The Selective mGlu$_5$ Receptor Agonist CHPG Inhibits Quinpirole-Induced Turning in 6-Hydroxydopamine-Lesioned Rats and Modulates the Binding Characteristics of Dopamine D$_2$ Receptors in the Rat Striatum

Interactions with Adenosine A$_{2a}$ Receptors

Patrizia Popoli, M.D., Antonella Pèzzola, Maria Torvinen, B.Sc., Rosaria Reggio, Annita Pintor, B.Sc., Laura Scarchilli, Kjell Fuxe, M.D., Ph.D., and Sergi Ferré, M.D., Ph.D.

In 6-hydroxydopamine-lesioned rats, the selective mGlu$_5$ receptor agonist (RS)-2-Cholro-5-Hydroxyphenylglycine (CHPG, 1-6 μg/10 μl intracerebroventricularly) significantly inhibited contralateral turning induced by quinpirole and, to a lesser extent, that induced by SKF 38393. The inhibitory effects of CHPG on quinpirole-induced turning were significantly potentiated by an adenosine A$_{2A}$ receptor agonist (CGS 21680, 0.2 mg/kg IP) and attenuated by an A$_{2A}$ receptor antagonist (SCH 58261, 1 mg/kg IP). In rat striatal membranes, CHPG (100–1,000 nM) significantly reduced the affinity of the high-affinity

state of D$_2$ receptors for the agonist, an effect potentiated by CGS 21680 (30 nM). These results show the occurrence of functional interactions among mGlu$_5$, adenosine A$_{2A}$, and dopamine D$_2$ receptors in the regulation of striatal functioning, and suggest that mGlu$_5$ receptors may be regarded as alternative/integrative targets for the development of therapeutic strategies in the treatment of Parkinson's disease.
[Neuropsychopharmacology 25:505–513, 2001]
© 2001 American College of Neuropsychopharmacology.
Published by Elsevier Inc. All rights reserved.

According to current theories of functional anatomy of basal ganglia (Albin et al. 1989), the neurotransmitters dopamine and glutamate closely interact in the regulation of normal movements; whereas, an altered balance between dopamine- and glutamate-mediated neurotransmissions is thought to play a major role in the pathogenesis of movement disorders (Carlsson and Carlsson 1990; Lipton and Rosenberg 1994; Starr 1995).

0893-133X/01/$–see front matter
PII S0893-133X(01)00256-1

In Parkinson's disease because of the reduction of dopamine D_2 receptor-mediated inhibition of striatopallidal neurones, glutamate pathways within the indirect circuit become overactive (Alexander and Crutcher 1990; Gerfen et al. 1990). Reduction of the overactivity of the indirect pathway, which generates from the striopallidal neurones, this, is the goal of antiparkinsonian treatments. Such a reduction may be achieved by potentiating the dopaminergic neurotrasmission and/or by inhibiting the glutamatergic tone. Indeed, several studies have shown that antagonists of ionotropic glutamate receptors possess antiparkinsonian effects (Starr et al. 1997).

Besides ionotropic receptors, metabotropic glutamate (mGlu) receptors also play a major role in the regulation of striatal functioning (Vezina and Kim 1999). In particular, group I mGlu receptors are highly expressed in the striatum (Romano et al. 1995; Tallaksen-Greene et al. 1998; Testa et al. 1994, 1995), where they contribute to neuronal plasticity (Calabresi et al. 1996), motor behavior (Ferré et al. 1999; Kearney et al. 1997) and excitotoxic injury (Calabresi et al. 1999). The intrastriatal injection of 1S-3R-ACPD (a group I and II mGlu receptor agonist) or of DHPG (a selective group I receptor agonist) induced delayed contralateral turning behavior, which seems to depend on an excessive activation of the subthalamic nucleus (Sacaan et al. 1992; Kaatz and Albin 1995; Kearney and Albin 1995; Kearney et al. 1997,1998). In 6-hydroxydopamine(6-OH-DA)-lesioned rats, which represent a rodent model of Parkinson's disease (Schwarting and Huston 1996; Ungerstedt 1971), the intracerebroventricular (ICV) injection of 1S,3R ACPD selectively counteracted the turning behavior elicited by a dopamine D_2 receptor agonist (Ferré et al. 1999). Both effects of mGlu receptor agonists (antagonism of D_2-dependent rotations and induction of contralateral rotations) seemed to involve adenosine A_{2A} receptors (Kearney and Albin 1995; Kearney et al. 1997; Ferré et al. 1999). Moreover, in rat striatal membranes, DHPG modulated the binding characteristics of dopamine D_2 receptors in a similar manner as the adenosine A_{2A} receptor agonist CGS 21680, with a significant decrease in the affinity of the high-affinity state of D_2 receptors for dopamine (Ferré et al. 1999; Rimondini et al. 1999). Thus, reciprocal interactions occurring among striatal group I mGlu, adenosine A_{2A} and dopamine D_2 receptors may play a role in the regulation of basal ganglia functions, and then of motor activity. Which of the group I mGlu receptor subtypes ($mGlu_1$ and/or $mGlu_5$) could be involved in such interactions is presently unknown.

Dopamine D_2 receptors and adenosine A_{2A} receptors are co-localized in the striatopallidal neurones (Fink et al. 1992; Schiffmann et al. 1991; Svenningsson et al. 1997), where the tonic, antagonistic A_{2A}/D_2 interaction mainly takes place (Ferré et al. 1993, 1997). Such neu-

rones, which are thought to be the main locus for the interaction occurring between group I mGlu receptors and adenosine A_{2A} receptors (Kearney and Albin 1995; Kearney et al. 1997), also show a high degree of expression of $mGlu_5$ receptor mRNA (Testa et al. 1995). An involvement of $mGlu_5$ receptor subtype in the modulation of the activity of striatopallidal neurones, thus, is conceivable.

The aim of the present work was to investigate the possible occurrence of functional interactions among $mGlu_5$, adenosine A_{2A}, and dopamine D_2 receptors in the regulation of striatal functioning. To this end, the effects of the selective $mGlu_5$ receptor agonist (RS)-2-Cholro-5-hydroxyphenylglycine (CHPG, Doherty et al. 1997) on the turning behavior elicited by a D_2 (quinpirole) dopamine receptor agonist in 6-OH-DA-lesioned rats, and on the binding characteristics of D_2 receptors in rat striatal membranes, were studied. The influence of adenosine A_{2A} receptor ligands on CHPG-induced effects was also evaluated. The effects of CHPG on the turning behavior elicited by a D_1 receptor agonist (SKF 38393) were tested for comparison. Finally, separate behavioral and in vivo microdialysis experiments were performed to verify whether the effects of CHPG could be mediated by a reduction of spontaneous motor activity and/or by a modulation of striatal dopamine release.

METHODS

Experimental Protocol (Lesion)

Male Sprague–Dawley rats (145–155 g) were used. The animals were kept under standardized temperature, humidity, and lighting conditions, with free access to water and food. Animal care and use followed the directives of the Council of the European Communities (86/609/EEC). Under Equithesin (3 ml/kg) anesthesia, animals were placed in a Kopf stereotaxic apparatus. Unilateral injections of 6-hydroxydopamine (6-OH-DA, 8μg/4μl of 0.2% ascorbic acid saline solution) were performed in the left nigrostriatal pathway (coordinates with respect to bregma: A = −2.4; L = +1.2; V = −7.8 mm) by means of an Hamilton syringe (mod. 701). Starting 3 weeks after the lesion, the animals' ability to rotate in response to apomorphine (0.05 mg/kg subcutaneously, SC) was tested. Contralateral rotations induced by apomorphine were measured 3 to 4 times at weekly intervals. Only animals showing at least 50 turns/5 min in the last test were included in the study. Thirty minutes before starting the experiments, the animals were placed in rotation bowls in a soundproof experimental room. The selective $mGlu_5$ receptor agonist CHPG was administered ICV at a rate of 1 μl/min by means of a microdrive pump (injection volume: 10 μl). The needle was then left in place for additional 5 min.

Ten minutes thereafter (i.e., 15 min after the end of drug infusion), the animals were treated with a D_2 (quinpirole) or D_1 (SKF 38393) dopamine receptor agonist. The number of contralateral rotations (only complete and uninterrupted 360° turns) over 60 min was then recorded. Each trial was analyzed by an observer unaware of the treatment received by the animals.

Placement of ICV Cannula

Under Equithesin anaesthesia, both 6-hydroxydopamine-lesioned and naive rats were stereotaxically implanted with stainless steel guide cannulae (22 G, Plastics ONE) 3 mm above the upper boundaries of lateral ventricle (A = +0.1, L = ±1.5; V = −2.9 mm from bregma, sagittal suture, and dura, respectively). Guide cannulae were fixed with dental acrylic to the skull surface. Stainless steel stylets were inserted into the cannulae to prevent occlusion. A recovery period of 5 to 6 days was allowed before testing. For ICCV administration, injection needles (28 G) extending 3 mm below the guide were inserted into the cannulae. Correct cannula placement was ascertained immediately after the animal's sacrifice (overdose of equithesin) by injecting 10 μl of China ink ICV. Only data from animals showing China ink diffusion to the ventricular system were included in the analysis.

Microdialysis Experiments

To verify whether CHPG could influence striatal dopamine release, microdialysis experiments were performed in naive animals (male Sprague–Dawley rats, 280–320 g). Under equithesin anesthesia, the animals were placed in a stereotaxic frame and implanted with a concentric dialysis probe (mod CMA/12, 4-mm length, Carnegie Medicine, Sweden) into the striatum. Stereotaxic coordinates in mm from bregma, sagittal suture, and dura, respectively, were as follows: A = +2.0, L = +2.5, V = −6.8. Perfusion was started 24 hours after probe implantation at a rate of 2 μl/min with Ringer's solution (NaCl 147, $CaCl_2$ 2.3 and KCl 4.0 mM, pH 7.0; Corsi et al. 1999; Pintor et al. 2000). Dialysates were collected every 20 min in 3 μl 0.01 M $HClO_4$ into a refrigerated fraction collector (mod CMA/170) and then frozen until assay. CHPG was infused through the probe for 30 min (250–1,000 μM, freshly dissolved in Ringer's solution). At the end of the experiments, each rat was sacrificed with an overdose of Equithesin, the brain was fixed with 4% paraformaldehyde, and coronal sections (20- μm thick) were cut to verify the probe location. Samples obtained from rats in which the probe was not correctly positioned (less than 5%) were not included in the analysis.

The dopamine content of all samples was assayed as previously described (Pintor et al. 2000) by reverse-phase high-performance liquid chromatography (HPLC) coupled to an electrochemical detector (ESA Coulochem II, Model 5200, Bedford, MA, USA) with a limit of detection of 0.5 to 2 pg (20 μl injection volume, 3:1 signal-to-noise ratio). Results were expressed as percentage changes of extracellular dopamine levels with respect to basal (predrug) values (mean of 4–5 samples).

To verify whether the effects exerted by ICV CHPG in 6-OH-DA-lesioned rats could be partially explained by an effect of the drug on striatal dopamine release, separate experiments were performed. In these experiments, CHPG was administered to naive animals at the same doses and by the same route of administration used in 6-OH-DA-lesioned rats. Thus, the animals were implanted with both the ICV cannula (to be used for drug administration) and the intrastriatal microdialysis probe (for the evaluation of extracellular dopamine levels in response to ICV CHPG) under the same surgical session.

Evaluation of the Effects of CHPG on Motor Activity

To evaluate whether CHPG could influence over-all motor activity, separate experiments were performed in naive rats. The animals were individually placed in an automated activity meter (mod. Automex II, Columbus Instruments, OH) and allowed to habituate to the cage for 30 min. CHPG (0, 1, 6, and 9 μg/10 μl ICV) was then administered, and activity counts recorded for 60 min.

Binding Experiments

The rats where killed by decapitation when weighing 200 to 250 g, the brain was rapidly removed, and the striata were dissected out. The tissue was weighed, placed in polypropylene vials, and sonicated for 30 s in ice-cold TRIS-HCl buffer (50 mM, pH 7.4) containing 5 mM EDTA. TRIS buffer (as above) was added, and the homogenate was centrifuged for 10 min at 45 g (4°C). The supernatant was discarded, and the pellet was resuspended by sonication in the same TRIS buffer. Adenosine deaminase (5 U/ml; Boehringer Mannheim Scandinavia AB, Bromma, Sweden) was added, and the preparation was preincubated for 30 min at 37°C (to activate adenosine deaminase and remove endogenous adenosine) and centrifuged again at 45 g (4°C). After two more washing steps (sonication and centrifugation at 45 g), the final membrane preparation was resuspended by sonication in the incubation buffer: TRIS-HCl buffer (50 mM, pH 7.4) containing 120 mM NaCl, 5 mM KCl, 5 mM $MgCl_2$, 1 mM $CaCl_2$ and 1 mM EDTA (final protein concentration, 0.2 mg/ml). Competition experiments with dopamine versus the dopamine D_2 receptor antagonist [³H]raclopride (90 Ci/mmol; NEN, Boston, MA) were performed by incubation with 20 concentrations (10 pM–1 mM) of dopamine and 2 nM

[³H]raclopride for 30 min at room temperature in the presence of the different drugs under study. These compounds were the adenosine A_{2A} receptor agonist 2-[*p*-(2-carboxyethyl)phenthylamino)-5'-*N*-ethylcarbox-amidoadenosine (CGS 21680) and the mGlu₅ receptor agonist CHPG. The concentration of CGS 21680 used (30 nM) had been previously found to induce a maximal effect on competitive-inhibition experiments of dopamine versus [³H]raclopride (Ferré et al. 1999). Two separate experiments were performed. To avoid the variability of the binding parameters associated with the assay conditions, one membrane preparation was used for each experiment. The first experiment analyzed the effect of CHPG (three different concentrations), and the second experiment analyzed the effect of CHPG and CGS 21680 alone and in combination. The incubation was stopped by automatically washing the membranes three times with 5 ml ice-cold TRIS buffer over What-man GF/B filters (Whatman International Ltd., Maid-stone, UK). The radioactivity content of the filters was detected by liquid scintillation spectrometry. Data from competition experiments were analyzed by nonlinear regression analysis for the determination of the dissociation constants of high- and low-affinity binding sites (K_H and K_L values, respectively) and the proportion of high-affinity binding sites (R_H values). To achieve homogeneity of variance and allow parametric statistical analysis, K_H and K_L values were logarithmically transformed and analyzed by repeated measures analysis of variance (ANOVA) followed by Dunnett's post hoc test.

Drugs

CHPG and the selective mGlu₅ receptor antagonist 2-Methyl-6-(phenylethynyl)-pyridine (MPEP, Gasparini et al. 1999) were purchased from Tocris Cookson (Bristol, UK); quinpirole, CGS 21680 and SKF 38393 from RBI (Natick, MA, USA), 6-hydroxidopamine hydrobro-mide from Sigma-Aldrich (Milan, Italy). SCH 58261 was a generous gift from Schering-Plough (Milan, Italy).

RESULTS

Influence of DHPG on D_2- and D_1-Dependent Contralateral Rotations in 6-OH-DA-lesioned lats

The doses of D_2 and D_1 dopamine receptor agonists were selected on the basis of previous studies (Ferré et al. 1999; Garrett and Holtzman 1995; Popoli et al. 2000) showing that 0.1 mg/kg quinpirole and 3 mg/kg SKF 38393 are comparable doses, because they are the minimal doses inducing a maximal effect in dose–response experiments. These doses of quinpirole and SKF 38393 are considered as selective for D_2 and D_1 receptors, respectively (Garrett and Holtzman 1994).

As shown in Figure 1A, CHPG (1–6 μg) clearly antagonized quinpirole-induced contralateral rotations in a statistically significant and dose-dependent way. A lower dose of CHPG (0.2 μg) was ineffective ($n = 4$, data not shown). Figure 1B shows the influence of CHPG on SKF 38393-induced contralateral rotations. Although a significant inhibition was observed at doses of 3 and 6 μg, the lowest dose of CHPG (1 μg) was totally ineffective. The inhibitory effect exerted by CHPG 6 μg was significantly lower toward SKF 38393 than quinpirole-induced rotations ($-60 \pm 4.5\%$ and $-82 \pm 4.7\%$, respectively, $p < .05$ according to Mann–Whitney test).

Administered alone up to the dose of 9 μg ICV, CHPG did not induce appreciable turning behavior in
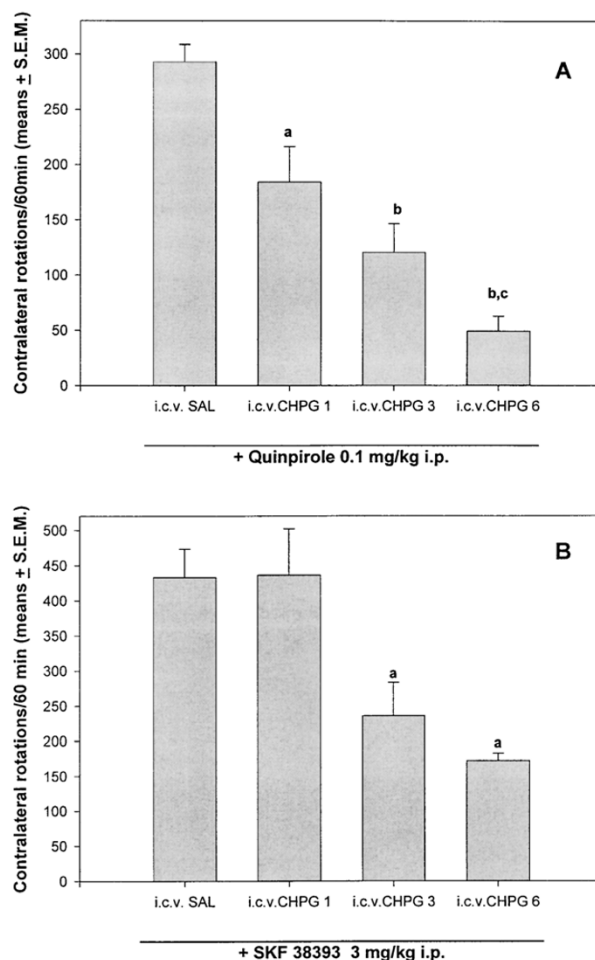
Figure 1. Influence of CHPG on quinpirole- and SKF 38393-induced contralateral rotations in 6-hydroxydopam-ine-lesioned rats. CHPG (0, 1, 3, and 6 μg/10 μl) was administered ICV 15 min before quinpirole (A) or SKF 38393 (B). Each group was made up of six to eight animals. a = $p < .01$ and b = $p < .005$ versus saline; c = $p < .05$ versus CHPG 1 and 3 μg (one-way ANOVA followed by post-hoc Dunnett's test).
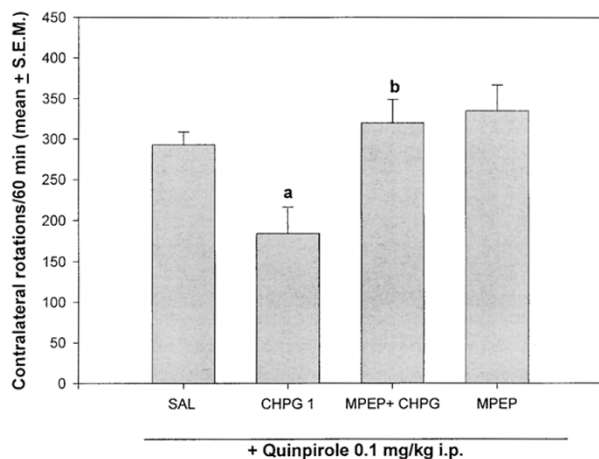
lesioned animals. Brief (lasting approximately 5 min) bouts of rotations contralateral with respect to the lesion were noticed in some rats immediately after the ICV injection, irrespective of the treatment (vehicle or CHPG).

The mGlu$_5$ receptor antagonist MPEP (1 μg), injected immediately before CHPG 1 μg (final volume 10 μl), abolished the inhibitory effects of the agonist toward quinpirole-induced rotations (Figure 2). MPEP 1 μg ICV had no effects per se on quinpirole-induced turning (Figure 2).
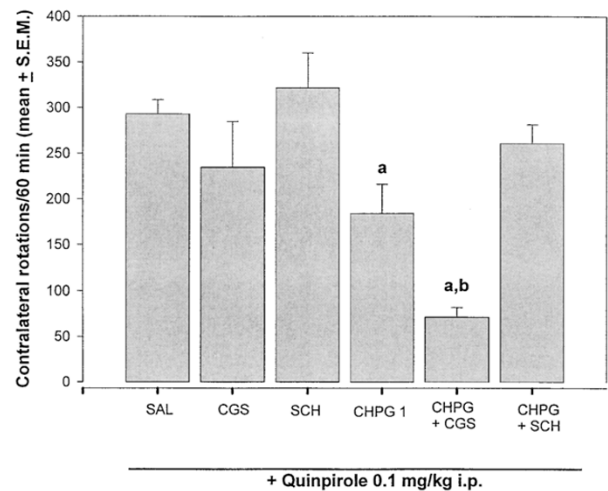
The adenosine A$_{2A}$ receptor agonist CGS 21680 (0.2 mg/kg, 15 min before CHPG injection) significantly potentiated the inhibitory effect exerted by CHPG on quinpirole-induced turning (Figure 3). The effects of CHPG on quinpirole-induced turning were conversely attenuated by the selective adenosine A$_{2A}$ receptor antagonist SCH 58261 (1 mg/kg IP, 15 min before CHPG injection, Figure 3). Administered alone at the same dose tested here, neither CGS 21680 nor SCH 58261 did significantly affect quinpirole-induced contralateral turning in lesioned rats (Figure 3).

Influence of CHPG on Striatal extracellular Levels of Dopamine

Basal dopamine levels were 4.28 ± 0.8 pg/20 μl. Neither the striatal perfusion of CHPG (250–1,000 μM over 30 min) through the microdialysis probe, nor the ICV injection of the drug (6 μg/10 μl) elicited significant effects on the extracellular levels of dopamine in intact

Figure 3. Influence of adenosine A$_{2A}$ receptor ligands on CHPG-mediated inhibition of contralateral rotations induced by quinpirole. CGS 21680 (0.2 mg/kg) and SCH 58261 (1 mg/kg) were administered IP either alone, 30 min before quinpirole (groups "CGS" and "SCH," $n = 5$), or 15 min before the ICV injection of 1 μg/10 μl CHPG (groups "CHPG+CGS" and "CHPG+SCH," $n = 6$–9). The group "CHPG 1" was the same shown in the previous figure. A = $p < .05$ versus saline; b = $p < .05$ versus CHPG (one-way ANOVA followed by post-hoc Dunnett's test).

rats (Figure 4). No overt behavioral effects were observed as a consequence of drug infusion.

### Influence of CHPG on over-all Motor Activity

In intact, habituated rats, the ICV injection of CHPG 9 μg significantly reduced over-all motor activity (as revealed by total motor counts/60 min in an automated activity meter) with respect to vehicle-injected animals (Figure 5). Motor activity was not significantly reduced (-29% in mean) by 6 μg CHPG. The lowest tested dose of CHPG (1 μg) did not induce any effect on motor activity (Figure 5).

Influence of CHPG on the Binding Characteristics of D$_2$ Receptors in Striatal Membranes

CHPH induced a significant decrease in the affinity of the high-affinity state of D$_2$ receptor for dopamine, which was maximal (three- to fourfold increase in K$_H$) at 100 nM. The other parameters, K$_L$ and R$_H$, were not significantly modified (Table 1). CGS 21680 also significantly increased K$_H$ values at 30 nM. The combined addition of CHPG 100 nM and CGS 21680 30 nM induced a more significant increase of K$_H$ values (Table 1).

Figure 2. The effects of CHPG on quinpirole-induced contralateral rotations are abolished by the mGlu$_5$ receptor antagonist MPEP. MPEP (1 μg) was administered ICV either alone ("MPEP," $n = 5$) or immediately before CHPG (1 μg, final volume : 10 μl, "MPEP + CHPG" $n = 6$). Groups "SAL" and "CHPG1" were made up of eight animals each. A = $p < .05$ versus saline; b = $p < .05$ versus CHPG (one-way ANOVA followed by post-hoc Dunnett's test).

DISCUSSION

The present results show that the selective mGlu$_5$ receptor agonist CHPG, when injected ICV, significantly and
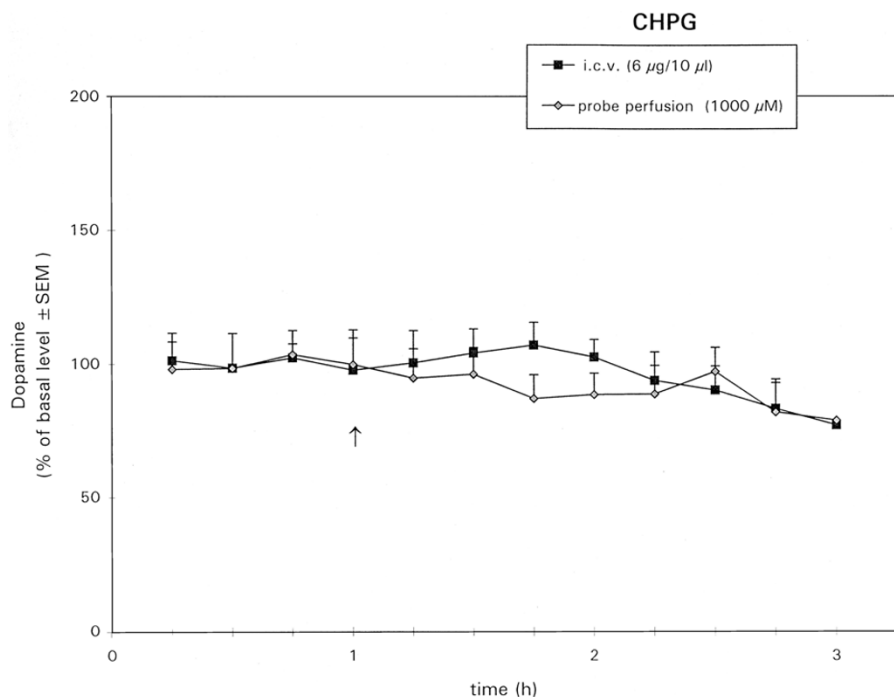
Figure 4. CHPG does not affect striatal extracellular levels of dopamine after either ICV injection or probe perfusion. CHPG (1,000 μM) was perfused through the microdialysis probe over 30 min (triangles) or administered ICV (6 μg/10μl, squares). The arrow indicates the time of ICV injection and the beginning of perfusion. Values are expressed as percentage of basal dopamine levels (mean of four samples). Groups were made up of four animals.

dose-dependently inhibits contralateral rotations induced by dopamine receptor agonists in 6-OHDA-lesioned rats. The occurrence of inhibitory effects on dopamine receptor agonist-induced contralateral rotations suggests that, at least under our experimental conditions (i.e., early after ICV administration), CHPG exerts antidopaminergic effects. This view is supported by the finding that ICV CHPG significantly reduced spontaneous motor activity in intact rats, an effect that is in line with a previous report showing that ICV 1S,
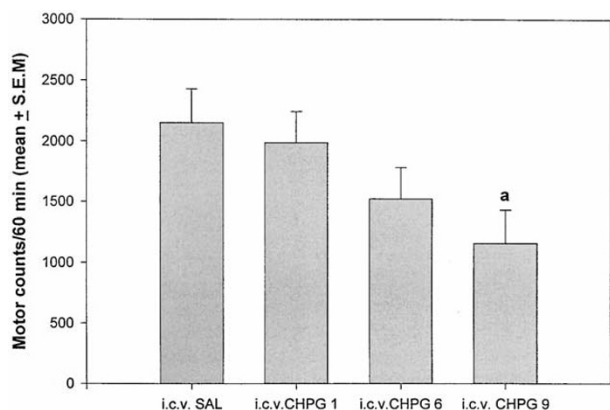
Figure 5. Influence of CHPG on spontaneous motor activity in intact rats. Groups of eight rats each were individually placed in an activity meter and habituated for 30 min before receiving ICV injections. Motor activity, expressed as motor counts, was then recorded over 60 min. CHPG doses are expressed as μg/10 μl ICV. A = $p < .05$ versus saline (one-way ANOVA followed by post-hoc Dunnett's test).

3R-ACPD induced motor depression and catalepsy and counteracted amphetamine-induced motor activation (Kronthaler and Schmidt 1996). These data seem to be at odds with the finding that intrastriatal injection of either 1S, 3R-ACPD or the selective group I mGlu receptor agonist DHPG induced contralateral rotations in both intact and 6-OH-DA-lesioned rats (Sacaan et al. 1992; Kaatz and Albin 1995; Kearney and Albin 1995; Kearney et al. 1997, 1998; Smith and Beninger 1996), which would imply an enhancement of the dopaminergic tone in the injected side. It should be noted, however, that although contralateral turning was not observed until 3 hours after the injection, significant ipsilateral turning was also reported during the first hour (Sacaan et al. 1992), which suggests the occurrence of antidopaminergic effects in early phases after the injection of mGlu receptor agonists.

Based on the examination of Fos-like immunoreactivity, a presumed indicator of neuronal activity, and on the effects elicited by lesioning the subthalamic nucleus (Kaatz and Albin 1995), it has been proposed that the appearance of delayed contralateral rotations by group I mGlu receptor agonists depend on the increased activity of nigrostriatal neurones on the injected side, secondary to the activation of the subthalamonigral projection. The excessive activation of the subthalamic nucleus, in turn, is thought to depend on an adenosine A_{2A} receptor-mediated increased activity of the striopallidal pathway (Kearney and Albin 1995; Kearney et al. 1997). Thus, the antidopaminergic effects reported here early after CHPG injection might reflect an early activation of the striopallidal pathway. Although

Table 1. Effects of the mGlu$_5$ Receptor Agonist CHPG on D$_2$ Receptor Agonist Binding Sites, as Shown in Competitive-Inhibition Experiments of Dopamine versus [^{3}H]Raclopride in Rat Striatal Membrane Preparation, and Its Modulation by the Adenosine A$_{2A}$ Receptor Agonist CGS 21680

Treatment	K$_H$ (nM)	K$_L$ (μM)	R$_H$ (%)
Control	15.8 (2.1–120.8)	2.3 (1.3–4.2)	30.6 ± 5.3
CHPG (10 nM)	18.7 (1.4–248.9)	1.7 (1.2–2.6)	30.8 ± 7.1
CHPG (100 nM)	86.4 (56.5–132.4)*	2.8 (1.8–4.5)	37.6 ± 3.1
CHPG (1000 nM)	86.1 (36.6–202.3)*	3.1 (1.7–5.6)	38.2 ± 4.9
Control	27.8 (11.9–65.3)	2.1 (1.4–2.9)	36.6 ± 6.0
CHPG (100 nM)	68.1 (22.8–202.8)*	2.8 (1.3–6.2)	39.0 ± 7.4
CGS 21680 (30 nM)	63.2 (28.3–140.9)*	1.9 (0.8–4.7)	31.6 ± 6.8
CHPG (100 nM) + CGS 21680 (30 nM)	104.1 (30.8–351.5)**	2.8 (0.7–12.4)	44.6 ± 8.1

K$_H$ and K$_L$ values are expressed as geometric means (antilogarithms of the logarithmically transformed data) and in parenthesis the 95% confidence limits of the geometric mean. R$_H$ values are expressed as means ± SEM ($n = 5$ per experiment). *$p < .05$ and **$p < .01$ compared to control (repeated measures ANOVA with Dunnett's post-hoc test).

the diffusion of ICV CHPG to brain areas other than the striatum must be considered, the high level of expression of mGlu$_5$ receptors in striatal neurones (Testa et al. 1995) suggests that these neurones may actually be a major locus where the effects of a selective mGlu$_5$ receptor agonist take place.

The finding that, at least in the dose range used here, CHPG does not influence striatal dopamine release suggests that the antagonistic effects exerted by CHPG on quinpirole- and SKF 38393-induced rotations are not attributable to an increase in dopamine release in the intact side (which would act in an opposite way with respect to the stimulation of supersensitive dopamine receptors in the lesioned side, thus reducing the directional bias induced by dopamine receptor agonists). Because microdialysis experiments were only performed in naive animals; however, we can argue that CHPG would have been effective in modulating dopamine release in lesioned rats. The fact that in lesioned rats CHPG failed to induce an appreciable turning in itself, however, clearly indicates that this is not the case. Thus, on the basis of the present results, we can conclude that CHPG does not directly stimulate dopamine release in the rat striatum. Because the group I mGlu receptor agonist DHPG has been reported to influence striatal dopamine release significantly (Bruton et al. 1999; Pintor et al. 2000), the inability of a selective mGlu$_5$ receptor agonist to mimic DHPG effects would suggest mGlu$_1$ receptors as being responsible, or mainly responsible, for the modulation of dopamine release in the rat striatum.

Although CHPG dose-dependently inhibited both quinpirole- and SKF 38393-induced contralateral rotations in 6-OH-DA-lesioned rats, a preferential effect toward quinpirole-induced turning was clearly seen. This view is supported by the following observations: (1) although the magnitude of the response elicited by quinpirole 0.1 mg/kg and SKF 3 mg/kg is not the same, these two doses can be viewed as fully comparable, because they are the minimal doses inducing a maximal effect, according to the dose–response curves performed in this model (Garrett and Holtzman 1995; Popoli et al. 2000); (2) only the contralateral rotations induced by quinpirole were significantly reduced by CHPG 1 μg, a dose that did not affect at all motor activity per se; and (3) the percentage of inhibition exerted by CHPG 6 μg was significantly greater ($p < .05$) toward quinpirole, than toward SKF 38393-induced turning. Thus, it can be concluded that CHPG preferentially inhibits D$_2$-dependent effects in 6-OH-DA-lesioned rats, an effect that is in line with the selective inhibition of quinpirole-induced turning by ICV 1S, 3R-ACPD (Ferré et al. 1999).

In striatal membranes, CHPG significantly decreased the affinity of D$_2$ receptors for dopamine, an interaction that may underlie the ability of CHPG to antagonize D$_2$-dependent turning. Because the same effect had been found in rat striatal membranes treated with DHPG (Ferré et al. 1999; Rimondini et al. 1999), the present findings indicate an involvement of mGlu$_5$ receptors in the effects of DHPG.

The effects of CHPG on D$_2$-dependent contralateral rotations are significantly potentiated by the adenosine A$_{2A}$ receptor agonist CGS 21680 and attenuated by the adenosine A$_{2A}$ receptor antagonist SCH 58261, thus suggesting an involvement of A$_{2A}$ receptors in CHPG-dependent inhibition. This finding is in full agreement with previous reports showing that striatal mGlu receptors and adenosine A$_{2A}$ receptors closely interact in the modulation of motor activity (Kearney and Albin 1995; Kearney et al. 1997). The involvement of adenosine A$_{2A}$ receptors in the effects elicited by CHPG is also supported by the results of the present binding studies, showing that CHPG exerts the same effects as the stimulation of adenosine A$_{2A}$ receptors; that is, a decrease in the affinity of D$_2$ receptors for the agonist in rat striatal membranes (Ferré et al. 1991), and that CGS 21680 potentiates the effects of CHPG. It should be noted, how-

ever, that although CGS 21680 markedly and significantly potentiates the effects of CHPG toward quinpirole-induced turning, only a weak, and not statistically significant, potentiation was observed in binding experiments. This discrepancy may indicate that the modulation of the binding characteristics of striatal dopamine D_2 receptors is not the only mechanism underlying the functional interaction occurring between mGlu$_5$ and A$_{2A}$ receptors at the behavioral level. In recent microdialysis experiments, we found, for example, that mGlu$_5$ and A$_{2A}$ receptors also interact at the presynaptic level in the modulation of striatal glutamate release (Pintor and Popoli unpublished results).

On the whole, these results show that mGlu$_5$ receptors functionally interact with adenosine A$_{2A}$ and dopamine D_2 receptors in the striatum. Such a functional interaction may be a critical factor in the regulation of the activity of striatopallidal pathway and then of motor activity. These findings suggest that mGlu$_5$ receptors may be regarded as alternative/integrative targets for the development of therapeutic strategies in the treatment of Parkinson's disease.

ACKNOWLEDGMENTS

REFERENCES

Albin RL, Young AB, Penney JB (1989): The functional anatomy of basal ganglia disorders. Trends Neurosci 12:366–375

Alexander GR, Crutcher MD (1990): Functional architecture and basal ganglia circuits: neural substrates of parallel processing. Trends Neurosci 13:266–271

Bruton RK, Ge J, Barnes NM (1999): Group I mGlu receptor modulation of dopamine release in the rat striatum in vivo. Eur J Pharmacol 369:175–181

Calabresi P, Pisani A, Mercuri NB, Bernardi G (1996): The corticostriatal projection: from synaptic plasticity to dysfunctions of the basal ganglia. TINS 19:19–24

Calabresi P, Centonze D, Pisani A, Bernardi G (1999): Metabotropic glutamate receptors and cell-type-specific vulnerability in the striatum: implication for ischemia and Huntington's disease. Exp Neurol 158:97–108

Carlsson M, Carlsson A (1990): Interactions between glutamatergic and monoaminergic systems within the basal ganglia—implications for schizophrenia and Parkinson's disease. TINS 13:272–276

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

Doherty AJ, Palmer MJ, Henley JM, Collingridge GL, Jane DE (1997): (RS)-2-cholro-5-hydroxyphenylglycine (CHPG) activates mGlu$_5$, but not mGlu$_1$, receptors expressed in CHO cells and potentiates NMDA responses in the hippocampus. Neuropharmacology 36:265–267

Ferré S, Von Euler G, Johansson B, Fredholm B, Fuxe K. (1991): Stimulation of high-affinity adenosine A-2 receptors decreases the affinity of dopamine D-2 receptors in rat striatal membranes. Proc Natl Acad Sci USA 88:7238–7241

Ferré S, O'Connor WT, Fuxe K, Ungerstedt U (1993): The striopallidal neuron: a main locus for adenosine–dopamine interactions in the brain. J Neurosci 13:5402–5406

Ferré S, Fredholm BB, Morelli M, Popoli P, Fuxe K (1997): Adenosine-dopamine receptor-receptor interactions as an integrative mechanism in the basal ganglia. TINS 20:482–487

Ferré S, Popoli P, Rimondini R, Reggio R, Kehr J, Fuxe K (1999): Adenosine A$_{2A}$ and group I metabotropic glutamate receptors synergistically modulate the binding characteristics of dopamine D_2 receptors in the rat striatum. Neuropharmacology 38:129–140

Fink JS, Weaver DR, Rivkees SA, Peterfreund RA, Pollack A, Adler DM, Reppert SM (1992): Molecular cloning of the rat A2 adenosine receptors: selective co-expression with D2 dopamine receptors in rat striatum. Mol Brain Res 14:186–195

Garrett BE, Holtzman SG (1994): Caffeine cross-tolerance to the selective dopamine D_1 and D_2 receptor agonists but not to their synergistic interaction. Eur J Pharmacol 262:65–75

Garrett BE, Holtzman SG (1995): The effects of dopamine agonists on rotational behavior in nontolerant and caffeine-tolerant rats. Behav Pharmacol 6:843–851

Gasparini F, Lingenhöhl K, Stoehr N, Flor PJ, Heinrich M, Vranesic I, Biollaz M, Allgeier H, Heckendorn R, Urwyeler S, Varney MA, Johnson EC, Hess SD, Rao SP, Sacaan AI, Santori EM, Veliçelebi G, Kuhn R (1999): 2-Methyl-6-(phenylethynyl)-pyridine (MPEP), a potent, selective, and systemically active mGlu5 receptor antagonists. Neuropharmacology 38:1493–1503

Gerfen CR, Engber TM, Mahan L, Susel Z, Chase TN, Monsma FJ, Sibley DR (1990): D1 and D2 dopamine receptor-regulated gene expression of strionigral and striopallidal neurons. Science 250:1429–1432

Kaatz KW, Albin RL (1995): Intrastriatal and intrasubthalamic stimulation of metabotropic glutamate receptors: A behavioral and FOS immunohistochemical study. Neuroscience 66:55–65

Kearney JAF, Albin RL (1995): Adenosine A$_2$ receptor-mediated modulation of contralateral rotation induced by metabotropic glutamate receptor activation. Eur J Pharmacol 287:115–120

Kearney JAF, Frey KA, Albin RL (1997): Metabotropic glutamate agonist-induced rotation: a pharmacologic, FOS immunohistochemical and [^{14}C]-2-deoxyglucose autoradiographic study. J Neurosci 17:4415–4425

Kearney JAF, Becker JB, Frey KA, Albin RL (1998): The role of nigrostriatal dopamine in metabotropic glutamate agonist-induced rotation. Neuroscience 87:881–891

Kronthaler UO, Schmidt WJ (1996): 1S-3R-ACPD has cataleptogenic effects and reverses MK-801 and less pronounced, d,l-amphetamine-induced locomotion. Eur J Pharmacol 316:129–136

Lipton SA, Rosenberg PA (1994): Excitatory amino acid as a final common pathway for neurologic disorders. N Engl J Med 330:613–622

Pintor A, Potenza RL, Domenici MR, Tiburzi F, Reggio R, Pèzzola A, Popoli P (2000): Age-related decline in the functional response of striatal group I mGlu receptors. NeuroReport 11:3033–3038

Popoli P, Reggio R, Pèzzola A (2000): Effects of SCH 58261, an adenosine A$_{2A}$ receptor antagonist, on quinpirole-induced turning in 6-hydroxydopamine-lesioned rats: lack of tolerance after chronic caffeine intake. Neuropsychopharmacology 22:522–529

Rimondini R, Fuxe K, Ferré S (1999): Multiple intramembrane receptor–receptor interactions in the regulation of striatal dopamine D$_2$ receptors. NeuroReport 10:2051–2054

Romano C, Sesma MA, McDonald CT, O'Malley K, Van Den Pol AN, Olney JW (1995): Distribution of metabotropic glutamate receptor mGluR5 immunoreactivity in rat brain. J Comp Neurol 355:455–469

Sacaan AI, Bymaster FP, Schoepp DD (1992): Metabotropic glutamate receptor activation produces extrapyramidal motor system activation that is mediated by striatal dopamine. J Neurochem 59:245–251

Schiffmann SN, Jacobs O, Vanderhaeghen J-J (1991): Striatal restricted adenosine A$_2$ receptor (RDC8) is expressed by enkephalin but not by substance P neurons: an in situ hybridization histochemistry study. J Neurochem 57:1062–1067

Schwarting PKW, Huston JP (1996): The unilateral 6-hydroxydopamine lesion model in behavioral brain research. Analysis of functional deficits, recovery, and treatments. Prog Neurobiol 50:275–331

Smith ID, Beninger RJ (1996): Contralateral turning caused by metabotropic glutamate receptor stimulation in the dorsal striatum is reversed by MCPG, TTX, and cis-Flupenthixol. Behav Neurosci 110:282–289

Starr MS (1995): Glutamate/dopamine D1/D2 balance in the basal ganglia and its relevance to Parkinson's disease. Synapse:264–293

Starr MS, Starr BS, Kaur S (1997): Stimulation of basal and L-DOPA-induced motor activity by glutamate antagonists in animal models of Parkinson's disease. Neurosci Biobehav Rev 21:437–446

Svenningsson P, Le Moine C, Kull B, Sunahara R, Bloch B, Fredholm BB (1997): Cellular expression of adenosine A$_{2A}$ receptor messenger RNA in the rat central nervous system with special reference to dopamine innervated areas. Neuroscience 80:1171–1185

Tallaksen-Greene SJ, Kaatz KW, Romano C, Albin RL (1998): Localization of mGluR1α-like immunoreactivity and mGlur5-like immunoreactivity in identified populations of striatal neurons. Brain Res 780:210–217

Testa CM, Standaert DG, Young AB, Penney JB (1994): Metabotropic glutamate receptor mRNA expression in the basal ganglia of the rat. J Neurosci 14:3005–3018

Testa CM, Standaert DG, Landwehrmeyer GB, Penney JB, Young AB (1995): Differential expression of mGluR5 metabotropic glutamate receptor mRNA by rat striatal neurones. J Comp Neurol 354:241–252

Ungerstedt U (1971): Postsynaptic supersensitivity after 6-hydroxydopamine induced degeneration of the nigrostriatal dopamine system in the rat brain. Acta Physiol Scand 82(Suppl. 367):69–93

Vezina P, Kim J-H (1999): Metabotropic glutamate receptors and the generation of locomotor activity: interactions with midbrain dopamine. Neurosci Biobehav Rev 23:577–589