Neuropharmacology xxx (2008) 1–8

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Functional interaction between adenosine A2A and group III metabotropic glutamate receptors to reduce parkinsonian symptoms in rats

Sebastien Lopez [a], Nathalie Turle-Lorenzo [a], Tom H. Johnston [b], Jonathan M. Brotchie [b], Stephan Schann [c], Pascal Neuville [c], Marianne Amalric [a,*]

[a] Laboratoire de Neurobiologie de la Cognition, CNRS UMR 6155, Aix-Marseille Université, Case C, 3 Place Victor Hugo, 13331 Marseille Cedex 3, Marseille, France
[b] Toronto Western Research Institute, Toronto Western Hospital, University Health Network, Toronto, Canada
[c] Faust Pharmaceuticals, BIOPARC, Boulevard Sebastien Brant, 67400 Illkirch, France

ARTICLE INFO

Article history:
Received 23 March 2008
Received in revised form 19 June 2008
Accepted 20 June 2008
Available online xxx

Keywords:
Metabotropic glutamate receptor
Adenosine A2A receptor
Parkinson's disease
Haloperidol
Catalepsy
6-OHDA
Basal ganglia
Rats

ABSTRACT

Non-dopaminergic drugs acting either on adenosine A2A or metabotropic glutamate (mGlu) receptors reduce motor impairment in animal models of Parkinson's disease (PD), suggesting a possible functional interaction between these receptors to regulate basal ganglia function. The present study therefore tested the behavioural effects of compounds acting selectively on A2A or on specific mGlu receptor subtypes, alone or in combination, in rodent models of PD. Acute administration of the adenosine A2A receptor antagonists CSC or MSX-3 at the highest doses tested (5 and 1.25 mg/kg, respectively) significantly reduces haloperidol-induced catalepsy. Furthermore, the anticataleptic effect of MSX-3 was enhanced by a 3-week treatment. Acute administration of the selective group III mGlu agonist ACPT-I produces potent anticataleptic effects and prolongs time on rotarod of 6-OHDA-lesioned rats. In contrast, acute or chronic administration of MPEP (mGlu5 receptor antagonist) has no anticataleptic action. Furthermore, the acute co-administration of ACPT-I 1 mg/kg, but not 5 mg/kg, with CSC markedly reduces catalepsy. Opposite effects are observed after a 3-week co-administration. The co-administration of ACPT-I with MSX-3 has anticataleptic effects both after acute or chronic treatment. In contrast, acute combination of subthreshold doses of CSC and MPEP has no effect. After a 3-week treatment, however, the combination of CSC and MPEP was found to reduce haloperidol-induced catalepsy. Altogether, these results show for the first time that systemic activation of group III mGlu receptors with ACPT-I provides benefits in parkinsonian rats and underlie a possible interaction with A2A receptors to regulate basal ganglia motor function.

1. Introduction

The altered balance between dopamine and glutamate transmission has been proposed to underlie the pathophysiology of Parkinson's disease (PD) (Albin et al., 1989). Because of their modulatory action on glutamate neurotransmission, the metabotropic glutamate (mGlu) receptors recently received much attention as potential targets in the treatment of PD (Conn et al., 2005). Based on primary sequence, second messenger coupling and pharmacological profiles, mGlu receptors are classified into three subgroups: group I (mGlu1, 5), group II (mGlu2, 3) and group III (mGlu4, 6, 7 and 8) (Pin and Duvoisin, 1995). In animal models of PD, blockade of group I or activation of group II mGlu receptors produces beneficial effects on parkinsonian symptoms by modulating the altered activity in the basal ganglia (BG) circuitry (Conn et al., 2005; Kearney and Albin, 2003). Since no group III mGlu receptor ligands were known to cross the blood–brain barrier, intracerebroventricular or intracerebral injections of group III mGlu receptor agonists have been used to assess their possible antiakinetic properties in various models of PD in rodents (Lopez et al., 2007; Marino et al., 2003; Valenti et al., 2003). One recent study, however, demonstrated that systemic administration of the mGlu4 receptor positive allosteric modulator PHCCC reduces reserpine-induced akinesia and exerts neuroprotection against MPTP-induced toxicity (Battaglia et al., 2006).

In line with this alternative antiparkinsonian strategy that bypasses the dopaminergic system, the blockade of adenosine A2A

Abbreviations: mGlu receptor, metabotropic glutamate receptor; 6-OHDA, 6-hydroxydopamine; ACPT-I, (1*S*,3*R*,4*S*)-1-aminocyclopentane-1,3,4-tricarboxylic acid; CSC, 1,3,7-trimethyl-8-(3-chlorostyryl)caffeine; MSX-3, 3,7-dihydro-8-[(1*E*)-2-(3-methoxyphenyl)ethenyl]-7-methyl-3-[3-(phosphonooxy)propyl-1-(2-propynyl)-1*H*-purine-2,6-dione disodium salt hydrate; MPEP, 2-methyl-6-(phenylethynyl) pyridine; PD, Parkinson's disease; DA, dopamine; BG, basal ganglia; GP, globus pallidus; SNr, substantia nigra pars reticulata.

* Corresponding author. Tel.: +33 4 88 57 68 73; fax: +33 4 88 57 68 72.
E-mail address: marianne.amalric@univ-provence.fr (M. Amalric).

0028-3908/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuropharm.2008.06.038

receptor subtypes, whose expression is largely restricted to the striatum, has been recently highlighted (Schwarzschild et al., 2006; Xu et al., 2005). Indeed, A2A receptors are coexpressed with dopamine D2 receptors on efferent striatopallidal GABAergic neurons, and functionally oppose the actions of dopamine on these receptors (Ferre et al., 1997; Schwarzschild et al., 2006). These findings suggest that A2A antagonists may improve parkinsonian motor symptoms by their direct inhibitory influence on striatopallidal GABAergic neurons. Recent findings support the presence of heteromeric complexes containing A2A and mGlu5 receptors in striatal output neurons and functional synergism between the two receptors (Ferre et al., 2002). In line with these results, behavioural studies demonstrated that blockade of A2A and mGlu5 receptors interacts synergistically at behavioural level to reduce akinetic symptoms in rat models of PD (Coccurello et al., 2004; Kachroo et al., 2005).

To date, interactions between A2A and mGlu receptors have only been demonstrated with mGlu5 subtypes. Considering the striking similar localization of A2A and group III mGlu receptors at the level of striatopallidal GABAergic neurons (Bradley et al., 1999), and the fact that A2A antagonists, as well as group III mGlu receptor agonists, have been shown to reduce striatopallidal GABAergic activity in vitro (Shindou et al., 2003; Valenti et al., 2003), we ask whether a possible interaction could occur between these two groups of receptors in vivo. The aim of the present study was thus to test the hypothesis of a possible interaction between A2A and group III mGlu receptors to reduce parkinsonian symptoms and to compare it to A2A and mGlu5 receptors' interaction in the same PD model.

2. Materials and methods

2.1. Experiment 1: haloperidol-induced catalepsy

2.1.1. Animals

Male Wistar rats ($n = 206$, Charles River, L'Arbresle, France), weighing 315–330 g at the beginning of the experiment, were housed in groups of two per cage and maintained in temperature-controlled conditions with a 12 h light/dark cycle (7:00 a.m.–7:00 p.m., lights off). Water and food were provided ad libitum. All procedures were conducted in accordance with the requirements of the French "Ministère de l'Agriculture et de la Pêche" Décret no. 87-848, October 19, 1987 and to the European Communities council directive of November 24th, 1986 (86/609/EEC).

2.1.2. Drugs

The mixed D1/D2 dopaminergic receptor antagonist haloperidol (Haldol injectable solution 5 mg/ml; Janssen, Boulogne, France) was dissolved in physiological 0.9% saline solution and injected systemically at a dose of 1 mg/kg. The selective group III metabotropic glutamate receptor agonist (1*S*,3*R*,4*S*)-1-aminocyclopentane-1,3,4-tricarboxylic acid (ACPT-I; Acher et al., 1997; Goudet et al., 2007) was freshly dissolved in a solution of Tween 80 (1–2%) and NaCl 0.9%. The ACPT-I solution was adjusted to a pH of 6.5–7.5 with NaOH 0.1 N. The mGluR5 antagonist 2-methyl-6-(phenylethynyl)pyridine hydrochloride (MPEP; a generous gift of F. Gasparini, Novartis, Basel, Switzerland) was dissolved in distilled water. The A2A receptor antagonist 1,3,7-trimethyl-8-(3-chlorostyryl)caffeine (CSC) was suspended in a solution of methylcellulose (0.3%) in distilled water. The selective A2A receptor antagonist 3,7-dihydro-8-[(1*E*)-2-(3-methoxyphenyl)ethenyl]-7-methyl-3-[3-(phosphonooxy)propyl-1-(2-propynyl)-1*H*-purine-2,6-dione disodium salt hydrate (MSX-3) (Sigma–Aldrich, France) was dissolved in distilled water (pH = 7). All compounds were injected intraperitoneally (i.p.) in a volume of 1 ml/kg.

2.1.3. Experimental procedure: catalepsy test

The different compounds and their respective vehicle were administered daily from d1 to d21. All groups received haloperidol (1 mg/kg, i.p.) only on the test day (d1 for the acute and d21 for the chronic test). On the basis of the results obtained in the acute experiment, we have chosen the lowest concentration of the different compounds used, and tested the hypothesis that 3-week treatment (daily injections) could produce a beneficial effect on the long term.

On the test day, catalepsy was measured 60 min after haloperidol injection, and every 30 min during the 2-h testing. For each measure of cataleptic state, animals were gently placed with their forepaws on a metal rod suspended 9 cm above the floor and the time elapsing before they climbed down from the bar was recorded in seconds (with a cutoff time of 120 s).

Concentrations and timing of injection of CSC, MSX-3 and MPEP were chosen on the basis of recent results showing that systemic injections of these compounds alleviate parkinsonian motor deficits in rats (Breysse et al., 2002; Coccurello et al., 2004; Ishiwari et al., 2007). CSC and MSX-3 were administered 50 and 20 min before the catalepsy test, respectively. MPEP was injected 40 min before the test. Doses and route of administration of ACPT-I were chosen according to previous results showing that 25 mg/kg has anxiolytic properties in rats (data not shown). ACPT-I was administered 90 min before the catalepsy test according to blood–brain barrier penetration data showing that peak micromolar concentrations are found in the brain 1–2 h after drug administration (Supplemental Fig. 1).

2.1.3.1. Single treatment. In this experiment, four groups of animals were tested on haloperidol-induced catalepsy after single systemic treatment with either the A2A receptor antagonist CSC or mGlu receptor ligands (mGlu5 antagonist MPEP and group III mGlu receptor agonist ACPT-I). To confirm the results obtained with CSC, additional experiment was conducted to assess the effects of acute and chronic treatment with another A2A receptor antagonist, MSX-3. Each subject was tested only once at a given concentration.

2.1.3.1.1. Single treatment with A2A receptor antagonists. Acute. On d1, animals received i.p. injections of various doses of CSC (0, 0.625, 1.25, and 5 mg/kg, $n = 6$ per group) or MSX-3 (0, 0.625, and 1.25 mg/kg, $n = 6$ per group) and were tested on haloperidol-induced catalepsy. An additional group received vehicle solutions of haloperidol and the respective vehicle solution of each compound ($n = 7$ each).

Chronic. Animals which received low doses of CSC (0.625 and 1.25 mg/kg, $n = 6$ per group) or MSX-3 (0.625 and 1.25 mg/kg, $n = 5$ and 6, respectively) were then treated chronically for 3 weeks and catalepsy was tested again on d21.

2.1.3.1.2. Single treatment with group III mGlu receptor agonist or mGlu5 receptor antagonist. Acute. On d1, animals received i.p. injections of ACPT-I (0, 1, 5, 15 or 25 mg/kg, $n = 6$–7 per group) or MPEP (0, $n = 6$; 1.5, 3 or 6 mg/kg, $n = 7$ per group) and were tested on haloperidol-induced catalepsy. The control groups received vehicle solution of haloperidol and the respective vehicle solution of each compound ($n = 7$ each).

Chronic. Rats which received low doses of ACPT-I (1 and 5 mg/kg, $n = 6$ per group) or MPEP 1.5 mg/kg ($n = 6$) were then treated for 3 weeks and catalepsy was tested again on d21.

2.1.3.2. Combined treatment

2.1.3.2.1. Combined administration of adenosine A2A receptor antagonists CSC or MSX-3 with group III mGlu receptor agonist ACPT-I. CSC + ACPT-I. Rats were divided into three subgroups (CSC 0.625 + ACPT-I 1, $n = 5$; CSC 1.25 + ACPT-I 1, $n = 6$; CSC 0.625 + ACPT-I 5, $n = 6$). The compounds and their respective vehicle were administered daily from d1 to d21. The effects of the different treatments in the catalepsy test were assessed on d1 (acute test) and d21 (chronic test) against their respective control (vehicle only, vehicle with haloperidol 1 mg/kg).

MSX-3 + ACPT-I. To confirm the results obtained with CSC, we conducted an additional experiment with another selective A2A receptor antagonist, MSX-3. The experiment was designed in the same manner as above with two groups (MSX-3 0.625 + ACPT-I 1, $n = 7$; MSX-3 1.25 + ACPT-I 1, $n = 6$).

2.1.3.2.2. Combined administration of the adenosine A2A receptor antagonist CSC with the mGlu5 receptor antagonist MPEP. Rats were divided into two groups depending on the dose of CSC tested (CSC 0.625 + MPEP 1.5, $n = 6$; CSC 1.25 + MPEP 1.5, $n = 6$). The compounds and their respective vehicle were administered daily from d1 to d21. In addition, another group of rats received vehicle of CSC + vehicle of MPEP solutions from d1 to d21. These control animals received haloperidol ($n = 11$) or its vehicle ($n = 7$) on the test days.

2.2. Experiment 2: rotarod performance in 6-hydroxydopamine-lesioned rats

2.2.1. Animals

Male Sprague–Dawley rats ($n = 24$, Charles River, Canada), weighing 320–350 g at the time of surgery, were housed in groups of two per cage and maintained in temperature-controlled conditions with a 12 h light/dark cycle (7:00 a.m.–7:00 p.m., lights off). Water and food were provided ad libitum. The studies were conducted according to guidelines set by the Canadian Council on Animal Care (CCAC).

2.2.2. 6-Hydroxydopamine lesioning

Animals were allowed a 2-week period of habituation between delivery and commencement of surgery. Thirty minutes prior to surgery animals were administered pargyline (5 mg/kg, i.p.) and desipramine (25 mg/kg, i.p.). Under isofluorane anaesthesia, rats were placed in a stereotaxic frame (Kopf, USA). A burr hole was drilled in the skull above the right median forebrain bundle (2.8 mm posterior, 2 mm lateral to bregma, according to the atlas of Paxinos and Watson, 1986). A 28 G Hamilton needle was then lowered 9 mm below the skull and injection (1 μl/min) of 6-hydroxydopamine hydrobromide (Sigma–Aldrich; 12.5 μg in 2.5 μl of sterile 0.9% saline containing 0.02% ascorbate) or vehicle was made. The needle was then left in place for a further 4 min. Animals were left untreated for 3 weeks to recover and for development and stabilization of the lesion.

Please cite this article in press as: Lopez, S., et al., Functional interaction between adenosine A2A and group III metabotropic glutamate receptors to reduce parkinsonian symptoms in rats, Neuropharmacology (2008), doi:10.1016/j.neuropharm.2008.06.038

2.2.3. Rotarod assessment

Performance on the accelerating rotarod was assessed using a four-station rat rotarod (MedAssociates, USA). The speed of rotation of the rotarod was increased from 2.5 to 25 rpm over 5 min and the time for which the animal remained on the rod was determined as the mean of three trials.

A total of three treatments were given, in a randomised way, using an incomplete Latin Square-type design. ACPT-I (10 and 30 mg/kg, i.p.), or vehicle, was administered 2 h prior to behavioural testing. All lesioned animals received all treatments ($n = 12$). A minimum of 48 h was left between treatments in the same animals. Rotarod performance was assessed 2 h post-drug administration. Sham-operated animals ($n = 12$) received vehicle and were tested following the same procedure.

2.2.4. Assessment of extent of 6-OHDA lesion

After completion of all behavioural assessments, animals were killed via carbon dioxide overdose, brains removed and frozen, and cryostat-cut striatal sections (20 μm) prepared. To define the extent of 6-OHDA lesion, the levels of striatal dopamine transporter (DAT) sites were measured using [^{125}I]RTI-121 autoradiography (2200 Ci/mmol; Perkin–Elmer Life Sciences, Boston, MA), as previously described (Quik et al., 2003). Densitometric analysis of autoradiograms was carried out using MCID software (Image Research Inc., Ontario, Canada). Non-specific binding was found to account for <1% of total. Lesioned and non-lesioned sides of three striata from each animal were analyzed. All 6-OHDA-lesioned animals included in the analysis had greater than 95% reduction in striatal DAT (96.0 ± 0.6% decrease in binding, cf. intact side).

2.3. Data and statistical analysis

Catalepsy data were analyzed by using a multiple Kruskal–Wallis "*H*" test. The mean latency was calculated for each dose and for each 30-min period. Individual comparisons were performed using the nonparametric Mann–Whitney *U* test. Rotarod data were analyzed using a repeated-measures one-way ANOVA with a Tukey's post hoc test.

3. Results

3.1. Experiment 1: haloperidol-induced catalepsy

3.1.1. A2A receptor antagonists

Haloperidol (1 mg/kg, i.p) produced a profound cataleptic state as shown by an increase in median latency to step down the rod compared with controls ($p < 0.01$, Mann–Whitney *U* test after a significant Kruskal–Wallis test; $p < 0.01$, $H = 88.05$ and 84.12 in the CSC and MSX-3 groups, respectively) (Fig. 1A). Acute administration of CSC significantly antagonized haloperidol-induced catalepsy at the highest concentration only (5 mg/kg) ($p < 0.05$, Mann–Whitney *U* test) (Fig. 1A). Similarly, acute administration of MSX-3, another A2A receptor antagonist, also counteracted the effects of haloperidol at a concentration of 1.25 mg/kg ($p < 0.01$, Mann–Whitney *U* test) (Fig. 1A). CSC and MSX-3 at lowest concentrations are ineffective after acute administration. After a chronic 3-week treatment, selected doses of CSC (0.625 and 1.25 mg/kg), that were ineffective in the acute test, failed to reverse haloperidol-induced catalepsy (Fig. 1B). In contrast, chronic administration of MSX-3 0.625 mg/kg, ineffective acutely, was found to significantly antagonize the effects of haloperidol ($p < 0.01$, Mann–Whitney *U* test after a significant Kruskal–Wallis test, $p < 0.01$, $H = 103.25$) (Fig. 1B). MSX-3 1.25 mg/kg, effective after acute administration, produced a strong anticataleptic effect after chronic treatment ($p < 0.01$, Mann–Whitney *U* test).

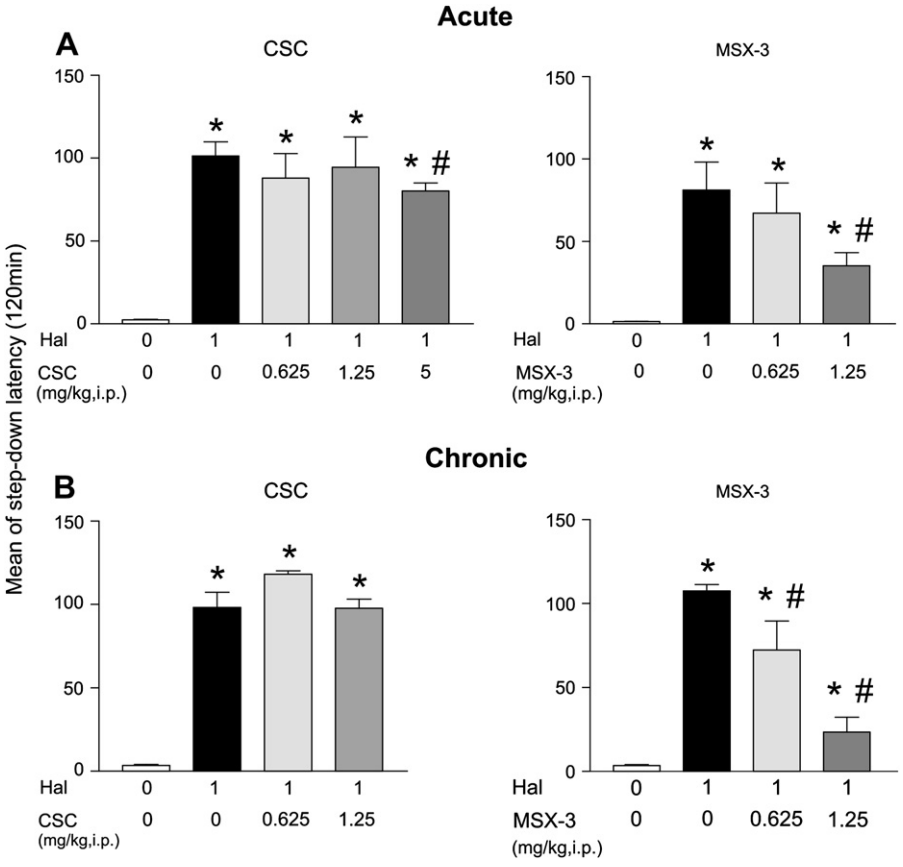

Fig. 1. Effects of acute (A) and chronic (B) administration of the A2A receptor antagonist CSC and MSX-3 on haloperidol-induced catalepsy. (A) On d1, the animals were tested 60 min after haloperidol injection (1 mg/kg), and every 30 min for the 2-h testing. CSC (0, 0.625, 1.25, and 5 mg/kg, i.p.; $n = 6$ per group) was injected i.p. 10 min after haloperidol administration. MSX-3 (0, 0.625, and 1.25 mg/kg, i.p.; $n = 5$–6 per group) was injected i.p. 40 min after haloperidol. Low doses of each compound tested in the acute experiment were selected to test their effect after a chronic treatment (3 weeks of daily injections). (B) Effects of chronic treatment with CSC (0, 0.625 and 1.25 mg/kg, i.p.; $n = 6$ per group) or MSX-3 (0, 0.625 and 1.25 mg/kg, i.p.; $n = 5$–6 per group) on haloperidol-induced catalepsy. Data are expressed as mean latency ± SEM to step down a rod during the total duration of the test. *Significantly different from control group ($p < 0.05$; significant Mann–Whitney *U* test). #Significantly different from haloperidol group ($p < 0.05$; significant Mann–Whitney *U* test).

Please cite this article in press as: Lopez, S., et al., Functional interaction between adenosine A2A and group III metabotropic glutamate receptors to reduce parkinsonian symptoms in rats, Neuropharmacology (2008), doi:10.1016/j.neuropharm.2008.06.038

Furthermore, the anticataleptic effects produced by acute administration of MSX-3 1.25 mg/kg were enhanced after chronic administration (inhibition of haloperidol-induced catalepsy by 56% and 78% for the acute and chronic treatment, respectively). The anticataleptic effects of chronic MSX-3 were significantly higher at 1.25 mg/kg than 0.625 mg/kg ($p < 0.01$, Mann–Whitney U test).

3.1.2. Group III mGlu receptor agonist or mGlu5 receptor antagonist

Acute administration of the highest dose of ACPT-I (25 mg/kg) significantly antagonized haloperidol-induced catalepsy ($p < 0.01$, Mann–Whitney U test after a significant Kruskal–Wallis test; $p < 0.01$, $H = 61.08$) (Fig. 2A), while MPEP had no effect, whatever the dose tested. Low doses of the two compounds [ACPT-I: 1, 5, and 15 mg/kg (15 mg/kg: data not shown); MPEP: 1.5 mg/kg] were ineffective in reversing the effects of haloperidol, neither after acute nor chronic treatment (Fig. 2B). After chronic administration, however, MPEP 1.5 mg/kg induced a slight decrease of haloperidol-induced catalepsy, but this effect failed to reach significant levels ($p = 0.08$, Mann–Whitney U test after a significant Kruskal–Wallis test; $p < 0.01$, $H = 95.01$).

3.1.3. Combined administration of adenosine A2A receptor antagonist CSC or MSX-3 with group III receptor agonist ACPT-I

CSC at a low dose (0.625 mg/kg) co-administered with ACPT-I 1 or 5 mg/kg did not modify haloperidol-induced catalepsy (Fig. 3A). In contrast, the dose of 1.25 mg/kg, ineffective as a single treatment, induced a robust decrease of the cataleptic state produced by haloperidol when co-administered with ACPT-I at 1 mg/kg ($p < 0.01$; Mann–Whitney U test after a significant Kruskal–Wallis test; $p < 0.01$, $H = 74.05$) (Fig. 3A). After a 3-week chronic treatment, combination of ACPT-I at a low dose (1 mg/kg) with CSC 0.625 and 1.25 mg/kg was ineffective against haloperidol-induced catalepsy (Fig. 3B). In contrast, chronic administration of highest dose of ACPT (5 mg/kg) with CSC 0.625 mg/kg, ineffective as a single treatment, significantly reduced haloperidol-induced catalepsy ($p < 0.01$; Mann–Whitney U test after a significant Kruskal–Wallis test; $p < 0.01$, $H = 83.15$) (Fig. 3B).

Co-administration of MSX-3 and ACPT-I confirmed the effects observed with CSC. Indeed, acute co-administration of MSX-3 0.625 mg/kg with ACPT-I 1 mg/kg, ineffective as a single treatment, was found to produce a significant decrease of haloperidol-induced catalepsy ($p < 0.01$, Mann–Whitney U test after a significant Kruskal–Wallis test; $p < 0.01$, $H = 86.22$) (Fig. 3A). Moreover, acute administration of MSX-3 1.25 mg/kg with ACPT-I 1 mg/kg significantly reduced catalepsy ($p < 0.01$, Mann–Whitney U test). After a chronic treatment, ACPT-I 1 mg/kg with either MSX-3 0.625 or MSX-3 1.25 mg/kg significantly antagonized haloperidol-induced catalepsy ($p < 0.01$, Mann–Whitney U test after a significant Kruskal–Wallis test; $p < 0.01$, $H = 113.42$) (Fig. 3B).

3.1.4. Combined administration of the adenosine A2A receptor antagonist CSC with the mGlu5 receptor antagonist MPEP

Acute combination of subthreshold doses of CSC (0.625 and 1.25 mg/kg) and MPEP (1.5 mg/kg) failed to reverse catalepsy (Fig. 4A). After chronic co-administration, however, CSC 1.25 + MPEP 1.5 significantly reduced haloperidol-induced catalepsy ($p < 0.01$, Mann–Whitney U test after a significant

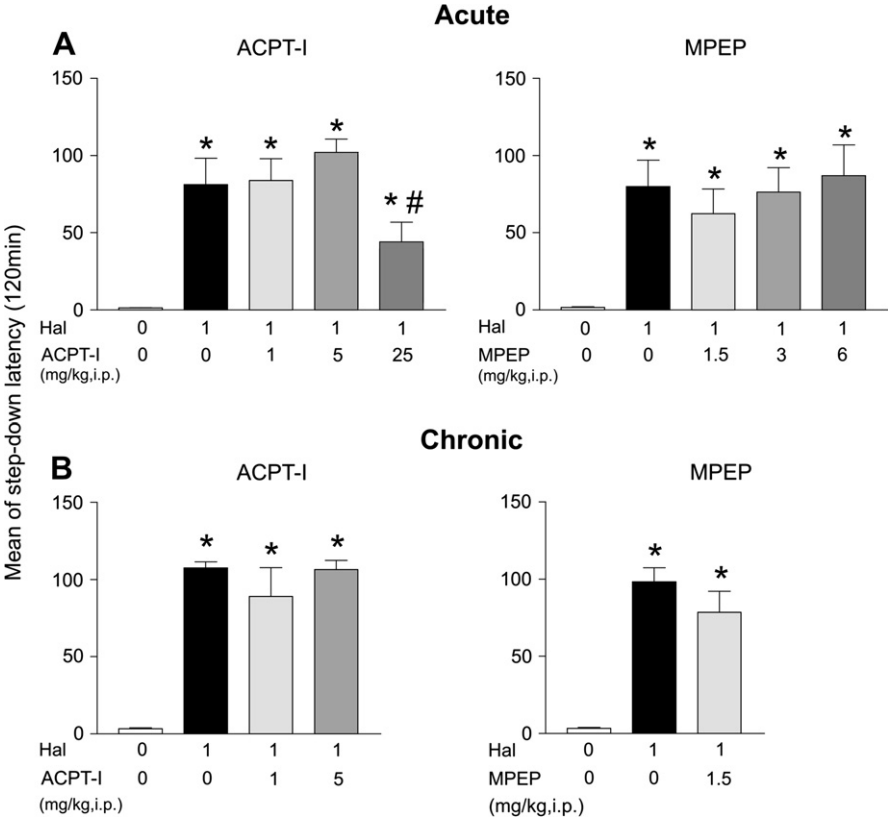

Fig. 2. Effects of acute (A) and chronic (B) administration of the group III mGlu receptor agonist ACPT-I and the mGlu5 receptor antagonist MPEP on haloperidol-induced catalepsy. (A) On d1, the animals were tested 60 min after haloperidol injection (1 mg/kg), and every 30 min for the 1-h testing. ACPT-I (0, 1, 5, and 25 mg/kg, $n = 6$–7 per group) was injected i.p. 30 min before haloperidol. MPEP (0, 1.5, 3, and 6 mg/kg, $n = 6$–7 per group) was injected i.p. 20 min after haloperidol. Low doses of each compound tested in the acute experiment were selected to test their effect after a chronic treatment (3 weeks of daily injections). (B) Effects of chronic treatment with ACPT-I (1 and 5 mg/kg, i.p.; $n = 6$ per group) or MPEP (1.5 mg/kg, i.p.; $n = 6$) on haloperidol-induced catalepsy. Data are expressed as mean latency ± SEM during the total duration of the test. *Significantly different from control group ($p < 0.05$; significant Mann–Whitney U test). #Significantly different from haloperidol group ($p < 0.05$; significant Mann–Whitney U test).

Please cite this article in press as: Lopez, S., et al., Functional interaction between adenosine A2A and group III metabotropic glutamate receptors to reduce parkinsonian symptoms in rats, Neuropharmacology (2008), doi:10.1016/j.neuropharm.2008.06.038

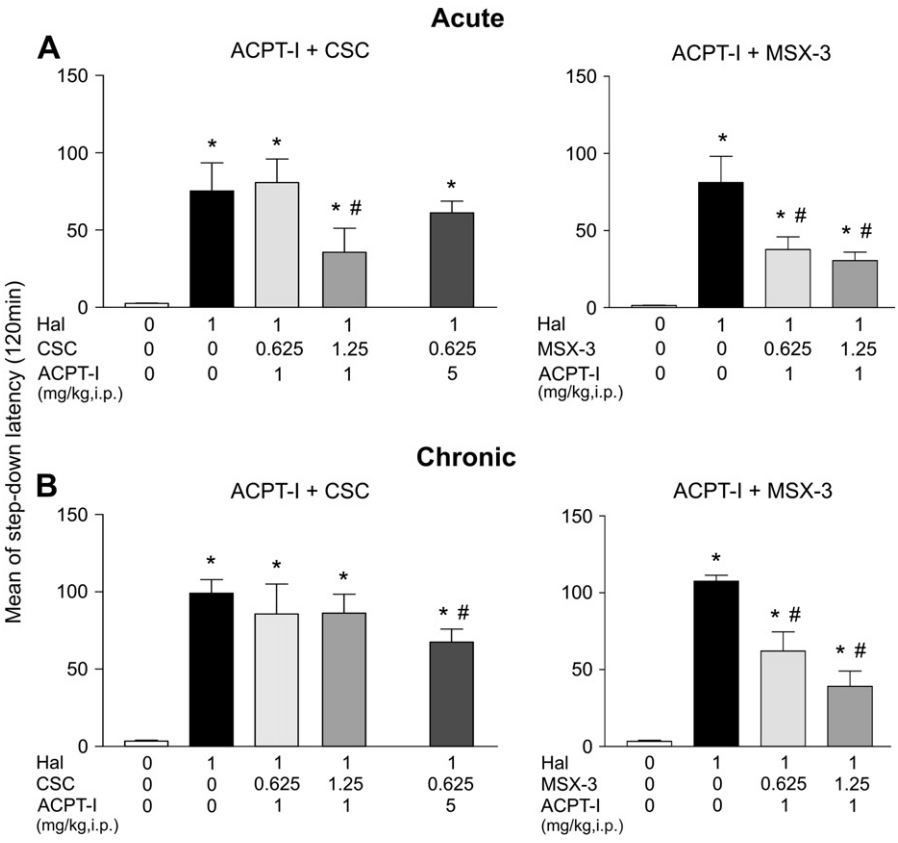

Fig. 3. Acute (A) and chronic (B) effects of combined treatment with ACPT-I and CSC or ACPT-I and MSX-3 on haloperidol-induced catalepsy. The animals were tested 60 min after haloperidol injection (1 mg/kg), and every 30 min for the 2-h testing. (A) Effects of acute injections (d1) of ACPT-I + CSC (0, 0.625, 1.25 and 0, 1, 5 mg/kg, i.p.; $n = 5$–7 per group) and ACPT-I + MSX-3 (0, 0.625, 1.25 and 0, 1 mg/kg, i.p., $n = 6$–7 per group) on haloperidol-induced catalepsy. (B) Effects of chronic injections (d21) of ACPT-I + CSC and ACPT-I + MSX-3 on catalepsy ($n = 5$–7 per group). The data are expressed as mean latency ± SEM during the total duration of the test. *Significantly different from control group ($p < 0.05$; significant Mann–Whitney *U* test). #Significantly different from haloperidol group ($p < 0.05$; significant Mann–Whitney *U* test).

Kruskal–Wallis test; $p < 0.01$, $H = 95.01$) (Fig. 4B), whereas CSC 0.625 + MPEP 1.5 had no effect.

3.2. Experiment 2: rotarod performance in 6-hydroxydopamine-lesioned rats

In the accelerating rotarod test, there was a significant effect of treatment on rotarod performance (ANOVA, $F_{(3,44)} = 6.10$, $p < 0.01$, Fig. 5). Post hoc Tukey's analysis revealed that performance of 6-OHDA-lesioned animals was significantly lower than sham-operated animals ($p < 0.01$). In lesioned animals, performance after administration of ACPT-I 30 mg/kg was significantly higher than after either vehicle or a low dose of ACPT-I (10 mg/kg) ($p < 0.01$). Indeed, the time-on-rod following treatment with ACPT-I 30 mg/kg was 37% greater than that seen following vehicle treatment (184.7 ± 19.9 *and* 134.8 ± 22.2 s for ACPT-I 30 mg/kg *and* vehicle, respectively).

4. Discussion

The present study shows for the first time that activation of group III mGlu receptors by acute systemic administration of the selective agonist ACPT-I counteracts haloperidol-induced catalepsy and reduces the motor impairment produced by unilateral 6-OHDA lesions on rotarod. In addition, our results show that concomitant acute and chronic blockade of A2A receptors and activation of group III mGlu receptors, with subthreshold doses of CSC or MSX-3 and ACPT-I, respectively, produce potent anticataleptic effect, thus demonstrating a functional interaction between these two groups of receptors. In line with our previous study in 6-OHDA-lesioned rats (Coccurello et al., 2004), we found a positive interaction with A2A and mGlu5 receptor antagonism in counteracting haloperidol-induced catalepsy, which is thought to reflect the parkinsonian akinesia.

4.1. Compounds' selectivity

As demonstrated recently, the A2A receptor antagonist CSC is also a potent inhibitor of monoamine oxidase-B (MAO-B) which could ultimately modify striatal DA and DA metabolite levels (Chen et al., 2002). However, our results show that the highly selective A2A antagonist MSX-3 (Sauer et al., 2000) at lower doses produces similar effects than CSC, thus confirming a selective action on A2A receptors. ACPT-I was chosen as a selective group III mGlu receptor agonist because it shows similar affinity for mGluR4 and mGluR8 than the classical agonist L-AP4, with higher concentrations required to activate mGluR7 (Goudet et al., 2007) and was recently found to cross the blood–brain barrier (Supplemental Fig. 1). The selectivity of MPEP for mGluR5 has recently been questioned. MPEP has nanomolar affinity for mGlu5 and also potentiates mGlu4 receptor activity at concentrations >50 μM (Gasparini et al., 1999; Mathiesen et al., 2003), concentrations which are not reached in the present study.

4.2. Functional interaction between A2A and mGlu receptors

4.2.1. A2A and group III mGlu receptors' interactions

In line with previous studies we found that the selective A2A antagonists CSC and MSX-3 decrease haloperidol-induced

Please cite this article in press as: Lopez, S., et al., Functional interaction between adenosine A2A and group III metabotropic glutamate receptors to reduce parkinsonian symptoms in rats, Neuropharmacology (2008), doi:10.1016/j.neuropharm.2008.06.038

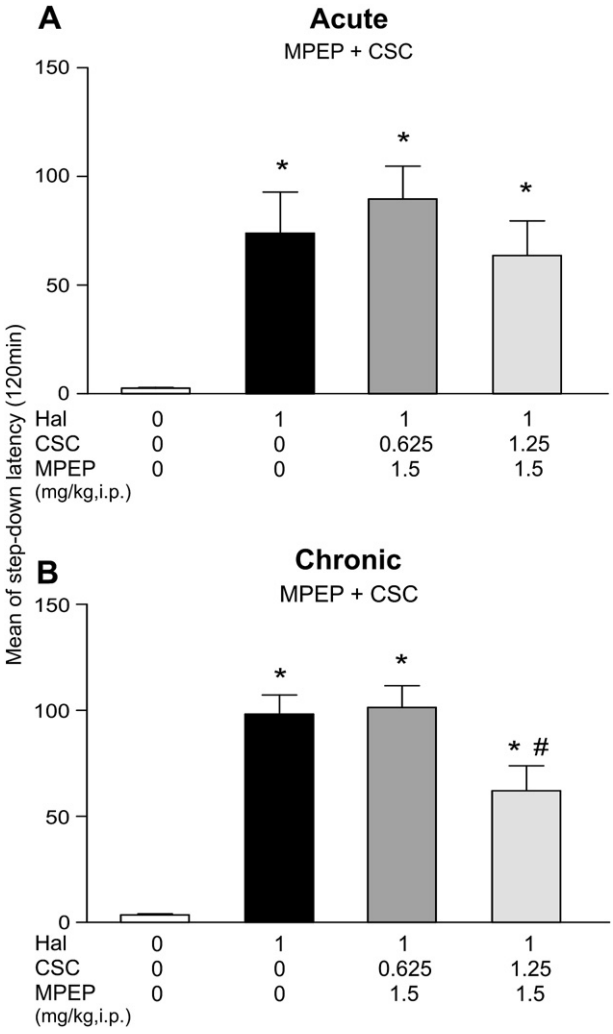

Fig. 4. Acute (A) and chronic (B) effects of combined treatment with subthreshold doses of CSC and MPEP on haloperidol-induced catalepsy. The animals were tested 60 min after haloperidol injections (1 mg/kg), and every 30 min for the 2-h testing. (A) Effects of acute injection (d1) of MPEP + CSC (0, 0.625, 1.25 and 0, 1.5 mg/kg, i.p., respectively) on haloperidol-induced catalepsy ($n = 6$–7 per group, CSC and MPEP were injected 10 and 20 min after haloperidol administration, respectively). (B) Effects of chronic injections (d21) of MPEP + CSC on catalepsy ($n = 6$–7 per group). The data are expressed as mean latency ± SEM during the total duration of the test. *Significantly different from control group ($p < 0.05$; significant Mann–Whitney U test). #Significantly different from haloperidol group ($p < 0.05$; significant Mann–Whitney U test).

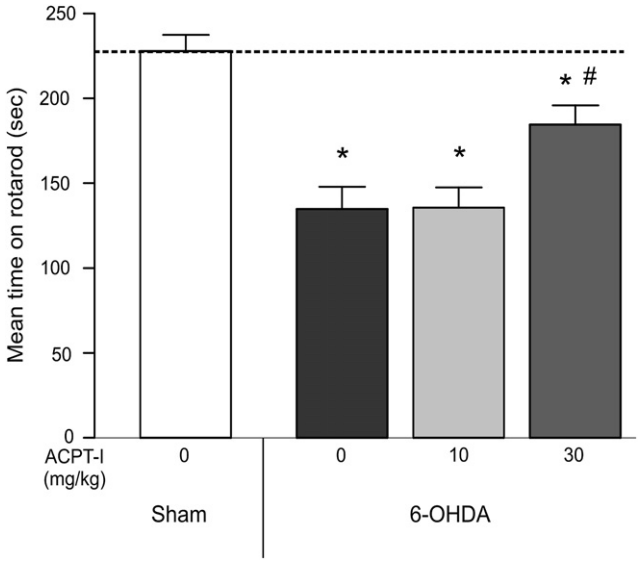

Fig. 5. Effect of ACPT-I on rotarod performance in 6-OHDA-lesioned rats. Two hours prior to behavioural testing, rats were treated with either vehicle or ACPT-I (10 or 30 mg/kg, i.p.). Time on accelerating rod (2.5–25 rpm over 300 s) was assessed 2 h following drug administration (sham, $n = 12$; 6-OHDA, $n = 12$). *Significantly different from sham-operated group ($p < 0.01$; significant Tukey's post hoc test). #Significantly different from 6-OHDA-lesioned group treated with vehicle solution ($p < 0.01$; significant Tukey's post hoc test).

catalepsy (Hauber et al., 2001; Shiozaki et al., 1999). These results confirm and extend the suggestion for A2A receptor antagonists to represent potent antiparkinsonian compounds (Schwarzschild et al., 2006; Xu et al., 2005), by showing that chronic administration for 3 weeks enhances their anticataleptic action. This antiparkinsonian action of A2A receptor antagonists is thought to reflect a preferential action on the overactive GABAergic striatopallidal neurons, as a consequence of the nigrostriatal DA neurons' degeneration. Interestingly, a similar site of action has recently been proposed to underline the antiparkinsonian effects of group III mGlu receptor selective agonists (Lopez et al., 2007; Marino et al., 2003; Valenti et al., 2003). Indeed, group III mGlu receptors are abundantly expressed presynaptically at different key synapses of the BG circuitry, including striatopallidal and corticostriatal synapses, and therefore may play a crucial role in regulating motor processes (Conn et al., 2005). To our knowledge, there is only one study that investigated the effect of systemic administration of group III mGlu positive allosteric modulator in the MPTP model of PD in mice. Indeed, the selective mGlu4 receptor positive allosteric modulator PHCCC reduces MPTP-induced nigrostriatal degeneration and reserpine-induced akinesia (Battaglia et al., 2006). The present results extend these findings and provide the first behavioural evidence for motor benefits induced by systemic administration of an orthosteric agonist selective for group III mGlu receptors in a model of PD.

In addition to the anticataleptic effect of systemic group III mGlu receptors' activation, the present results show that concomitant acute and chronic injections of subthreshold doses of ACPT-I and the A2A receptor antagonists CSC or MSX-3 reduce drastically the akinetic deficit induced by haloperidol. These new findings support the idea that activation of group III mGlu and blockade of A2A receptors potentiate their effects to produce the behavioural recovery. The functional interaction between these two groups of receptors may involve direct receptors interaction or additive effects on different neuronal pathways. The similar pattern of group III mGlu and A2A receptors expression on striatopallidal and corticostriatal nerve terminals could represent the morphological basis for these interactions.

First, at GP level, A2A receptors are present on presynaptic terminals of the striatopallidal neurons (Rosin et al., 2003) and the A2A agonist CGS21680 enhances GABA activity at GP level by a presynaptic mechanism (Ochi et al., 2000; Shindou et al., 2003). Similarly, activation of presynaptic group III mGlu receptors reduces GABAergic activity at striatopallidal synapses (Marino et al., 2003; Valenti et al., 2003), and reverses akinesia in various models of PD (Konieczny et al., 2007; Lopez et al., 2007). It has been suggested that mGlu4 receptor subtypes mediate these antiparkinsonian effects, since the preferential mGlu8 agonist, (*S*)-DCPG, does not modulate striatopallidal GABAergic activity, and that the effects of L-AP4 at these synapses are not found in mGlu4 knock-out mice (Valenti et al., 2003). Together with the differential affinity of ACPT-I for the various group III mGlu receptor subtypes (mGlu4, 6, 7 and 8), our results point to a specific involvement of mGlu4 receptor subtypes. Indeed, after acute i.p. administration at a dose of 30 mg/kg, the brain concentrations of ACPT-I reach

Please cite this article in press as: Lopez, S., et al., Functional interaction between adenosine A2A and group III metabotropic glutamate receptors to reduce parkinsonian symptoms in rats, Neuropharmacology (2008), doi:10.1016/j.neuropharm.2008.06.038

a maximum concentration of 3.71 μM, which is too low to activate mGlu7 receptors (Supplemental Fig. 1).

Second, at striatal level, A2A receptors are expressed at presynaptic level onto the striatal synapses, where they regulate glutamate, GABAergic and cholinergic striatal transmission (Rosin et al., 2003). Furthermore, activation of striatal presynaptic A2A receptors enhances glutamatergic transmission in rats (Rodrigues et al., 2005), while concomitant blockade of A2A and D2 receptors decreases it (Tozzi et al., 2007). Similarly, the group III mGlu receptor agonist L-AP4 was found to reduce glutamatergic activity at corticostriatal synapses (Pisani et al., 1997), which is supposed to be hyperactive in different models of PD (Calabresi et al., 1993; Lindefors and Ungerstedt, 1990), and intrastriatal injections of ACPT-I produce potent anticataleptic effects (Konieczny et al., 2007). A concomitant blockade of presynaptic A2A and activation of group III mGlu receptors may therefore potentiate their effects in decreasing glutamatergic corticostriatal activity. As detailed above the doses of ACPT-I used in the present study suggest that mGlu4 receptors should mediate the anticataleptic effects observed after ACPT-I treatment. However, at striatal level, the expression and function of mGlu8 receptors remains to be clarified, and a possible involvement of these receptors cannot be ruled out.

While ACPT-I and CSC or MSX-3 acute cotreatment produces robust anticataleptic effects, their chronic administration alleviates haloperidol-induced motor deficits to a lesser extent. The desensitisation of A2A receptors can be ruled out since 6-OHDA-lesioned rats and MPTP-treated monkeys show no development of tolerance after repeated treatment with A2A receptor antagonists (Kanda et al., 1998; Pinna et al., 2001). Desensitisation of group III mGlu receptors is unlikely to occur in our experimental conditions, since our results show that chronic administration of ACPT-I 5 mg/kg with CSC 0.625 mg/kg significantly antagonizes haloperidol-induced catalepsy. Together with preliminary studies showing positive effects of ACPT-I administered for 3 weeks on 6-OHDA-induced akinesia, this suggests that a subtle equilibrium in the degree of the blockade/activation of these two receptors rather than a desensitisation phenomenon appears to be crucial for chronic action of these compounds.

4.2.2. A2A and group I mGlu receptors' interactions

It has been shown that striatal mGlu5 receptors interact synergistically with A2A receptors to modulate striatopallidal activity (Diaz-Cabiale et al., 2002; Ferre et al., 1999). The behavioural studies emphasize the functional crosstalk that links striatal A2A and mGlu5 receptors by demonstrating that antagonists of these receptors interact synergistically to reduce akinetic symptoms in 6-OHDA and reserpine models of PD (Coccurello et al., 2004; Kachroo et al., 2005). Interactions between A2A and mGlu5 receptors may also occur at a presynaptic level on corticostriatal nerve terminal, where these receptors colocalize and interact synergistically to facilitate glutamate release (Rodrigues et al., 2005). Studies in striatal tissue and transfected cells in culture show that A2A and mGlu5 receptor agonists act synergistically to increase phosphorylation of the signal transduction molecule DARPP-32 (dopamine and cAMP-regulated phosphoprotein-32) (Nishi et al., 2003) and c-fos expression in striatal output neurons (Ferre et al., 2002). The mechanisms by which these two receptors interact after chronic treatment only are suggestive of synaptic adaptation and/or enhancement of these signalling pathways into the striatum.

5. Conclusion

Altogether, these results underlie the functional interaction that occurs between A2A and mGlu receptors in reducing parkinsonian symptoms, either by an antagonistic or synergistic action for group III and group I mGluR, respectively. To date, these results are the first to demonstrate a possible functional interaction between A2A and group III mGlu receptors. We suggest that a possible antagonistic interaction between the two groups of receptors may occur at presynaptic level into the GP and the striatum.

Acknowledgements

This research was supported by the CNRS, Aix-Marseille University, and research grants of the Fondation de France, French Ministry of Education and Research (ACI No. 04 2 91), National Research Agency (ANR-05-NEUR-021-01). S.L. was supported by a grant from the Ministry of Education and Research and from France Parkinson Association.

Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neuropharm.2008.06.038.

References

Acher, F.C., Tellier, F.J., Azerad, R., Brabet, I.N., Fagni, L., Pin, J.P., 1997. Synthesis and pharmacological characterization of aminocyclopentanetricarboxylic acids: new tools to discriminate between metabotropic glutamate receptor subtypes. J. Med. Chem. 40, 3119–3129.

Albin, R.L., Young, A.B., Penney, J.B., 1989. The functional anatomy of basal ganglia disorders. Trends Neurosci. 12, 366–375.

Battaglia, G., Busceti, C.L., Molinaro, G., Biagioni, F., Traficante, A., Nicoletti, F., Bruno, V., 2006. Pharmacological activation of mGlu4 metabotropic glutamate receptors reduces nigrostriatal degeneration in mice treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. J. Neurosci. 26, 7222–7229.

Bradley, S.R., Standaert, D.G., Levey, A.I., Conn, P.J., 1999. Distribution of group III mGluRs in rat basal ganglia with subtype-specific antibodies. Ann. N. Y. Acad. Sci. 868, 531–534.

Breysse, N., Baunez, C., Spooren, W., Gasparini, F., Amalric, M., 2002. Chronic but not acute treatment with a metabotropic glutamate 5 receptor antagonist reverses the akinetic deficits in a rat model of parkinsonism. J. Neurosci. 22, 5669–5678.

Calabresi, P., Mercuri, N.B., Sancesario, G., Bernardi, G., 1993. Electrophysiology of dopamine-denervated striatal neurons. Implications for Parkinson's disease. Brain 116 (Pt. 2), 433–452.

Chen, J.F., Steyn, S., Staal, R., Petzer, J.P., Xu, K., Van Der Schyf, C.J., Castagnoli, K., Sonsalla, P.K., Castagnoli Jr., N., Schwarzschild, M.A., 2002. 8-(3-Chlorostyryl)caffeine may attenuate MPTP neurotoxicity through dual actions of monoamine oxidase inhibition and A2A receptor antagonism. J. Biol. Chem. 277, 36040–36044.

Coccurello, R., Breysse, N., Amalric, M., 2004. Simultaneous blockade of adenosine A2A and metabotropic glutamate mGlu5 receptors increase their efficacy in reversing Parkinsonian deficits in rats. Neuropsychopharmacology 29, 1451–1461.

Conn, P.J., Battaglia, G., Marino, M.J., Nicoletti, F., 2005. Metabotropic glutamate receptors in the basal ganglia motor circuit. Nat. Rev. Neurosci. 6, 787–798.

Diaz-Cabiale, Z., Vivo, M., Del Arco, A., O'Connor, W.T., Harte, M.K., Muller, C.E., Martinez, E., Popoli, P., Fuxe, K., Ferre, S., 2002. Metabotropic glutamate mGlu5 receptor-mediated modulation of the ventral striopallidal GABA pathway in rats. Interactions with adenosine A(2A) and dopamine D(2) receptors. Neurosci. Lett. 324, 154–158.

Ferre, S., Fredholm, B.B., Morelli, M., Popoli, P., Fuxe, K., 1997. Adenosine–dopamine receptor–receptor interactions as an integrative mechanism in the basal ganglia. Trends Neurosci. 20, 482–487.

Ferre, S., Karcz-Kubicha, M., Hope, B.T., Popoli, P., Burgueno, J., Gutierrez, M.A., Casado, V., Fuxe, K., Goldberg, S.R., Lluis, C., Franco, R., Ciruela, F., 2002. Synergistic interaction between adenosine A2A and glutamate mGlu5 receptors: implications for striatal neuronal function. Proc. Natl. Acad. Sci. U.S.A. 99, 11940–11945.

Ferre, S., Popoli, P., Rimondini, R., Reggio, R., Kehr, J., Fuxe, K., 1999. Adenosine A2A and group I metabotropic glutamate receptors synergistically modulate the binding characteristics of dopamine D2 receptors in the rat striatum. Neuropharmacology 38, 129–140.

Gasparini, F., Lingenhohl, K., Stoehr, N., Flor, P.J., Heinrich, M., Vranesic, I., Biollaz, M., Allgeier, H., Heckendorn, R., Urwyler, S., Varney, M.A., Johnson, E.C., Hess, S.D., Rao, S.P., Sacaan, A.I., Santori, E.M., Velicelebi, G., Kuhn, R., 1999. 2-Methyl-6-(phenylethynyl)-pyridine (MPEP), a potent, selective and systemically active mGlu5 receptor antagonist. Neuropharmacology 38, 1493–1503.

Goudet, C., Chapuy, E., Alloui, A., Acher, F., Pin, J.P., Eschalier, A., 2007. Group III metabotropic glutamate receptors inhibit hyperalgesia in animal models of inflammation and neuropathic pain. Pain.

Hauber, W., Neuscheler, P., Nagel, J., Muller, C.E., 2001. Catalepsy induced by a blockade of dopamine D1 or D2 receptors was reversed by a concomitant blockade of adenosine A(2A) receptors in the caudate-putamen of rats. Eur. J. Neurosci. 14, 1287–1293.

Please cite this article in press as: Lopez, S., et al., Functional interaction between adenosine A2A and group III metabotropic glutamate receptors to reduce parkinsonian symptoms in rats, Neuropharmacology (2008), doi:10.1016/j.neuropharm.2008.06.038

Ishiwari, K., Madson, L.J., Farrar, A.M., Mingote, S.M., Valenta, J.P., DiGianvittorio, M. D., Frank, L.E., Correa, M., Hockemeyer, J., Muller, C., Salamone, J.D., 2007. Injections of the selective adenosine A2A antagonist MSX-3 into the nucleus accumbens core attenuate the locomotor suppression induced by haloperidol in rats. Behav. Brain Res. 178, 190–199.

Kachroo, A., Orlando, L.R., Grandy, D.K., Chen, J.F., Young, A.B., Schwarzschild, M.A., 2005. Interactions between metabotropic glutamate 5 and adenosine A2A receptors in normal and parkinsonian mice. J. Neurosci. 25, 10414–10419.

Kanda, T., Jackson, M.J., Smith, L.A., Pearce, R.K., 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.

Kearney, J.A.F., Albin, R.L., 2003. mGluRs: a target for pharmacotherapy in Parkinson disease. Exp. Neurol. 184 (Suppl. 1), S30–S36.

Konieczny, J., Wardas, J., Kuter, K., Pilc, A., Ossowska, K., 2007. The influence of group III metabotropic glutamate receptor stimulation by (1*S*,3*R*,4*S*)-1-aminocyclopentane-1,3,4-tricarboxylic acid on the parkinsonian-like akinesia and striatal proenkephalin and prodynorphin mRNA expression in rats. Neuroscience.

Lindefors, N., Ungerstedt, U., 1990. Bilateral regulation of glutamate tissue and extracellular levels in caudate-putamen by midbrain dopamine neurons. Neurosci. Lett. 115, 248–252.

Lopez, S., Turle-Lorenzo, N., Acher, F., De Leonibus, E., Mele, A., Amalric, M., 2007. Targeting group III metabotropic glutamate receptors produces complex behavioral effects in rodent models of Parkinson's disease. J. Neurosci. 27, 6701–6711.

Marino, M.J., Williams Jr., D.L., O'Brien, J.A., Valenti, O., McDonald, T.P., Clements, M.K., Wang, R., DiLella, A.G., Hess, J.F., Kinney, G.G., Conn, P.J., 2003. Allosteric modulation of group III metabotropic glutamate receptor 4: a potential approach to Parkinson's disease treatment. Proc. Natl. Acad. Sci. U.S.A. 100, 13668–13673.

Mathiesen, J.M., Svendsen, N., Brauner-Osborne, H., Thomsen, C., Ramirez, M.T., 2003. Positive allosteric modulation of the human metabotropic glutamate receptor 4 (hmGluR4) by SIB-1893 and MPEP. Br. J. Pharmacol. 138, 1026–1030.

Nishi, A., Liu, F., Matsuyama, S., Hamada, M., Higashi, H., Nairn, A.C., Greengard, P., 2003. Metabotropic mGlu5 receptors regulate adenosine A2A receptor signaling. Proc. Natl. Acad. Sci. U.S.A. 100, 1322–1327.

Ochi, M., Koga, K., Kurokawa, M., Kase, H., Nakamura, J., Kuwana, Y., 2000. Systemic administration of adenosine A(2A) receptor antagonist reverses increased GABA release in the globus pallidus of unilateral 6-hydroxydopamine-lesioned rats: a microdialysis study. Neuroscience 100, 53–62.

Paxinos, G., Watson, C., 1986. The Rat Brain in Stereotaxic Coordinates. Academic Press, Sydney.

Pin, J.P., Duvoisin, R., 1995. The metabotropic glutamate receptors: structure and functions. Neuropharmacology 34, 1–26.

Pinna, A., Fenu, S., Morelli, M., 2001. Motor stimulant effects of the adenosine A2A receptor antagonist SCH 58261 do not develop tolerance after repeated treatments in 6-hydroxydopamine-lesioned rats. Synapse 39, 233–238.

Pisani, A., Calabresi, P., Centonze, D., Bernardi, G., 1997. Activation of group III metabotropic glutamate receptors depresses glutamatergic transmission at corticostriatal synapse. Neuropharmacology 36, 845–851.

Quik, M., Sum, J.D., Whiteaker, P., McCallum, S.E., Marks, M.J., Musachio, J., McIntosh, J.M., Collins, A.C., Grady, S.R., 2003. Differential declines in striatal nicotinic receptor subtype function after nigrostriatal damage in mice. Mol. Pharmacol. 63, 1169–1179.

Rodrigues, R.J., Alfaro, T.M., Rebola, N., Oliveira, C.R., Cunha, R.A., 2005. Co-localization and functional interaction between adenosine A(2A) and metabotropic group 5 receptors in glutamatergic nerve terminals of the rat striatum. J. Neurochem. 92, 433–441.

Rosin, D.L., Hettinger, B.D., Lee, A., Linden, J., 2003. Anatomy of adenosine A2A receptors in brain: morphological substrates for integration of striatal function. Neurology 61, S12–S18.

Sauer, R., Maurinsh, J., Reith, U., Fulle, F., Klotz, K.N., Muller, C.E., 2000. Water-soluble phosphate prodrugs of 1-propargyl-8-styrylxanthine derivatives, A(2A)-selective adenosine receptor antagonists. J. Med. Chem. 43, 440–448.

Schwarzschild, M.A., Agnati, L., Fuxe, K., Chen, J.F., Morelli, M., 2006. Targeting adenosine A2A receptors in Parkinson's disease. Trends Neurosci. 29, 647–654.

Shindou, T., Richardson, P.J., Mori, A., Kase, H., Ichimura, M., 2003. Adenosine modulates the striatal GABAergic inputs to the globus pallidus via adenosine A2A receptors in rats. Neurosci. Lett. 352, 167–170.

Shiozaki, S., Ichikawa, S., Nakamura, J., Kitamura, S., Yamada, K., Kuwana, Y., 1999. Actions of adenosine A2A receptor antagonist KW-6002 on drug-induced catalepsy and hypokinesia caused by reserpine or MPTP. Psychopharmacology (Berl.) 147, 90–95.

Tozzi, A., Tscherter, A., Belcastro, V., Tantucci, M., Costa, C., Picconi, B., Centonze, D., Calabresi, P., Borsini, F., 2007. Interaction of A2A adenosine and D2 dopamine receptors modulates corticostriatal glutamatergic transmission. Neuropharmacology 53, 783–789.

Valenti, O., Marino, M.J., Wittmann, M., Lis, E., DiLella, A.G., Kinney, G.G., Conn, P.J., 2003. Group III metabotropic glutamate receptor-mediated modulation of the striatopallidal synapse. J. Neurosci. 23, 7218–7226.

Xu, K., Bastia, E., Schwarzschild, M., 2005. Therapeutic potential of adenosine A(2A) receptor antagonists in Parkinson's disease. Pharmacol. Ther. 105, 267–310.

Please cite this article in press as: Lopez, S., et al., Functional interaction between adenosine A2A and group III metabotropic glutamate receptors to reduce parkinsonian symptoms in rats, Neuropharmacology (2008), doi:10.1016/j.neuropharm.2008.06.038