385:205–208.

Collingridge GL, Kehl SJ, McLennan H (1983) Excitatory amino acids in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus. J Physiol (Lond) 334:33–46.

Cotzias GC, Papavasiliou PS, Gellene R (1969) Modification of parkinsonism: chronic treatment with L-DOPA. N Engl J Med 280:337–345.

Craig CG, White TD (1993) NMDA-evoked adenosine release from rat cortex does not require the intermediate formation of nitric oxide. Neurosci Lett 158:167–169.

Craig CG, Temple SD, White TD (1994) Is cyclic AMP involved in excitatory amino acid-evoked adenosine release from rat cortical slices? Eur J Pharmacol 269:79–85.

Crossman AR (1989) Neural mechanisms in disorders of movement. Comp Biochem Physiol [A] 93:141–149.

Crossman AR, Mitchell IJ, Sambrook MA (1985) Regional brain uptake of 2-deoxyglucose in *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced parkinsonism in the macaque monkey. Neuropharmacology 24:587–591.

Delaney SM, Geiger JD (1998) Levels of endogenous adenosine in rat striatum. II. Regulation of basal and *N*-methyl-D-aspartate-induced levels by inhibitors of adenosine transport and metabolism. J Pharmacol Exp Ther 285:568–572.

Garthwaite G, Garthwaite J (1986) Neurotoxicity of excitatory amino acid receptor agonists in rat cerebellar slices: dependence on calcium concentration. Neurosci Lett 66:193–198.

Garthwaite G, Garthwaite J (1989) Differential dependence on Ca2+ of *N*-methyl-D-aspartate and quisqualate neurotoxicity in young rat hippocampal slices. Neurosci Lett 97:316–322.

Griffiths PD, Sambrook MA, Perry R, Crossman AR (1990) Changes in benzodiazepine and acetylcholine receptors in the globus pallidus in Parkinson's disease. J Neurol Sci 100:131–136.

Hauber W, Munkle M (1995) Stimulation of adenosine A2a receptors in the rat striatum induces catalepsy that is reversed by antagonists of *N*-methyl-D-aspartate receptors. Neurosci Lett 196:205–208.

Henselmans JM, Stoof JC (1991) Regional differences in the regulation of acetylcholine release upon D2 dopamine and *N*-methyl-D-aspartate receptor activation in rat nucleus accumbens and neostriatum. Brain Res 566:1–7.

Hide I, Padgett WL, Jacobson KA, Daly JW (1992) 2A adenosine receptors from rat striatum and rat pheochromocytoma PC12 cells: characterization with radioligand binding and by activation of adenylate cyclase. Mol Pharmacol 41:352–359.

Hogan YH, Hawkins R, Alkadhi KA (1998) Adenosine A1 receptor activation inhibits LTP in sympathetic ganglia. Brain Res 807:19–28.

Hornykiewicz O (1975) Brain monoamines and parkinsonism. Natl Inst Drug Abuse Res Monogr Ser 00:13–21.

Jin S, Fredholm BB (1997) Electrically-evoked dopamine and acetylcholine release from rat striatal slices perfused without magnesium: regulation by glutamate acting on NMDA receptors. Br J Pharmacol 121:1269–1276.

Kanda T, Jackson MJ, Smith LA, Pearce RK, Nakamura J, Kase H, Kuwana Y, Jenner P (1998) Adenosine A2A antagonist: a novel antiparkinsonian agent that does not provoke dyskinesia in parkinsonian monkeys. Ann Neurol 43:507–513.

Kessey K, Trommer BL, Overstreet LS, Ji T, Mogul DJ (1997) A role for adenosine A2 receptors in the induction of long-term potentiation in the CA1 region of rat hippocampus. Brain Res 756:184–190.

Kincaid AE, Wilson CJ (1996) Corticostriatal innervation of the patch and matrix in the rat neostriatum. J Comp Neurol 374:578–592.

Klockgether T, Turski L (1993) Toward an understanding of the role of glutamate in experimental parkinsonism: agonist-sensitive sites in the basal ganglia. Ann Neurol 34:585–593.

Klockgether T, Loschmann PA, Wullner U (1994) New medical and surgical treatments for Parkinson's disease. Curr Opin Neurol 7:346–352.

Loschmann PA (1997) The NMDA2B antagonist Ro 25-6981 is an antiparkinsonian agent. Mov Disord 12:525.

Loschmann PA, Lange KW, Kunow M, Rettig KJ, Jahnig P, Honore T, Turski L, Wachtel H, Jenner P, Marsden CD (1991) Synergism of the AMPA-antagonist NBQX and the NMDA-antagonist CPP with L-dopa in animal models of Parkinson's disease. J Neural Transm Parkinson's Dis Dementia Sect 3:203–213.

Maneuf YP, Mitchell IJ, Crossman AR, Brotchie JM (1994) On the role of enkephalin cotransmission in the GABAergic striatal efferents to the globus pallidus. Exp Neurol 125:65–71.

Marsden CA, Joseph SA (1989) HPLC of macromolecules: a practical approach. Oxford: IRL.

Menegoz M, Lau LF, Herve D, Huganir RL, Girault JA (1995) Tyrosine phosphorylation of NMDA receptor in rat striatum: effects of 6-OH-dopamine lesions. NeuroReport 7:125–128.

Miller WC, DeLong MR (1987) Altered tomic activity of neurons in the globus pallidus and subthalamic nucleus in the primate MPTP model of parkinsonism. In: The basal ganglia II (Carpenter MB, Jayaraman A, eds), pp 415–427. New York: Plenum.

Mitchell IJ, Crossman AR (1989) Neural mechanisms in Parkinson's disease and related disorders. Drug News Perspect 2:146–149.

Mitchell IJ, Cross AJ, Sambrook MA, Crossman AR (1986) Neural mechanisms mediating 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced parkinsonism in the monkey: relative contribution of the striatopallidal and striatonigral pathways are suggested by 2-deoxyglucose uptake. Neurosci Lett 63:61–65.

Mons N, Cooper DM (1994) Selective expression of one $Ca(^{2+})$-inhibitable adenylyl cyclase in dopaminergically innervated rat brain regions. Mol Brain Res 22:236–244.

Mons N, Harry A, Dubourg P, Premont RT, Iyengar R, Cooper DM (1995) Immunohistochemical localization of adenylyl cyclase in rat brain indicates a highly selective concentration at synapses. Proc Natl Acad Sci USA 92:8473–8477.

Nash JE, Fox SH, Henry B, Hill MP, Hille CJ, Maneuf Y, Peggs D, Mcguire S, Crossman AR, Brotchie JM (1997) Anti-Parkinsonian effect of NR2B-selective NMDA antagonists in animal models of Parkinson's disease: a striatal mechanism of action? Soc Neurosci Abstr 23:79.5.

Nash JE, Hill MP, Brotchie JM (1999) Antiparkinsonian actions of blockade of NR2B-containing NMDA receptors in the reserpine-treated rat. Exp Neurol 155:42–48.

Nash JE, Fox SH, Henry B, Hill MP, Peggs D, McGuire S, Maneuf Y, Hille C, Crossman AR, Brotchie JM 2000 Anti-parkinsonian actions of ifenprodil in the MPTP-lesioned marmoset model of Parkinson's disease. Exp Neurol, in press.

Oh JD, Russell D, Vaughan CL, Chase TN (1998) Enhanced tyrosine phosphorylation of striatal NMDA receptor subunits: effect of dopaminergic denervation and L-DOPA administration. Brain Res 813:150–159.

Oh JD, Vaughan CL, Chase TN (1999) Effect of dopamine denervation and dopamine agonist administration on serine phosphorylation of striatal NMDA receptor subunits. Brain Res 821:433–442.

Pan HS, Penney JB, Young AB (1985) Gamma-aminobutyric acid and benzodiazepine receptor changes induced by unilateral 6-OHDA lesions of the medial forebrain bundle. J Neurochem 45:1396–1404.

Papa SM, Engber TM, Kask AM, Chase TN (1994) Motor fluctuations in levodopa treated parkinsonian rats relation to lesion extent and treatment duration. Brain Res 662:69–74.

Paxinos G, Watson CP (1998) The rat brain in stereotaxic co-ordinates. New York: Academic.

Pearce RK, Jackson M, Smith L, Jenner P, Marsden CD (1995) Chronic L-DOPA administration induces dyskinesias in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated common marmoset (Callithrix Jacchus). Mov Disord 10:731–740.

Perrier ML, Benavides J (1995) Pharmacological heterogeneity of NMDA receptors in cerebellar granule cells in immature rat slices. A microfluorimetric study with the $[Ca^{2+}]_i$ sensitive dye Indo-1. Neuropharmacology 34:35–42.

Pycock CJ, Carter CJ, Kerwin RW (1980) Effect of 6-hydroxydopamine lesions of the medial prefrontal cortex on neurotransmitter systems in subcortical sites in the rat. J Neurochem 34:91–99.

Quinn N, Critchley P, Marsden CD (1987) Young onset Parkinson's disease. Mov Disord 2:73–91.

Rascol O (2000) Medical treatment of levodopa-induced dyskinesias. Ann Neurol 474[Suppl 1]:S179–188.

Rascol A, Guiraud B, Montastruc JL, David J, Clanet M (1979) Long-term treatment of Parkinson's disease with bromocriptine. J Neurol Neurosurg Psychiatry 42:143–150.

Ravenscroft P, Nash JE, Brotchie JM (1998) Striatal NR2B NMDA receptor subunit expression is elevated following D2, but not D1 dopamine receptor antagonism in rat. Eur J Neurosci 10:000.

Robertson RG, Clarke CA, Boyce S, Sambrook MA, Crossman AR (1990) The role of striatopallidal neurones utilizing gamma-aminobutyric acid in the pathophysiology of MPTP-induced parkinsonism in the primate: evidence from [3H]flunitrazepam autoradiography. Brain Res 531:95–104.

Robertson RG, Graham WC, Sambrook MA, Crossman AR (1991) Further investigations into the pathophysiology of MPTP-induced parkinsonism in the primate: an intracerebral microdialysis study of gamma-aminobutyric acid in the lateral segment of the globus pallidus. Brain Res 563:278–280.

Schmidt WJ, Bubser M, Hauber W (1990) Excitatory amino acids and Parkinson's disease. Trends Neurosci 13:46–47.
Schmidt WJ, Bubser M, Hauber W (1992) Behavioural pharmacology of glutamate in the basal ganglia. J Neural Transm [Suppl] 38:65–89.
Schulz JB, Matthews RT, Jenkins BG, Ferrante RJ, Siwek D, Henshaw DR, Cipolloni PB, Mecocci P, Kowall NW, Rosen BR (1995) Blockade of neuronal nitric oxide synthase protects against excitotoxicity in vivo. J Neurosci 15:8419–8429.
Sebastiao AM, Ribeiro JA (1996) Adenosine A2 receptor-mediated excitatory actions on the nervous system. Prog Neurobiol 48:167–189.
Semba K, White TD (1997) M3 muscarinic receptor-mediated enhancement of NMDA-evoked adenosine release in rat cortical slices in vitro. J Neurochem 69:1066–1072.
Steece-Collier K, Chambers LK, Jaw-Tsai SS, Menniti FS, Greenamyre JT (2000) Antiparkinsonian actions of CP-101,606, an antagonist of NR2B subunit-containing *N*-methyl-D-aspartate receptors. Exp Neurol 163:239–243.
Sullivan GW, Linden J, Hewlett EL, Carper HT, Hylton JB, Mandell GL (1990) Adenosine and related compounds counteract tumor necrosis factor-alpha inhibition of neutrophil migration: implication of a novel cyclic AMP-independent action on the cell surface. J Immunol 145:1537–1544.
Thompson CL, Drewery DL, Atkins HD, Stephenson FA, Chazot PL (2000) Immunohistochemical localization of *N*-methyl-D-aspartate receptor NR1, NR2A, NR2B, NR2C/D subunits in the adult mammalian cerebellum. Neurosci Lett 283:85–88.
Ulas J, Weihmuller FB, Brunner LC, Joyce JN, Marshall JF, Cotman CW (1994) Selective increase of NMDA-sensitive glutamate binding in the striatum of Parkinson's disease, Alzheimer's disease, and mixed Parkinson's disease/Alzheimer's disease patients: an autoradiographic study. J Neurosci 14:6317–6324.
Ungerstedt U (1968) 6-Hydroxy-dopamine induced degeneration of central monoamine neurons. Eur J Pharmacol 5:107–110.
Weihmuller FB, Ulas J, Nguyen L, Cotman CW, Marshall JF (1992) Elevated NMDA receptors in parkinsonian striatum. NeuroReport 3:977–980.
White TD (1996) Potentiation of excitatory amino acid-evoked adenosine release from rat cortex by inhibitors of adenosine kinase and adenosine deaminase and by acadesine. Eur J Pharmacol 303:27–38.
Widmann R, Sperk G (1986) Topographical distribution of amines and major amine metabolites in the rat striatum. Brain Res 367:244–249.
Wullner U, Kupsch A, Arnold G, Renner P, Scheid C, Scheid R, Oertel W, Klockgether T (1992) The competitive NMDA antagonist CGP40.116 enhances L-dopa response in. Neuropharmacology 31:713–715.
Yu GD, Rusak B, Piggins HD (1993) Regulation of melatonin-sensitivity and firing-rate rhythms of hamster suprachiasmatic nucleus neurons: constant light effects. Brain Res 602:191–199.