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A Dual Role of Adenosine A_{2A} Receptors in 3-Nitropropionic Acid-Induced Striatal Lesions: Implications for the Neuroprotective Potential of A_{2A} Antagonists

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Reduction of A_{2A} receptor expression is one of the earliest events occurring in both Huntington's disease (HD) patients and mice overexpressing the N-terminal part of mutated huntingtin. Interestingly, increased activity of A_{2A} receptors has been found in striatal cells prone to degenerate in experimental models of this neurodegenerative disease. However, the role of A_{2A} receptors in the pathogenesis of HD remains obscure. In the present study, using $A_{2A}{}^{-/-}$ mice and pharmacological compounds in rat, we demonstrate that striatal neurodegeneration induced by the mitochondrial toxin 3-nitropropionic acid (3NP) is regulated by A_{2A} receptors. Our results show that the striatal outcome induced by 3NP depends on a balance between the deleterious activity of presynaptic A_{2A} receptors and the protective activity of postsynaptic A_{2A} receptors. Moreover, microdialysis data demonstrate that this balance is anatomically determined, because the A_{2A} presynaptic control on striatal glutamate release is absent within the posterior striatum. Therefore, because blockade of A_{2A} receptors has differential effects on striatal cell death *in vivo* depending on its ability to modulate presynaptic over postsynaptic receptor activity, therapeutic use of A_{2A} antagonists in Huntington's as well as in other neurodegenerative diseases could exhibit undesirable biphasic neuroprotective–neurotoxic effects.

Key words: 3-nitropropionic; Huntington's disease; striatum; A_{2A} receptors; neuroprotection; cell death

Introduction

Adenosine is a purinergic messenger that modulates diverse neuronal functions, principally through the activation of A_1 and A_{2A} G-protein-coupled receptors (for review, see Svenningsson et al., 1999). Both A_1 receptor stimulation and A_{2A} receptor blockade have been widely recognized to be neuroprotective in models of neurodegenerative diseases (Jones et al., 1998; Chen et al., 1999; Svenningsson et al., 1999; Kase, 2001; Blum et al., 2002b, 2003; Popoli et al., 2002). In excitotoxic-related models [quinolinic acid (QA)-induced striatal lesions, cerebral ischemia], A_{2A} receptor blockade has been suggested to be neuroprotective through inhibition of glutamate release (Gao and Phillis, 1994; Popoli et al., 1995, 2002; Monopoli et al., 1998b; Chen et al., 1999; Reggio et al., 1999).

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder characterized by motor, cognitive, and emotional impairments (Brouillet et al., 1999). The responsible mutation is a CAG expansion within the gene encoding huntingtin (The Huntington's Disease Study Group, 1993). For unclear reasons, it leads primarily to the preferential degeneration of medium-sized spiny GABAergic neurons of the striatum. Interestingly, one of the earliest neurochemical features observed in HD is a reduction of striatal A_{2A} binding sites (Glass et al., 2000). In the R6/2 transgenic model of HD, A_{2A} receptor mRNA decreases even before the appearance of motor symptoms (Cha et al., 1999). Conversely, other studies suggest an upregulation of A_{2A} receptor activity in either regions prone to degeneration in a neurotoxic model of the disease (Blum et al., 2002a) or clonal striatal cells overexpressing mutated huntingtin (Varani et al., 2001). Together, these data strongly implicate A_{2A} receptors in the physiology of the disease through mechanisms that are yet unknown.

Mitochondrial metabolic inhibition is one of the earliest events that could lead to striatal degeneration in HD, presumably through secondary excitotoxicity (Browne et al., 1997; Tabrizi et al., 1999; Beal, 2000). The increased susceptibility to apoptosis, the defect in membrane potential of lymphoblast mitochondria from HD patients, and the direct interaction of mutated hunting-

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tin with mitochondrial membranes (Sawa et al., 1999; Panov et al., 2002) also support the involvement of a metabolic compromise. Accordingly, treatment of either rodents or monkeys with 3-nitropropionic acid (3NP), a complex II irreversible inhibitor, provides reliable models of HD both at the behavioral and histopathological levels (Beal et al., 1993; Brouillet et al., 1995, 1999; Ouary et al., 2000; Blum et al., 2001, 2002a; El Massioui et al., 2001). In the present study, we sought to determine whether modifications of A_{2A} receptor activity would influence the 3NP-induced neurodegenerative process using A_{2A} receptor knockout ($A_{2A}{}^{-/-}$) animals or pharmacological compounds. Our results suggest that A_{2A} receptors have a dual effect toward 3NP-induced neurotoxicity depending on a balance between presynaptic and postsynaptic sites of action and on the considered anatomical level within the striatum.

Materials and Methods

Animals

We used 3-month-old wild-type and $A_{2A}{}^{-/-}$ mice generated as described previously on a CD1 background (Ledent et al., 1997), adult male Lewis rats, 12 weeks of age for the 3NP model, and adult Wistar rats for microdialysis. Animals were housed three per cage and maintained in a temperature- and humidity-controlled room on a 12 hr light/dark cycle with food and water *ad libitum*. The number of animals was kept to a minimum, and all efforts to avoid animal suffering were made in accordance with the standards of the Institutional Ethical Committee.

Treatments

3NP (Fluka, Buchs, Switzerland) was dissolved in 0.1 M PBS, pH 7.4, adjusted to pH 7.3–7.4 with 5N NaOH.

Mice studies. The survival study was performed by one daily intraperitoneal injection of 3NP to both wild-type and $A_{2A}{}^{-/-}$ mice ($n = 19$ per group) with an increasing dosage (starting dosage, 25 mg · kg^{-1} · d^{-1}, increased by 15% each day) until all of the animals died. The appearance of motor disabilities, the body weight of surviving mice, and the number of deaths were monitored each day.

Lesional experiments in mice were performed by two daily intraperitoneal injections of 3NP (70 mg/kg for each injection; 250 μl/50 gm). A first series of 8 wild-type and 23 $A_{2A}{}^{-/-}$ mice were injected at 9 A.M. and 7 P.M. (mild protocol), and a second series of 13 wild-type and 15 knockout mice were injected at 9 A.M. and 11 A.M. (severe protocol). Injections were stopped and animals were killed when lethality reached ~40% in wild-type animals.

Rat studies. Chronic treatments with 3NP (56 mg · kg^{-1} · d^{-1}), using 2ML1 Alzet minipumps (10 μl/h for 7 d; IFFA Credo, Arbresle, France), were performed as described previously (Blum et al., 2001, 2002a,b). Rats were anesthetized with a mixture containing xylazine hydrochloride (Rompun; Bayer, Wuppertal, Germany; 4.5 mg/kg) and ketamine hydrochloride (Imalgene; Merial; 90 mg/kg). In the 3NP-treated animals, an incision was made below the base of the neck and a 2ML1 Alzet osmotic minipump (delivering 10 μl/h for 7 d) containing 3-nitropropionic acid (Fluka) was positioned under the skin. The final concentration of 3NP in the pump was adjusted to the weight of the rats on the day of implantation to exactly deliver 56 mg · kg^{-1} · d^{-1}. Sham rats and animals treated with pharmacological compounds alone underwent all of the surgical procedures (without minipump implantation).

All of the rats were killed after 5 d of 3NP subcutaneous infusion. It is noteworthy that the time of killing was chosen according to the known kinetics of striatal lesion occurrence in this particular model, because it was described previously that macroscopic lesions induced by 3NP are detected 5 d after the beginning of the toxic treatment (Ouary et al., 2000; Blum et al., 2001, 2002a,b).

In a first experiment, 41 rats [sham, $n = 6$; vehicle/3NP, $n = 8$; 3-(3-hydroxypropyl)-8-(3-methoxystyryl)-7-methy-1-propargylxanthine (MSX-3) (1 mg/kg), $n = 6$; MSX-3 (1 mg/kg)/3NP, $n = 7$; MSX-3 (5 mg/kg), $n = 6$; and MSX-3 (5 mg/kg)/3NP, $n = 8$] were treated with the A_{2A} receptor antagonist MSX-3 (Hauber et al., 2001). Before each injection, MSX-3 was dissolved in water by the addition of drops of NaOH (10N), and pH was corrected to 7.4. Concentrations of MSX-3 were chosen on the basis of the fact that MSX-3 provides a maximal locomotor stimulation without A_1-related effect at the dose of 5 mg/kg and a submaximal effect (~60% of the maximal effect) at 1 mg/kg (Karcz-Kubicha et al., 2003). In a second experiment, 37 rats [sham, $n = 5$; vehicle/3NP, $n = 8$; 2-*p*-(2-carboxyethyl)phenethylamino-5-*N*-ethylcarboxamido-adenosine (CGS21680) (0.3 mg/kg), $n = 5$; CGS21680 (0.3 mg/kg)/3NP, $n = 8$; CGS21680 (1 mg/kg), $n = 5$; and CGS21680 (1 mg/kg)/3NP, $n = 6$] were treated with the A_{2A} receptor agonist CGS21680 dissolved in heated NaCl (0.9%). At the concentrations used, CGS21680 does not induce A_1-related effects (Rimondini et al., 1997). In both experiments, rats were treated with a total of three injections of MSX-3 or CGS21680 on the fourth (injections at 9 A.M. and 7 P.M.) and fifth (injection at 9 P.M.) days after minipump implantation and killed 10 hr after the last administration. For each compound, the volume injected was adjusted according to the body weight of each rat (100 μl/100 gm, i.p.).

To verify whether doses of MSX-3 and CGS21680 used here were behaviorally active in Lewis rats, animals injected with only MSX-3 (5 mg/kg) or CGS21680 (1 mg/kg) were tested for spontaneous locomotion in a simple open-field paradigm (1 min habituation, 5 min counting in semiobscurity; open field, 6 × 6 squares, 9 × 9 cm each), 15 min or 2 hr after injection, respectively.

Tissue postprocessing

All of the animals were killed by decapitation; their brains were quickly removed and frozen in 2-methylbutane cooled by dry ice (−40°C). The tissue was cut at 16 μm thickness on a cryostat (Leitz, Wetzlar, Germany), and serial coronal sections were mounted onto poly-L-lysine- or gelatin-coated slides and stored at −20°C until use. Hematoxylin staining was used to reveal striatal lesions. The latter were measured every 144 μm (mice) or 240 μm (rats) along the anteroposterior axis of the striatum (bregma +1.18 to −0.4 mm for mice; bregma +1.6 to −0.8 mm for rats). In mice, the anterior striatum was considered to be between bregma +1.18 and +0.3 mm, and the posterior striatum was considered to be between +0.3 and −0.4 mm. In rat, the anterior striatum was considered to be between bregma +1.6 and +0.4 mm, and the posterior striatum was considered to be between +0.4 and −0.8 mm. Lesional volume was calculated for each animal using Cavalieri's method.

Semiquantitative measurement of succinate dehydrogenase and cytochrome oxidase activities

Measurement and analysis of succinate dehydrogenase (SDH) and cytochrome oxidase (CO) activity in control and 3NP-treated rats were performed as described previously (Brouillet et al., 1998; Blum et al., 2002a,b).

A_{2A} mRNA in situ *hybridization*

The hybridization technique was adapted from previous reports (Schiffmann and Vanderhaeghen, 1993; Dassesse et al., 2001). The sections mounted on RNase-free poly-L-lysine-coated slides were fixed in 4% freshly prepared paraformaldehyde for 30 min and rinsed in PBS (0.1 M). All of the sections were dehydrated and dipped for 3 min in chloroform. After air drying, the sections were incubated overnight at 42°C with 0.35×10^6 cpm/section ^{35}S-labeled probes diluted in hybridization buffer, which consisted of 50% formamide, 4× SSC [1× SSC (in M): 0.15 NaCl and 0.015 sodium citrate, pH 7.4], 1× Denhardt's solution (0.02% each of polyvinylpyrrolidone, bovine serum albumin, and Ficoll), 1% sarcosyl, 0.02 M sodium phosphate, pH 7.4, 10% dextran sulfate, yeast tRNA (500 μg/ml), salmon sperm DNA (100 μg/ml), and 60 mM dithiothreitol. Compounds were provided by Sigma (St. Louis, MO). After hybridization, the sections were rinsed for 15 min four times in 1×SSC at 55°C, dehydrated, and covered with Hyperfilm-βmax film (Amersham Biosciences, Arlington Heights, IL) for 2 or 3 weeks. The oligonucleotide probe (5′-CCGCTCCCCTGGCA-GGGGCTGGCTCTCCATCTGCTTCAGCTG-3′) was synthesized on an Applied Biosystems (Foster City, CA) 381A DNA synthesizer. It was labeled with [α-^{35}S]deoxyATP (DuPont NEN, Boston, MA) at its 3′ end by terminal DNA deoxynucleotidylexotransferase (Invitrogen, San Diego, CA) and purified with a G50 column (Amersham Biosciences) according to the manufacturer's instructions.

A_{2A} receptor autoradiography
A_{2A} receptor binding autoradiography was performed as described previously (Dassesse et al., 2001; Blum et al., 2002a) using a single concentration of radioligand. The gelatin-coated slides, stored at −20°C until use, were brought to room temperature 30 min before the autoradiographic experiments. The sections were incubated 90 min at room temperature in a buffer containing 50 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$, 2.5 IU/ml adenosine deaminase (Roche), and 10 nM [^{3}H]CGS21680 (47.0 Ci/mmol; DuPont NEN). Nonspecific binding of the ^{3}H-labeled ligand was assessed by the addition of 1 μM CGS21680. Slides were washed four times for 5 min in ice-cold 50 mM Tris-HCl, pH 7.4, buffer, dipped in ice-cold distilled water, dried under a stream of cold air, and exposed to ^{3}H-Hyperfilms (Amersham Biosciences) for 5 weeks.

Image analysis
Digitalized images with 256 gray levels were generated from the autoradiograms with the public domain NIH Image 1.61 program (National Institutes of Health, Bethesda, MD), a Power Macintosh G3, and a CCD video camera (Dage-MTI) with fixed gain and black level as described previously (Blum et al., 2002a,b). For the quantification of *in situ* hybridization and binding, depending on the marker studied, the average optical densities were measured on x-ray film autoradiograms. For *in situ* hybridization analysis, on each section, an averaged optical density of the background level was subtracted from that of the measured areas to obtain corrected values. For quantification of binding autoradiography, specific binding was determined by subtracting the nonspecific from the total binding. Quantification of binding site density and of mRNA level was performed along the rostrocaudal axis of the striatum in a squared area in the dorsolateral part to avoid confusion between the striatum and the globus pallidus at the most caudal planes. Results were expressed as the percentage mean ± SEM of mean sham value.

Microdialysis experiments
Microdialysis and determination of glutamate concentrations by HPLC (electrochemical detection) were performed as we reported previously (Popoli et al., 2002). Under Equithesin anesthesia (3 ml/kg, i.p.), naive Wistar rats were placed in a stereotaxic frame and implanted with a concentric dialysis probe (model CMA/12; 3 mm length; Carnegie Medicine, Solna, Sweden) into the striatum. Stereotaxic coordinates were as follows: anterior, −0.8; lateral, +3.5; ventral, −7 (posterior striatum); or anterior, +1; lateral, +3; ventral, −6.5 (anterior striatum), according to the atlas of Paxinos and Watson (1986). Twenty-four hours later, the probe was perfused at a rate of 2 μl/min with a Ringer's solution (in mM: 147 NaCl, 2.3 $CaCl_2$, and 4.0 KCl). After a washout period of at least 90 min, samples were collected every 5 min into a refrigerated fraction collector (model CMA/170) and then frozen until assay. Evoked glutamate release was induced by QA as shown previously (Popoli et al., 2002). Because the intracerebral injection of QA induces tremors and convulsions in rodents, these experiments were performed under general anesthesia (Equithesin). Results were expressed as percentage changes of extracellular glutamate levels induced by probe perfusion with QA (5 mM over 30 min) with respect to basal (predrug) values (mean of three to four samples collected after the induction of general anesthesia). The A_{2A} antagonist 5-amino-7-(2-phenylethyl)-2-(2-furyl)pyrazolo[4,3-e]-1,2,4-triazolo[1,5-c]pyramine, 61 (SCH 58261) (0.01 mg/kg) was administered intraperitoneally 20 min before starting QA perfusion. At the end of the experiments, each rat was killed with an overdose of Equithesin, the brain was fixed with 4% paraformaldehyde, and coronal sections were cut to verify the probe location. The glutamate content of all of the samples was measured by reverse-phase HPLC coupled to a fluorometric detector [PerkinElmer Instruments (Norwalk, CT) LC240 at wavelength of 335 nm and emission cutoff filter of 425 nm], using a 15 min gradient elution program (methanol from 20 to 80% with 50 mM NaH_2PO_4 and CH_3COONa) and automatic precolumn derivatization with *o*-phthalaldehyde and β-mercaptoethanol. Cysteic acid was used as the internal standard. The concentration of the standard was linear ($r^2 = 0.99$) between 0.2 and 25 ng/10 μl. Basal glutamate levels were calculated by comparison of sample peak height with external standard peak height, both corrected for the internal standard peak height and expressed as micromolar concentrations without probe recovery correction.

Primary striatal cultures
Primary cultures of striatal neurons were obtained from 17- to 18-d-old Wistar rat embryos as described previously (Blum et al., 2002b). Cells were exposed for 3 d to 100 μM 3NP with or without 100 μM forskolin (Sigma). To provide a reliable physiologic environment to cells, the latter were cultured in the presence of a subtoxic concentration of glutamic acid (10 μM; pH adjusted to 7.2) (Research Biochemicals, Natick, MA). Cell viability was assessed by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; Sigma] assay as follows. Cells were cultured for 1 hr in the presence of 5 mg/ml MTT. Formazan crystals were solubilized in DMSO. The optical density was measured at 540 nm on a Titertek Multiskan MCC/340 (ICN Biomedicals, Costa Mesa, CA).

Macrophage antigen complex-1 reverse transcription-PCR
Total striatal RNA was extracted, using TRIzol reagent, from the striatum of rats treated either with vehicle ($n = 3$), vehicle/3NP ($n = 3$), CGS21680 (1 mg/kg) ($n = 3$), or 3NP/CGS21680 (1 mg/kg) ($n = 3$). Reverse transcription (RT)-PCR analysis was performed as described previously (Wu et al., 2002) using the following primers: for macrophage antigen complex-1 (MAC-1), 5′-CAG ATC AAC AAG GTG ACC ATA TGG-3′ (forward) and 5′-CAT CAT GTC CTT GTA CTG-3′ (reverse); and for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 5′-TCC ACC ACC CTG TTG CTG TA-3′ (forward) and 5′-ACC ACA GTC CAT GCC ATC AC-3′ (reverse). PCR amplification was performed for 40 cycles for MAC-1 and 28 cycles for GADPH.

Analysis and statistics
Results were expressed as means ± SEM. Depending on the parameter studied, comparisons among groups were made using either the Mantel–Cox log rank test, unpaired Student's *t* test, the χ^2 test with Yates correction, one-way ANOVA followed by a Newman–Keuls *post hoc* test, or two-way ANOVA.

Results

Effects of 3NP in A_{2A}-deficient mice

We first determined the susceptibility of both wild-type and A_{2A}-deficient mice to increasing daily doses of 3NP. Although the mean weight of both groups was similar at the beginning of the experiment (38.6 ± 0.4 and 39.3 ± 0.9 for $A_{2A}{}^{+/+}$ and $A_{2A}{}^{-/-}$ mice, respectively; NS, Student's *t* test), during the treatment the weight loss was higher in knock-out mice (Fig. 1*A*) (genotype × days, $F_{(14,476)} = 10.26$; $p < 0.001$; repeated measures ANOVA). At day 12, when we first observed motor symptoms (gait abnormalities and/or dystonia of both hindlimbs), behavioral changes were more frequent in $A_{2A}{}^{-/-}$ mice (8 of 17) than in wild-type animals (1 of 19; $p < 0.0122$; Yates-corrected χ^2 test). The percentage of knock-out mice presenting motor symptoms was always higher than for wild-type animals all through the experiment (data not shown). The survival curve (Fig. 1*B*) indicated that $A_{2A}{}^{-/-}$ mice were more susceptible to 3NP intoxication ($p < 0.001$; Mantel–Cox log rank test).

$A_{2A}{}^{-/-}$ mice display cerebral transcriptional alterations (Dassesse et al., 2001) as well as changes in blood flow and heart rate (Ledent et al., 1997). Therefore, to rule out a possible difference in the basal metabolic activity and an eventual alteration of 3NP bioavailability as a cause of the observed differential susceptibility, we determined whether A_{2A} receptor deficiency induced a change in basal SDH and CO activities and also in the ability of 3NP to inhibit its target. We found that A_{2A} deficiency did not modify the basal activities of SDH and CO either in the striatum, the outer layers of the cortex (Fig. 2), or the liver (data not shown). Six hours after a single injection of 3NP (120 mg/kg), SDH activity was significantly and similarly inhibited within wild-type and $A_{2A}{}^{-/-}$ striatum ($A_{2A}{}^{+/+}$, −43.1 ± 2.6% of the control; $A_{2A}{}^{-/-}$, −47.6 ± 3.4%) (Fig. 2*A*), outer cortical layers ($A_{2A}{}^{+/+}$, −38.2 ± 4.2% of the control; $A_{2A}{}^{-/-}$, −39.9 ± 5.9%)

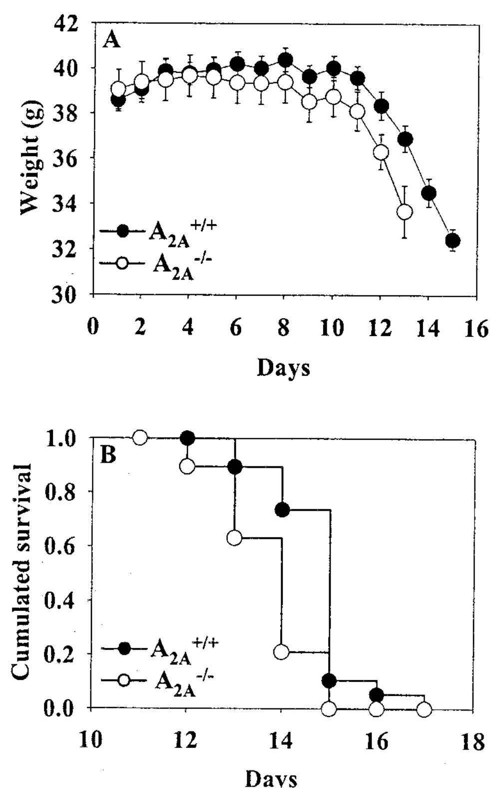

Figure 1. Survival and body weight of $A_{2A}{}^{+/+}$ and $A_{2A}{}^{-/-}$ mice challenged with daily injections of 3NP (intraperitoneal injection; starting dose, 25 mg/kg; increased by 15% every day). *A*, Effect on body weight. *B*, Kaplan–Meier probability of survival analysis.

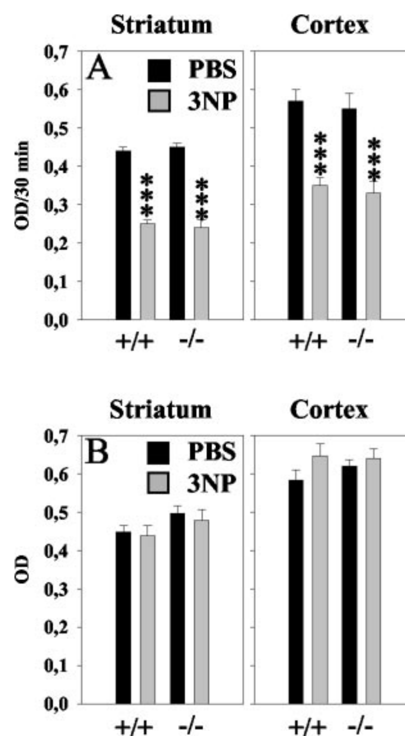

Figure 2. SDH (*A*) and CO (*B*) activities in the striatum and the outer layers of the cortex of mice ($n = 5$ per group) treated 6 hr with a single injection of 120 mg/kg 3NP. ***$p < 0.001$ versus sham rats, using Newman–Keuls ANOVA *post hoc* test. +/+, $A2A^{+/+}$; −/−, $A2A^{-/-}$.

(Fig. 2*A*), and liver ($A_{2A}{}^{+/+}$, −38.4 ± 4.7% of the control; $A_{2A}{}^{-/-}$, −47.2 ± 2.0%) (data not shown). Under 3NP treatment, CO activity remained unaltered in both genotypes (Fig. 2*B*).

To obtain animals in which a striatal lesion could be accurately evaluated, we submitted a new series of mice to two distinct protocols consisting of two daily injections of 3NP (two times 70 mg/kg per day). In a first (mild) protocol, injections were performed at a 10 hr interval (9 A.M. and 7 P.M.). In the second (severe) protocol, injections were spaced by 2 hr (9 A.M. and 11 A.M.). In wild-type animals, the mild protocol led to ~40% mortality after nine injections (4.5 d), whereas the severe protocol produced similar effects within six injections (3 d) (Table 1). Only the second protocol led to the formation of macroscopic striatal lesions in wild-type mice (Fig. 3, Table 1). At the end of the mild protocol (9 A.M./7 P.M.; nine injections), mortality in $A_{2A}{}^{-/-}$ mice was similar to that of wild-type mice (Table 1). However, throughout the 3NP administration, weight loss was found to be significantly higher for $A_{2A}{}^{-/-}$ mice (genotype × days, $F_{(4,64)} = 3.5$; $p < 0.012$; repeated measures ANOVA). Interestingly, wild-type mice that survive to 3NP treatment did not display macroscopic striatal lesions (Fig. 3*A*), whereas 70% of the surviving $A_{2A}{}^{-/-}$ animals showed obvious histological marks of striatal degeneration ($p = 0.044$ vs wild type; Yates-corrected χ^2 test) (Fig. 3, Table 1). In the severe 3NP protocol, mortality in $A_{2A}{}^{-/-}$ and wild-type mice was similar (Table 1). Throughout the 3NP treatment, weight loss was not significantly different between the two groups (data not shown). In contrast to what we have found in the mild protocol experiment, the proportion of animals with macroscopic striatal lesions was similar in wild-type and $A_{2A}{}^{-/-}$ groups (Table 1). However, the mean volume of striatal lesions in the $A_{2A}{}^{-/-}$ mice was twofold smaller compared with that of $A_{2A}{}^{+/+}$ animals ($p = 0.038$) (Fig. 3*B*, Table 1). Noteworthy, this effect was more pronounced within the anterior part (−68.6 ± 12.5%; $p = 0.0068$) (Table 1) compared with the posterior part of the striatum (−35.2 ± 26.3%; NS). This observation was not attributable to a greater SDH inhibition by 3NP within the anterior part of the striatum, because the neurotoxin induced a similar decrease of SDH activity along the rostrocaudal axis of the structure (data not shown).

Effects of A_{2A} receptor antagonist (MSX-3) and agonist (CGS21680) on the striatal lesions induced by 3NP in Lewis rats

To further evaluate the effects of A_{2A} receptor modulation on the striatal impairments induced by 3NP, we performed experiments in a rat model of chronic intoxication known to produce reproducible macroscopic striatal lesions (Ouary et al., 2000; Blum et al., 2001, 2002a,b).

In a first experiment, we determined the effects of the antagonist MSX-3 at the doses of 1 and 5 mg/kg. According to the expected locomotor effects of A_{2A} receptor antagonists, MSX-3 (5 mg/kg) increased spontaneous locomotion by 31 ± 9.4% 15 min after injection ($p = 0.013$ vs sham group; unpaired Student's *t* test). As shown in Table 2 and Figure 4A, treatment with MSX-3 at the dose of 5 mg/kg significantly increased the volume of the striatal lesion induced by 3NP (+40.1 ± 12.9%; $p = 0.039$). This effect was greater in the posterior part of the striatum (+57.9 ± 14.9%; $p = 0.0069$) compared with the anterior part (+22.9 ± 11.9%; NS). This effect was not observed using a concentration of 1 mg/kg (−26.6 ± 9.9% of the 3NP group; $p = 0.11$) (Fig. 4*A*, Table 2), and compared with the 3NP/MSX-3 group, such a treatment produces a reduction of lesion size by 47.7 ± 6.9% ($p < 0.01$).

Table 1. Effect of 3NP on $A_{2A}{}^{+/+}$ and $A_{2A}{}^{-/-}$ mice challenged with the mild or severe protocols of injection

Protocol	S	L	T (mm^3)	A (mm^3)	P (mm^3)
Mild					
$A_{2A}{}^{+/+}$	5/8	0/5	0	0	0
$A_{2A}{}^{-/-}$	10/23	7/10*	4.10 ± 1.25	2.35 ± 0.55	1.65 ± 0.67
Severe					
$A_{2A}{}^{+/+}$	8/13	7/8	2.66 ± 0.43	1.75 ± 0.24	0.91 ± 0.25
$A_{2A}{}^{-/-}$	7/15	5/7	1.14 ± 0.45**	0.55 ± 0.22***	0.59 ± 0.24

S, Survival; L, lesioned animals; T, total volume of the striatal lesion (bregma +1.18 to −0.3 mm); A, volume of the lesion in the anterior striatum (bregma +1.18 to +0.3 mm); P, volume of the lesion in the posterior striatum (bregma +0.3 to −0.4 mm).

*p = 0.044 versus wild-type animals (Yates-corrected χ^2 test); **p = 0.0389, ***p = 0.0068 versus wild-type animals (Student's t test).

Figure 3. Typical hematoxylin staining of $A_{2A}{}^{+/+}$ and $A_{2A}{}^{-/-}$ mice submitted to either mild (*A*) or severe (*B*) 3NP intoxication. Note that macroscopic striatal lesions are not present in the wild-type mice that were submitted to the mild protocol.

Table 2. Effects of MSX-3 and CGS21680 on the striatal lesions induced by 3NP in Lewis rats

Treatment	T (mm^3)	A (mm^3)	P (mm^3)
MSX-3			
3NP	21.7 ± 2.1	10.9 ± 1.3	10.7 ± 1.4
+MSX-3 (1 mg/kg)	15.9 ± 2.1	7.9 ± 0.9	7.9 ± 1.3
+MSX-3 (5 mg/kg)	30.4 ± 2.8*	13.4 ± 1.3	16.9 ± 1.6**
CGS21680			
3NP	17.9 ± 0.9	6.8 ± 0.5	11.1 ± 0.7
+CGS21680 (0.3 mg/kg)	20.6 ± 1.2	8.5 ± 0.5	12.1 ± 0.7
+CGS21680 (1.0 mg/kg)	29.4 ± 4.8***	13.6 ± 2.4****	15.8 ± 2.6

T, Total volume of the striatal lesion (bregma +1.6 to −0.8 mm); A, volume of the lesion in the anterior striatum (bregma +1.6 to +0.4 mm); P, volume of the lesion in the posterior striatum (bregma +0.4 to −0.8 mm).

*p = 0.039, **p = 0.0069, ***p = 0.043, ****p = 0.016 versus 3NP-treated animals (Newman–Keuls *post hoc* test).

The observed effects, especially at the highest concentration, could be related to either an interference of MSX-3 with the ability of 3NP to inhibit SDH or to a modification of the bioavailability of the neurotoxin via hemodynamic changes (Ledent et al., 1997; Monopoli et al., 1998a). To rule out such effects, we determined cortical and striatal levels of SDH inhibition after 3NP treatment in the presence of MSX-3. In the outer cortical layers, where there is no neuronal death in this model (Ouary et al., 2000; Blum et al., 2002a), 3NP similarly alters SDH activity in vehicle- (−55.5 ± 1.71%; p = 0.0001 vs sham group; Newman–Keuls *post hoc* test) and MSX3-treated rats (1 mg/kg, −54.3 ± 1.89%; p = 0.82; 5 mg/kg, −58.9 ± 1.41; p = 0.49 vs 3NP/vehicle group; Newman–Keuls *post hoc* test). 3NP also greatly decreased SDH activity in the striatum (−85.6 ± 1.4%; p = 0.00017 vs sham group; Newman–Keuls *post hoc* test). This reduction was similar in the 3NP/MSX-3 (1 mg/kg) (−80.2 ± 1.63%; p = 0.085 vs 3NP/vehicle group; Newman–Keuls *post hoc* test) and the 3NP/MSX-3 (5 mg/kg) groups (−88.5 ± 1.14%; p = 0.44 vs 3NP/vehicle group; Newman–Keuls *post hoc* test). Treatment with MSX-3 alone did not modify basal SDH activity (data not shown). Finally, binding autoradiography experiments demonstrated that the antagonist, in the absence of 3NP, did not modify the apparent striatal density of A_{2A} receptor binding sites [MSX-3 (1 mg/kg), 91.9 ± 5.1% of the control; NS vs sham group; MSX3 (5 mg/kg), 106.7 ± 5.7% of the control; NS vs sham group; Newman–Keuls *post hoc* test].

In a second experiment, we determined the effects of the agonist CGS21680 at the doses of 0.3 and 1 mg/kg. As expected, CGS21680 (1 mg/kg) decreased spontaneous locomotion by 42.6 ± 10.9% 2 hr after injection (p = 0.021 vs sham group; unpaired Student's t test). As shown in Table 2 and Figure 4*B*, CGS21680 increased the volume of the striatal lesion induced by 3NP. This increase was found to be significant at the dose of 1 mg/kg (+64.6 ± 26.8%; p = 0.043). It is noteworthy that this phenomenon was more pronounced within the anterior part of the striatum (+100.0 ± 35.2%; p = 0.016) compared with the posterior part (+42.9 ± 23.6%; NS). The lesional outcome was not attributable to an effect of CGS21680 on the basal SDH activity or on the ability of the neurotoxin to inhibit SDH activity (data not shown). Moreover, the agonist, in the absence of 3NP, did not modify the apparent striatal density of A_{2A} receptor binding sites [CGS21660 (0.3 mg/kg), 92.1 ± 7.4% of the control; NS vs sham group; CGS21680 (1 mg/kg), 99.9 ± 2.5% of the control; NS vs sham group; Newman–Keuls *post hoc* test].

Anteroposterior density and expression of A_{2A} receptors

Given the differential anteroposterior effects provided by both A_{2A} receptor agonist and antagonist and the results obtained in $A_{2A}{}^{-/-}$ mice (9 A.M./11 A.M. severe protocol), we determined whether the apparent density of binding sites and the expression of the receptor could be different at different rostrocaudal planes of the striatum. Binding experiments were performed on five coronal planes of the striatum (+1.1 to −0.8 mm from bregma) separated by 480 μm from five Lewis rats. The apparent density of A_{2A} binding sites decreased at the two last caudal planes compared with the first rostral plane (Fig. 5). Conversely, *in situ* hybridization, performed on adjacent sections, revealed that the level of A_{2A} receptor mRNA did not change along the rostrocaudal axis of the striatum (Fig. 5). These results thus suggest the existence of a decreased density of A_{2A} presynaptic sites within the caudal striatum.

Ability of A_{2A} blockade to inhibit evoked striatal glutamate release in the anterior and posterior striatum

To functionally assess the latter findings, we compared the effects of A_{2A} receptor blockade toward QA-evoked glutamate outflow in the anterior and the posterior striatum (+1 and −0.8 mm from bregma). Basal glutamate levels were similar at the two locations (2.56 ± 0.14 and 2.41 ± 0.56 μM at bregma −0.8 and +1.0, respectively). The administration of SCH 58261 (0.01 mg/kg, i.p.) decreased basal glutamate levels in the anterior (Fig. 6*A*)

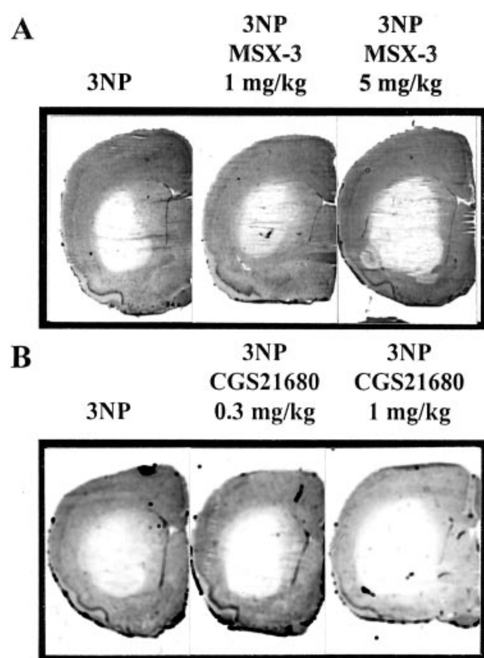

Figure 4. Typical hematoxylin staining of 3NP-treated rats treated with either MSX-3 (*A*) or CGS21680 (*B*).

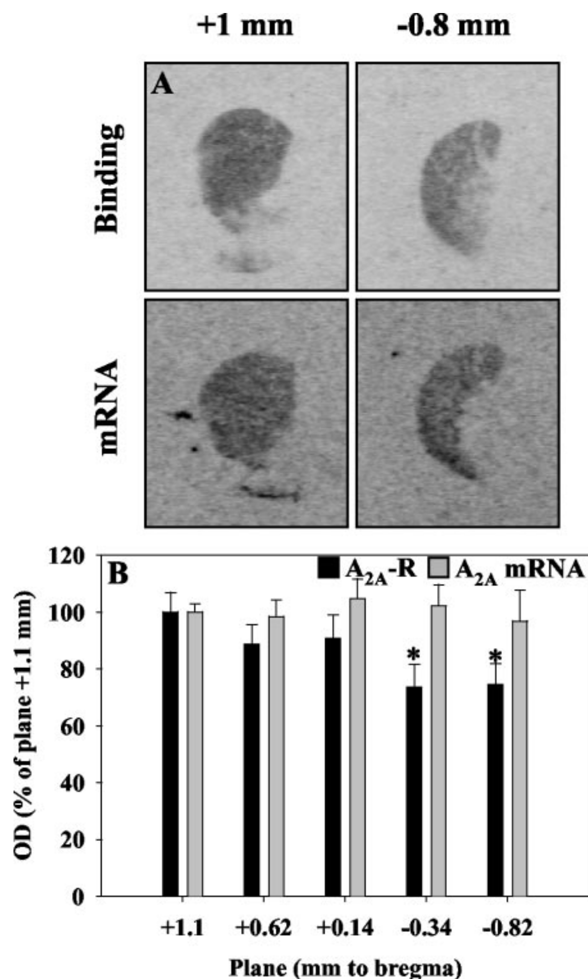

Figure 5. Anteroposterior density and mRNA expression of A_{2A} receptor in the striatum of naive rats. *A*, Typical receptor binding (top) and *in situ* hybridization (bottom) observed at planes +1.1 and −0.8 mm from bregma. *B*, Quantification of these data from five animals. *$p = 0.03$ versus value at plane +1.1 mm, Student's *t* test.

but not in the posterior striatum (*B*). Interestingly, although SCH 58261 fully prevented the effects of QA perfusion at the rostral coordinate (Fig. 6*A*), it did not at all influence the glutamate outflow evoked by QA perfusion in the caudal striatum (*B*).

Although a fraction of A_{2A} receptors are thought to be located presynaptically in the striatum (Corsi et al., 2000; Hettinger et al., 2001; Marchi et al., 2002; Popoli et al., 2002; the present results), they are mostly located postsynaptically on striatal efferent neurons (Schiffmann and Vanderhaeghen, 1993), and in specific conditions, they may also be present on activated inflammatory cells (Mayne et al., 2001).

Because the former population of neuronal A_{2A} receptors could also play a role in the susceptibility to 3NP, we attempted to determine whether the activation of A_{2A} receptors would modulate the toxic effect of 3NP on primary cultured striatal cells.

Activation of A_{2A} receptor-dependent transduction pathway protects against 3NP-induced striatal cell death *in vitro*

Although A_{2A} receptor mRNA is expressed in our primary striatal cells as assessed by RT-PCR (data not shown), stimulation with up to 1 μM CGS21680 failed to activate protein kinase A (PKA) activity (data not shown) as shown previously (Hillion et al., 2002). We therefore directly activated the A_{2A} receptor-related PKA transduction pathway using 100 μM forskolin. In our culture conditions, 100 μM 3NP led to 44.4 ± 5.1% of striatal cell death ($p < 0.001$ vs control; Newman–Keuls *post hoc* test). Cotreatment with forskolin provided an almost complete protection against 3NP toxicity (4.14 ± 2.8% of cell death; NS vs control). This effect was not observed in the presence of the inactive forskolin analog 1,9-dideoxyforskolin (data not shown). These results suggest that, *in vivo*, a postsynaptic activation of the A_{2A}-related transduction pathway could protect striatal cells against 3NP-induced neurotoxicity.

Modulation of inflammatory processes by A_{2A} receptors

Because CGS21680 has been described *in vivo* as a potential antiinflammatory compound (Cassada et al., 2001; Mayne et al., 2001; Fozard et al., 2002) and A_{2A} receptor invalidation has been described as producing increased liver inflammation (Ohta and Sitkovsky, 2001), we checked whether, in our experimental conditions and despite its deleterious effect, this agonist could downregulate potential inflammatory processes induced by 3NP. Although no activated— but only resting—microglial cells could be detected by immunohistochemistry in the striatum of both 3NP- and 3NP/CGS21680-treated rats (data not shown), semiquantitative RT-PCR showed that MAC-1 expression was significantly increased in 3NP animals ($n = 3$; 411.6 ± 12.6% of control; $p < 0.01$ vs sham; Newman–Keuls *post hoc* test) compared with sham animals ($n = 3$), suggesting a preparatory inflammatory reaction. CGS21680, in conditions leading to increased striatal lesion (1 mg/kg; $n = 3$), did not change the increased MAC-1 mRNA expression induced by 3NP (422.9 ± 107.2% of control; $p < 0.01$ vs sham and NS vs 3NP-treated animals; Newman–Keuls *post hoc* test). This suggests that, under our experimental conditions, inflammatory mechanisms are not involved in the effects of 3NP or of A_{2A} receptor ligands.

Discussion

Our results demonstrate that A_{2A} receptors exhibit a dual effect on the 3NP-induced striatal neuronal death that is qualitatively similar to the dual neuroprotective–neurotoxic action of NMDA

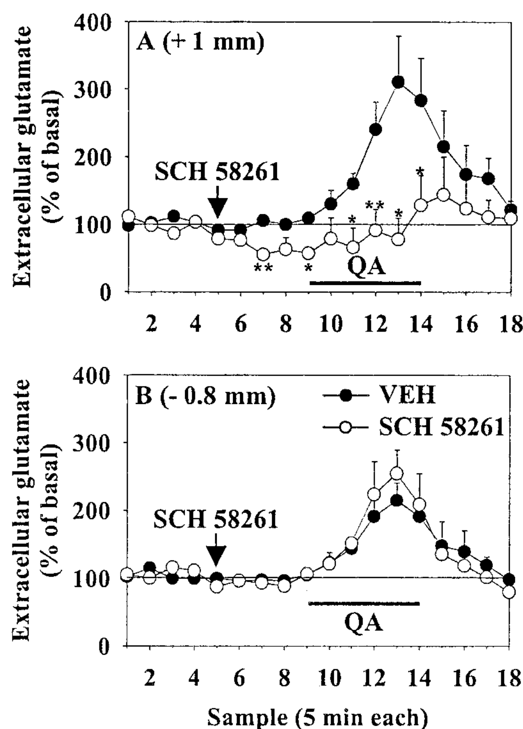

Figure 6. Influence of SCH 58261 on the basal and QA-evoked glutamate outflow in the rat anterior (*A*) or posterior (*B*) striatum. The bar indicates the period of QA perfusion through the probe. The time of injection of SCH 58261 or vehicle (VEH) is indicated by the arrow. Each experimental group was made up of four to six animals. $^{*}p < 0.05$; $^{**}p < 0.01$ versus group not treated with SCH 58261. The horizontal line indicates a glutamate basal level of 100%.

antagonists in rat models of 3NP intoxication (Ikonomidou et al., 2000).

Under severe 3NP intoxication that leads to rapid lethality and to striatal lesions in surviving wild-type mice, A_{2A} inactivation provides a significant striatal protection. Consistent results were found in the rat model, because the agonist CGS21680 potentiated 3NP-induced neurotoxicity. These findings are in agreement with previous *in vivo* studies reporting neuroprotection after either A_{2A} gene inactivation or pharmacological A_{2A} blockade in various models of neuronal cell death (Jones et al., 1998; Chen et al., 1999; Popoli et al., 2002; Blum et al., 2003). In these conditions, protective effects of A_{2A} blockade have been suggested to arise from a presynaptic inhibition of glutamate release. Indeed, in the striatum, besides their main postsynaptic localization on the medium-sized GABAergic projecting neurons (Schiffmann and Vanderhaeghen, 1993), A_{2A} receptors have also been morphologically and functionally localized on terminals of glutamatergic corticostriatal neurons (Popoli et al., 1995; Hettinger et al., 2001; Popoli et al., 2002; present results). Although 3NP, unlike quinolinic acid, is unable to evoke glutamate release in the striatum (Beal et al., 1993; Sanchez-Carbente and Massieu, 1999), inhibition of glutamate release decreased the severity of 3NP-induced striatal lesions through a reduction in the secondary excitotoxicity (Beal et al., 1993; Guyot et al., 1997; Blum et al., 2002b). Therefore, similarly to A_1 receptor activation (Blum et al., 2002b), genetic or pharmacological A_{2A} inhibition provides neuroprotection by inhibiting glutamate release within the striatum.

Conversely, in mice under a less severe 3NP intoxication (9 A.M./7 P.M. protocol), genetic inactivation of A_{2A} receptor led to the formation of macroscopic striatal lesions, whereas there was an absence of lesions in similarly treated wild-type mice. Because striatal lesions induced by an acute treatment with 3NP involve excitotoxicity (Beal et al., 1993; Brouillet et al., 1999; Ikonomidou et al., 2000), this suggests that, the mild intoxication did not involve glutamate-related secondary excitotoxicity. Similar unexpected results were observed with a high dose of the antagonist MSX-3, because it worsened 3NP-induced lesions rather than afforded protection. These results demonstrate that blockade of non-presynaptic A_{2A} sites leads to deleterious effects on striatal cells when glutamate outflow is poorly implicated or not implicated.

Because a very large proportion of striatal A_{2A} receptors are expressed postsynaptically by striatal efferent neurons (Schiffmann and Vanderhaeghen, 1993), we tested their putative involvement in this effect. Our *in vitro* data on striatal neurons in primary cultures strongly support such a postsynaptic role of A_{2A} receptor, because PKA activation almost completely protects striatal cells against 3NP. This is in agreement with the trophic/beneficial effects provided by A_{2A} receptor activation on neurons *in vitro* (Lee and Chao, 2001; Cheng et al., 2002; Popoli et al., 2002) and with the inhibition by PKA of the 3NP-induced NMDA long-term potentiation in corticostriatal slices (Calabresi et al., 2001). Activation of non-neuronal A_{2A} receptors may also provide antiinflammatory activity (Mayne et al., 2001; Ohta and Sitkovsky, 2001). However, the involvement of such a mechanism was not supported by our data in the rat model.

This postsynaptic hypothesis is reinforced by the effect observed with MSX-3 on rats. Indeed, MSX-3 given at 5 mg/kg provides the maximal effect on locomotion (Karcz-Kubicha et al., 2003), supporting that all of the postsynaptic receptors are blocked. This results in a worsening of the lesion possibly because of an overriding of the presynaptic protection. Given that MSX-3 induces a nonmaximal increase of locomotor activity at 1 mg/kg (~60%) (Karcz-Kubicha et al., 2003), it would not block all of the postsynaptic sites, resulting in a reduced postsynaptic influence and hence in the absence of potentiation of a 3NP-induced deleterious effect.

Together, the present results support that the A_{2A} receptor regulation of striatal susceptibility to 3NP-induced metabolic compromise depends on a balance between presynaptic and postsynaptic sites of action. This hypothesis is reinforced by the anatomical observations that this balance is anatomically determined. Indeed, an A_{2A} antagonist inhibited basal or QA-evoked glutamate release in the anterior striatum (Popoli et al., 2002; present results), whereas it did not exhibit this effect in the posterior striatum. On the basis of these observations, one may expect that the relative contributions of presynaptic and postsynaptic effects under 3NP intoxication would be differentially balanced in the anterior and posterior striatum. This is in complete agreement with our results, because the protection afforded by the A_{2A} receptor gene inactivation in the severe excitotoxic condition or the aggravating effect of CGS21680 were significantly observed only in the anterior striatum. Conversely, the deleterious effect of the higher dose of MSX-3 was greater in the posterior striatum.

In conclusion, our results suggest that modulation of 3NP-induced striatal damage by A_{2A} receptors may lead to opposite outcomes depending on the relative proportion of activated presynaptic and postsynaptic sites, being itself dependent also on the anatomical distribution of the receptors. Therefore, the use of A_{2A} receptor antagonists in the treatment of HD should be considered with caution. Although A_{2A} antagonists are thought to

have a potential therapeutic interest in other neurodegenerative disorders such as Parkinson's disease (Kase, 2001), a biphasic neuroprotective–neurotoxic effect could be expected depending on the dose used and the relative functional importance of presynaptic and postsynaptic A_{2A} receptors in the degenerating brain area.

References

Beal MF (2000) Energetics in the pathogenesis of neurodegenerative diseases. Trends Neurosci 23:298–304.

Beal MF, Brouillet E, Jenkins BG, Ferrante RJ, Kowall NW, Miller JM, Storey E, Srivastava R, Rosen BR, Hyman BT (1993) Neurochemical and histologic characterization of striatal excitotoxic lesions produced by the mitochondrial toxin 3-nitropropionic acid. J Neurosci 13:4181–4192.

Blum D, Gall D, Cuvelier L, Schiffmann SN (2001) Topological analysis of striatal lesions induced by 3-nitropropionic acid in the Lewis rat. NeuroReport 12:1769–1772.

Blum D, Galas MC, Gall D, Cuvelier L, Schiffmann SN (2002a) Striatal and cortical neurochemical changes induced by chronic metabolic compromise in the 3-nitropropionic model of Huntington's disease. Neurobiol Dis 10:410–426.

Blum D, Gall D, Galas MC, d'Alcantara P, Bantubungi K, Schiffmann SN (2002b) The adenosine A_1 receptor agonist adenosine amine congener exerts a neuroprotective effect against the development of striatal lesions and motor impairments in the 3-nitropropionic acid model of neurotoxicity. J Neurosci 22:9122–9133.

Blum D, Hourez R, Galas MC, Popoli P, Schiffmann SN (2003) Adenosine receptors in Huntington's disease. Lancet Neurol 2:366–374.

Brouillet E, Hantraye P, Ferrante RJ, Dolan R, Leroy-Willig A, Kowall NW, Beal MF (1995) Chronic mitochondrial energy impairment produces selective striatal degeneration and abnormal choreiform movements in primates. Proc Natl Acad Sci USA 92:7105–7109.

Brouillet E, Guyot MC, Mittoux V, Altairac S, Conde F, Palfi S, Hantraye P (1998) Partial inhibition of brain succinate dehydrogenase by 3-nitropropionic acid is sufficient to initiate striatal degeneration in rat. J Neurochem 70:794–805.

Brouillet E, Conde F, Beal MF, Hantraye P (1999) Replicating Huntington's disease phenotype in experimental animals. Prog Neurobiol 59:427–468.

Browne SE, Bowling AC, MacGarvey U, Baik MJ, Berger SC, Muqit MM, Bird ED, Beal MF (1997) Oxidative damage and metabolic dysfunction in Huntington's disease: selective vulnerability of the basal ganglia. Ann Neurol 41:646–653.

Calabresi P, Gubellini P, Picconi B, Centonze D, Pisani A, Bonsi P, Greengard P, Hipskind RA, Borrelli E, Bernardi G (2001) Inhibition of mitochondrial complex II induces a long-term potentiation of NMDA-mediated synaptic excitation in the striatum requiring endogenous dopamine. J Neurosci 21:5110–5120.

Cassada DC, Tribble CG, Laubach VE, Nguyen BN, Rieger JM, Linden J, Kaza AK, Long SM, Kron IL, Kern JA (2001) An adenosine A_{2A} agonist, ATL-146e, reduces paralysis and apoptosis during rabbit spinal cord reperfusion. J Vasc Surg 34:482–488.

Cha JH, Frey AS, Alsdorf SA, Kerner JA, Kosinski CM, Mangiarini L, Penney Jr JB, Davies SW, Bates GP, Young AB (1999) Altered neurotransmitter receptor expression in transgenic mouse models of Huntington's disease. Philos Trans R Soc Lond B Biol Sci 354:981–989.

Chen JF, Huang Z, Ma J, Zhu J, Moratalla R, Standaert D, Moskowitz MA, Fink JS, Schwarzschild MA (1999) A_{2A} adenosine receptor deficiency attenuates brain injury induced by transient focal ischemia in mice. J Neurosci 19:9192–9200.

Cheng HC, Shih HM, Chern Y (2002) Essential role of cAMP-response element-binding protein activation by A_{2A} adenosine receptors in rescuing the nerve growth factor-induced neurite outgrowth impaired by blockage of the MAPK cascade. J Biol Chem 277:33930–33942.

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.

Dassesse D, Massie A, Ferrari R, Ledent C, Parmentier M, Arckens L, Zoli M, Schiffmann SN (2001) Functional striatal hypodopaminergic activity in mice lacking adenosine A_{2A} receptors. J Neurochem 78:183–198.

El Massioui N, Ouary S, Cheruel F, Hantraye P, Brouillet E (2001) Perseverative behavior underlying attentional set shifting deficit in rats chronically treated with the neurotoxin 3-nitropropionic acid. Exp Neurol 172:172–181.

Fozard JR, Ellis KM, Villela Dantas MF, Tigani B, Mazzoni L (2002) Effects of CGS 21680, a selective adenosine A_{2A} receptor agonist, on allergic airways inflammation in the rat. Eur J Pharmacol 438:183–188.

Gao Y, Phillis JW (1994) CGS 15943, an adenosine A_2 receptor antagonist, reduces cerebral ischemic injury in the Mongolian gerbil. Life Sci 55:L61–L65.

Glass M, Dragunow M, Faull RL (2000) The pattern of neurodegeneration in Huntington's disease: a comparative study of cannabinoid, dopamine, adenosine and $GABA_A$ receptor alterations in the human basal ganglia in Huntington's disease. Neuroscience 97:505–519.

Guyot MC, Palfi S, Stutzmann JM, Maziere M, Hantraye P, Brouillet E (1997) Riluzole protects from motor deficits and striatal degeneration produced by systemic 3-nitropropionic acid intoxication in rats. Neuroscience 81:141–149.

Hauber W, Neuscheler P, Nagel J, Muller CE (2001) Catalepsy induced by a blockade of dopamine D_1 or D_2 receptors was reversed by a concomitant blockade of adenosine A_{2A} receptors in the caudate-putamen of rats. Eur J Neurosci 14:1287–1293.

Hettinger BD, Lee A, Linden J, Rosin DL (2001) Ultrastructural localization of adenosine A_{2A} receptors suggests multiple cellular sites for modulation of GABAergic neurons in rat striatum. J Comp Neurol 431:331–346.

Hillion J, Canals M, Torvinen M, Casado V, Scott R, Terasmaa A, Hansson A, Watson S, Olah ME, Mallol J, Canela EI, Zoli M, Agnati LF, Ibanez CF, Lluis C, Franco R, Ferre S, Fuxe K (2002) Coaggregation, cointernalization, and codesensitization of adenosine A_{2A} receptors and dopamine D_2 receptors. J Biol Chem 277:18091–18097.

The Huntington's Disease Study Group (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. The Huntington's Disease Collaborative Research Group. Cell 72:971–983.

Ikonomidou C, Stefovska V, Turski L (2000) Neuronal death enhanced by *N*-methyl-D-aspartate antagonists. Proc Natl Acad Sci USA 97:12885–12890.

Jones PA, Smith RA, Stone TW (1998) Protection against hippocampal kainate excitotoxicity by intracerebral administration of an adenosine A_{2A} receptor antagonist. Brain Res 800:328–335.

Karcz-Kubicha M, Antoniou K, Terasmaa A, Quarta D, Solinas M, Justinova Z, Pezzola A, Reggio R, Muller CE, Fuxe K, Goldberg SR, Popoli P, Ferre S (2003) Involvement of adenosine A_1 and A_{2A} receptors in the motor effects of caffeine after its acute and chronic administration. Neuropsychopharmacology, in press.

Kase H (2001) New aspects of physiological and pathophysiological functions of adenosine A_{2A} receptor in basal ganglia. Biosci Biotechnol Biochem 65:1447–1457.

Ledent C, Vaugeois JM, Schiffmann SN, Pedrazzini T, El Yacoubi M, Vanderhaeghen JJ, Costentin J, Heath JK, Vassart G, Parmentier M (1997) Aggressiveness, hypoalgesia and high blood pressure in mice lacking the adenosine A_{2A} receptor. Nature 388:674–678.

Lee FS, Chao MV (2001) Activation of Trk neurotrophin receptors in the absence of neurotrophins. Proc Natl Acad Sci USA 98:3555–3560.

Marchi M, Raiteri L, Risso F, Vallarino A, Bonfanti A, Monopoli A, Ongini E, Raiteri M (2002) Effects of adenosine A_1 and A_{2A} receptor activation on the evoked release of glutamate from rat cerebrocortical synaptosomes. Br J Pharmacol 136:434–440.

Mayne M, Fotheringham J, Yan HJ, Power C, Del Bigio MR, Peeling J, Geiger JD (2001) Adenosine A_{2A} receptor activation reduces proinflammatory events and decreases cell death following intracerebral hemorrhage. Ann Neurol 49:727–735.

Monopoli A, Casati C, Lozza G, Forlani A, Ongini E (1998a) Cardiovascular pharmacology of the A_{2A} adenosine receptor antagonist, SCH 58261, in the rat. J Pharmacol Exp Ther 285:9–15.

Monopoli A, Lozza G, Forlani A, Mattavelli A, Ongini E (1998b) Blockade of adenosine A_{2A} receptors by SCH 58261 results in neuroprotective effects in cerebral ischaemia in rats. NeuroReport 9:3955–3959.

Ohta A, Sitkovsky M (2001) Role of G-protein-coupled adenosine receptors in downregulation of inflammation and protection from tissue damage. Nature 414:916–920.

Ouary S, Bizat N, Altairac S, Menetrat H, Mittoux V, Conde F, Hantraye P, Brouillet E (2000) Major strain differences in response to chronic systemic administration of the mitochondrial toxin 3-nitropropionic acid in rats: implications for neuroprotection studies. Neuroscience 97:521–530.

Panov AV, Gutekunst CA, Leavitt BR, Hayden MR, Burke JR, Strittmatter WJ, Greenamyre JT (2002) Early mitochondrial calcium defects in Huntington's disease are a direct effect of polyglutamines. Nat Neurosci 5:731–736.

Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates. Orlando, FL: Academic.

Popoli P, Betto P, Reggio R, Ricciarello G (1995) Adenosine A_{2A} receptor stimulation enhances striatal extracellular glutamate levels in rats. Eur J Pharmacol 287:215–217.

Popoli P, Pintor A, Domenici MR, Frank C, Tebano MT, Pezzola A, Scarchilli L, Quarta D, Reggio R, Malchiodi-Albedi F, Falchi M, Massotti M (2002) Blockade of striatal adenosine A_{2A} receptor reduces, through a presynaptic mechanism, quinolinic acid-induced excitotoxicity: possible relevance to neuroprotective interventions in neurodegenerative diseases of the striatum. J Neurosci 22:1967–1975.

Reggio R, Pezzola A, Popoli P (1999) The intrastriatal injection of an adenosine A_2 receptor antagonist prevents frontal cortex EEG abnormalities in a rat model of Huntington's disease. Brain Res 831:315–318.

Rimondini R, Ferre S, Ogren SO, Fuxe K (1997) Adenosine A_{2A} agonists: a potential new type of atypical antipsychotic. Neuropsychopharmacology 17:82–91.

Sanchez-Carbente MR, Massieu L (1999) Transient inhibition of glutamate uptake *in vivo* induces neurodegeneration when energy metabolism is impaired. J Neurochem 72:129–138.

Sawa A, Wiegand GW, Cooper J, Margolis RL, Sharp AH, Lawler Jr JF, Greenamyre JT, Snyder SH, Ross CA (1999) Increased apoptosis of Huntington disease lymphoblasts associated with repeat length-dependent mitochondrial depolarization. Nat Med 5:1194–1198.

Schiffmann SN, Vanderhaeghen JJ (1993) Adenosine A_2 receptors regulate the gene expression of striatopallidal and striatonigral neurons. J Neurosci 13:1080–1087.

Svenningsson P, Le Moine C, Fisone G, Fredholm BB (1999) Distribution, biochemistry and function of striatal adenosine A_{2A} receptors. Prog Neurobiol 59:355–396.

Tabrizi SJ, Cleeter MW, Xuereb J, Taanman JW, Cooper JM, Schapira AH (1999) Biochemical abnormalities and excitotoxicity in Huntington's disease brain. Ann Neurol 45:25–32.

Varani K, Rigamonti D, Sipione S, Camurri A, Borea PA, Cattabeni F, Abbracchio MP, Cattaneo E (2001) Aberrant amplification of A_{2A} receptor signaling in striatal cells expressing mutant huntingtin. FASEB J 15:1245–1247.

Wu DC, Jackson-Lewis V, Vila M, Tieu K, Teismann P, Vadseth C, Choi DK, Ischiropoulos H, Przedborski S (2002) Blockade of microglial activation is neuroprotective in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson disease. J Neurosci 22:1763–1771.