D1 and D2 dopamine receptors contribute to the locomotor response induced by Group II mGluRs activation in the rat nucleus accumbens

Nathalie Breysse1, Céline Risterucci1, Marianne Amalric*

CNRS and Université de la Méditerranée, France

Received 11 June 2001; received in revised form 23 January 2002; accepted 8 February 2002

Abstract

Whereas the involvement of ionotropic glutamate receptors (iGluRs) in the functional interaction between glutamate and dopamine (DA) systems in the nucleus accumbens (N. Acc.) is well established, the role of metabotropic glutamate receptors (mGluRs) is less clear. This study was thus aimed to investigate the mechanisms involving DA and glutamate systems via mGluRs in the generation of motor activity in rats. Intra-accumbens infusion of the Group II agonist (2S,3S,4S)-2-(carboxycyclopropyl)glycine (L-CCG-I; 25, 50, 100 nmol) increased locomotor activity, whereas the Group I agonist (S)-3,5-dihydroxyphenylglycine (S-3,5-DHPG) at the same doses had no effect. The effects of L-CCG-I were blocked by a selective Group II mGluRs antagonist (2S,3S,4S)-2-methyl-2-(carboxypropyl)glycine (MCCG; 50 nmol). The locomotor stimulant effect induced by L-CCG-I might be partly DA mediated, as it is abolished by a pretreatment with the DA receptor antagonist haloperidol (0.1 mg/kg ip) and potentiated by D-amphetamine systemic injection (0.5 mg/kg sc). Furthermore, selective D1 (SCH 23390; 0.005, 0.01 and 0.02 mg/kg) or D2 (raclopride; 0.05, 0.1 and 0.2 mg/kg) antagonists injected systemically were also effective in decreasing L-CCG-I induced hyperactivity. Taken together, these results demonstrate that stimulation of Group II but not Group I mGluRs contributes to the regulation of motor behavior in the N. Acc. and that this increased activity requires the activation of both D1 and D2 DA receptors. © 2002 Elsevier Science Inc. All rights reserved.

Keywords: Metabotropic glutamate receptors; Dopamine; L-CCG-I; DHPG; Amphetamine; Locomotor activity; Nucleus accumbens

1. Introduction

The nucleus accumbens (N. Acc.) located in the ventral part of the striatum is involved in the control of motor behavior, mood disorders and drug abuse. The mesolimbic dopamine (DA) system, which originates in the ventral tegmental area and projects heavily to the N. Acc., is known to interact with the glutamatergic system to modulate these functions. The N. Acc. receives extensive glutamatergic projections from the prefrontal cortex, the subiculum of the hippocampus, the amygdala and the intralaminar nuclei of the thalamus (Berendse et al., 1992; Christie et al., 1987; Sesack et al., 1989; Meredith et al., 1993). Considerable evidences now indicate that agonists of ionotropic glutamate receptors (iGluRs), such as N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA), increase extracellular levels of DA and locomotor activity in rodents when infused into the N. Acc. (for review, see Schmidt et al., 1992). These effects are reversed by DA receptor antagonists either systemically or intracerebrally administered. Conversely, competitive NMDA receptor antagonists also produced stimulant effects that are DA dependent (Carlsson and Carlsson, 1989; Schmidt et al., 1992; Ouagazzal and Amalric, 1995).

While the functional role of iGluRs and their interaction with DA receptors has been addressed in numerous studies since the past decade, the more recent development of selective ligands for the second class of receptors activated by glutamate, the metabotropic glutamate receptors (mGluRs), has given the opportunity to study these interactions in light of the modulatory action of glutamate on second-messenger coupled receptors. Molecular cloning has...
indeed established the existence of at least eight mGluRs subtypes with different patterns of expression in the brain with a relatively high concentration in the basal ganglia including the N. Acc. (Testa et al., 1995; Tallaksen-Greene et al., 1998). Based on primary sequence, second-messenger coupling and pharmacological profiles, mGluRs can be classified into three subgroups: Group I (mGluR1 and mGluR5), Group II (mGluR2 and mGluR3) and Group III (mGluR4, 6, 7 and 8) (Conn and Pin, 1997 for review). Group I receptors are predominantly located postsynaptically whereas Groups II and III are thought to act mainly on presynaptic receptors to regulate glutamate transmission (Testa et al., 1995; Shigemoto and Mizuno, 2000), although mGluR2/3 receptors may also be found postsynaptically (Neki et al., 1996). Initial studies, using nonsel ective agonists or antagonists, investigated the functional interaction between DA and glutamate systems via mGluRs in motor behavior regulation at the level of the N. Acc. (Attarian and Amalric, 1997; Kim and Vezina, 1997, 1998; Vezina and Kim, 1999). For example, the bilateral infusion of the Group I/II mGluR agonist 1S,3R-1-amino-cyclopentane-1,3-dicarboxylic acid (1S,3R-ACPD) into the N. Acc. has been shown to increase the locomotor activity in rats, a behavioral effect reversed by the nonsel ective antagonist α-methyl-4-carboxyphenylglycine (MCPG) (Attarian and Amalric, 1997; Kim and Vezina, 1997). These effects were DA-dependent since the blockade of DA receptors with DA antagonists reduced the 1S,3R-ACPD induced locomotor hyperactivity, whereas d-amphetamine potentiated this motor activation (Attarian and Amalric, 1997). Furthermore, microdialysis studies also reported that 1S,3R-ACPD in the striatum increased extracellular levels of DA in the N. Acc. (Ohno and Watanabe, 1995; Taber and Fibiger, 1995; Verma and Moghaddam, 1998). The recent development of selective mGluRs ligands allowed further investigations on the specific contribution of each group of mGluRs to these effects. Unfortunately, the results obtained report apparently conflicting results (Swanson and Kalivas, 2000; Kronthaler and Schmidt, 2000; David and Abraini, 2001a,b). Thus, although these data provide strong evidence for functional interactions between mGluRs and DA transmission in the N. Acc., the exact role of the different mGluRs subtypes and the mechanisms underlying this interaction remain to be clarified. In this study, we investigated whether the selective activation of Group I and/or Group II could modify the spontaneous locomotor activity in rats and if these effects were modulated by the DA D1 or D2 receptor subtypes.

2. Materials and methods

2.1. Animals and surgery

Male albino Wistar rats weighing 280–300 g on arrival from Iffa-Credo (Lyon, France) were used. They were housed in groups of two per cage with food and water available ad libitum and were maintained in temperature-controlled conditions with an alternating 12 h light–12 h darkness cycle (lights on at 0700 h). All experiments were performed during the light cycle. The animals were anesthetized by intramuscular injection of xylazine (15 mg/kg; Bayer, Leverkusen) and ketamine (100 mg/kg; Merial, Lyon, France). Once anesthetized, animals were mounted on a stereotaxic apparatus (Kopf Instrument, Tujunga, CA) with the tooth bar set 5 mm above the interaural line, and were implanted with 10 mm bilateral stainless steel guide cannulae (23 gauge) positioned 3 mm above the N. Acc. The coordinates used for the N. Acc. were according to the atlas of Pellegrino et al. (1979), and were as follows: AP + 3.2 mm; L ± 1.7 mm from the bregma; DV − 4.8 mm from the skull surface. The cannulae were anchored to the skull with four stainless steel screws and dental cement. Stainless steel wire styles (10 mm) were inserted into the cannulae to prevent occlusion. A recovery period of 1 week was allowed before testing. All animal-use 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.

2.2. Drugs and intracranial microinjections

The drugs used in the present study to target the mGluRs were: the Group I mGluR agonist (RS)-3,5-dihydroxyphenylglycine (DHPG); the Group II mGluR agonist (S)-3,5-dihydroxyphenylglycine (MCCG) (Tocris Neuramin, UK). The compounds used to stimulate or inactivate the dopaminergic system were: the indirect dopaminergic receptor agonist d-amphetamine; the mixed D1/D2 dopaminergic receptor antagonist haloperidol (Haldol injectable solution; Janssen, Boulogne, France); the D2-like receptor antagonist SCH 23390 (Tocris Neuramin, UK). The compounds used to stimulate or inactivate the dopaminergic system were: the indirect dopaminergic receptor agonist d-amphetamine; the mixed D1/D2 dopaminergic receptor antagonist haloperidol (Haldol injectable solution; Janssen, Boulogne, France); the D1-like receptor antagonist SCH 23390 (Tocris Neuramin, UK). and the D2-like receptor antagonist raclopride (a generous gift from Astra laboratories, Sweden).

(S)-3,5-DHPG and MCCG were dissolved in a physiological 0.9% saline solution with a minimal quantity of NaOH (0.5 N). t-CCG-I was dissolved with a few drops of NaOH (0.5 N) and was diluted with phosphate buffer 0.2M (PB 0.2M). The pH was adjusted to 7.0 with HCl for all mGluRs agonist and antagonist solutions. Haloperidol, d-amphetamine, SCH 23390 and raclopride were dissolved in physiological 0.9% saline solution and were injected systemically in a volume of 1 ml/kg. According to previous experiments (Ouagazzal et al., 1993), haloperidol, SCH 23390 and raclopride were injected intraperitoneally 30 min before testing. d-Amphetamine was injected immediately before testing.

On the test day, a bilateral 30-gauge injection needle was inserted through the guide to 3 mm beyond its tip and (S)-3,5-DHPG, MCCG and t-CCG-I solution were bilaterally in-
fused in a volume of 0.5 μl per side at the rate of 0.16 μl/min with a Hamilton syringe mounted on a microdrive pump (CMA/100; CMA, Stockholm, Sweden). After drug injection, injection needles were left in situ for another 2 min to allow diffusion of the drug away from the tips. Immediately afterwards, the animals were placed in the test apparatus.

2.3. Measurement of locomotor activity

A bank of 16 individual wire (top, floor and front door) and Plexiglas (side walls) photocell cages was used to measure the locomotor activity. Each cage (40 × 25 × 23 cm) was fitted with two parallel horizontal infrared beams, 1 cm above the floor, located across the long axis of the cage (Imetrionic, Pessac, France). Beam interruptions were accumulated over 1-min intervals and recorded in bins of 1 min by an one-line input to a microcomputer. The animals were familiarized with the experimental cages during a 3-h session, 1 day before the test session. On the day of testing, the spontaneous locomotor activity was monitored for 120 min prior to any drug treatment. The compounds were then administered intracerebrally and/or systemically to the animals, and their locomotor activity was monitored for 180 min.

2.4. Experimental design

Prior to any drug treatment, all the animals were first infused with a vehicle control solution (0.9% NaCl) in order to familiarize them with the experimental procedure.

In a first series of experiments, we investigated the functional involvement of Group I and Group II mGluRs in motor behavior. The effects of the Group I mGluRs agonist (DHPG) in the N. Acc. were tested on a first group of animals in a dose range of 0, 25, 50, 100 nmol/0.5 μl per side (corresponding to 0.27, 0.46 and 0.92 μg/0.5μl; n = 8). In view of the lack of effects produced by these low doses, another group of animals (n = 10) was given a bilateral infusion of higher doses of DHPG in a dose range of 0, 25, 50, 100 nmol/0.5 μl per side (corresponding to 2.29, 4.58 and 9.16 μg/0.5μl). All the animals received the different doses of DHPG injected following a Latin square design. Injections were performed once a week for 4 weeks. A second group of rats (n = 11) received a bilateral infusion of the Group II mGluRs agonist (l-CCG-I) into the N. Acc. (0, 25, 50, 100 nmol/0.5 μl per side corresponding to 1.99, 3.95 and 7.96 μg/0.5μl). Each animal received the different doses following a Latin square design.

In a second series of experiments, we verified the specificity of action of l-CCG-I on Group II mGluRs by coadministering l-CCG-I with a selective Group II antagonist (MCCG). A first group of animals (n = 10) was administered with l-CCG-I (50 nmol/0.5 μl) immediately prior to the microinfusion of MCCG (50 nmol/0.5 μl). A second group (n = 8) received a bilateral microinfusion of vehicle (PB 0.2M, i.e., l-CCG-I solvent) prior to a bilateral microinfusion of the other vehicle solution (NaCl 0.9%, i.e., MCCG solvent). A third group (n = 10) received the PB 0.2M solution prior to MCCG (50 nmol/0.5 μl) infusion. A fourth group (n = 8) received a microinfusion of l-CCG-I (50 nmol/0.5 μl) prior to NaCl 0.9% intracerebrally. In these experiments, each subject was used only once. MCCG treatment was further tested during the dark cycle to examine any nonspecific sedative effect on a higher level of spontaneous locomotor activity.

In a third series of experiments, the functional interaction between glutamate and DA systems was assessed using Group II mGluRs agonist l-CCG-I and simultaneous injections of compounds known to stimulate or inactivate the dopaminergic system. The first experiment tested the behavioral effects induced by the coactivation of the two systems. A first group of animals (n = 10) received a microinfusion of l-CCG-I (50 nmol/0.5 μl) in the N. Acc. followed by a subcutaneous injection of d-amphetamine (0.5 mg/kg). A first control group (n = 11) was given a vehicle intracerebral injection (i.e., l-CCG-I solvent) and NaCl 0.9% systemically (i.e., d-amphetamine solvent). A second control group (n = 10) was injected with an intra-accumbal infusion of vehicle and received an injection of d-amphetamine. The effects of l-CCG-I (50 nmol/0.5 μl) infused into the N. Acc. with a systemic injection of NaCl 0.9% were also tested in an additional group of rats (n = 9). The next experiments were aimed at testing the effects of Group II mGluRs activation and DA receptors blockade. In a first experiment, one group of rats was pretreated with the D1/D2 DA receptor antagonist haloperidol (0.1 mg/kg ip) 25 min before infusion into the N. Acc. of 50 nmol l-CCG-I (n = 5). A second group (n = 5) received an injection of haloperidol solvent prior to infusion of l-CCG-I (50 nmol/0.5 μl). A third group was injected with both solvent (n = 5). Each animal was used only once. In a second experiment, one group of rats (n = 14) was pretreated with the D1 DA receptor antagonist SCH 23390 (0.005, 0.01, 0.02 mg/kg) which was injected intraperitoneally 25 min before infusion of l-CCG-I (50 nmol). All animals received either l-CCG-I only or the coadministration of l-CCG-I and SCH 23390 at the three doses tested, in different orders, following a Latin square design. In a third experiment, one group of rats (n = 12) was pretreated with the D2 DA receptor antagonist raclopride (0.05, 0.1, 0.2 mg/kg) which was injected intraperitoneally 25 min before infusion of l-CCG-I (50 nmol), and in the same fashion than with SCH 23390 injection. Concentrations of haloperidol, raclopride and SCH 23390 were chosen on the basis of earlier studies showing no sedative effects of these compounds on catalepsy or locomotor activity test (Amalric et al., 1993; Attarian and Amalric, 1997) or in an operant reaction time task (Amalric et al., 1993).

2.5. Histology

At the end of the experiment, animals were killed by decapitation. The brains were then removed and frozen.
Brain coronal sections were cut (20 μm) with a cryostat and stained with Cresyl violet to check the accuracy of the injection sites and the morphological structure of the surrounding tissue. Only rats showing the appropriate injection sites were used for data analysis.

2.6. Statistical analysis

Analysis of the data was carried out using a two-factor analysis of variance (ANOVA). When animals were only injected once, the different groups (doses of the various experimental treatments) were the independent factor and time was considered the repeated measure. When the effect was found to be significant, post hoc comparisons between the different groups were carried out using the Newman–Keuls test. The significance level was taken to be $P < .05$. When animals were injected according to a Latin square design, we first analysed the effect of the order of injections between the different groups tested. The subjects that did not receive all the injections within a given order (loss of cannula for example) were excluded from statistical analysis. If the order of injections did not produce any significant effect, all the animals injected with the same treatment were pooled into one group and the differences between the various doses over time were subjected to the same two-factor ANOVA as described above. When the ANOVA revealed a significant effect, post hoc comparisons were carried out using the Newman–Keuls test.

3. Results

3.1. Histology

The location sites of the cannula tips placed into the N. Acc. are illustrated in Fig. 1A and B. Most placements fell within the N. Acc. in the anterior planes from +4.0 to +3.0 mm from the bregma according to the atlas of Pellegrino et al. (1979), as illustrated in Fig. 1B for 17 subjects of the first set of experiments. Eight animals with injector misplacements were discarded. No evidence of excitotoxicity was found in the tissue surrounding the cannula tracks after intra-accumbens infusion of DHPG, 1-CCG-I and MCCG. The morphological structure of the N. Acc. was preserved and was not different from that in vehicle-infused animals.

3.2. Involvement of Group I and Group II mGluRs in the N. Acc. in the generation of locomotor activity

3.2.1. Effect of Group I mGluR agonist on locomotor activity

Activation of Group I mGluRs in the N. Acc. with the receptor agonist DHPG at low concentrations (3, 5 and 10 nmol/0.5 μl) did not produce any significant effect on locomotor behavior (Table 1). When tested in higher concentrations (25, 50 and 100 nmol/0.5 μl), DHPG did not produce any effect either, although a nonsignificant tendency to an overall increased activity was observed after 90 min at the highest doses (Fig. 2A).
3.2.2. Effect of Group II mGluR agonist on locomotor activity

Activation of Group II mGluRs in the N. Acc. with the receptor agonist L-CCG-I produced a significant effect on locomotor activity. As can be seen in Fig. 2B, the locomotor activity was enhanced within the first minutes following the intra-accumbens infusion of L-CCG-I. Following a non-significant effect of the order of injections \[ F(3,7) = 1.33, P > .05 \], the ANOVA testing the effects of L-CCG-I over time demonstrated a significant main effect of dose \[ F(3,30) = 6.43, P < .05 \] and a significant main effect of time \[ F(17,170) = 33.37, P < .001 \]. In addition, a significant Dose × Time \[ F(51,510) = 1.65, P < .05 \] was found. Post hoc comparisons showed that the locomotor activity after the highest doses of L-CCG-I (50 and 100 nmol) was significantly increased compared with the control vehicle infusion \( P < .05 \), Newman–Keuls test). Since no significant difference on locomotor activity was found between these two doses, we thus selected the dose of 50 nmol for subsequent experiments, testing the functional interactions between Group II mGluRs and DA activity.

3.2.3. Locomotor response to Group II mGluRs antagonist MCCG infused in the N. Acc. as a single treatment or in combination with L-CCG-I

In order to assess the specificity of L-CCG-I on Group II mGluRs in producing a locomotor response, the effects of the phenylglycine derivative MCCG, a competitive antagonist of Group II mGluRs, on baseline locomotion and on L-GGG-I induced locomotor stimulation were further studied. Pretreatment with MCCG (50 nmol) decreased the locomotor hyperactivity induced by the subsequent infusion of 50 nmol L-CCG-I into the N. Acc. (Fig. 3). The overall ANOVA revealed a significant main effect of drug treatment \[ F(3,32) = 5.88, P < .05 \] and time \[ F(17,544) = 45.91, P < .001 \]. MCCG injected alone at the dose of 50 nmol did not modify the basal level of locomotion when compared to control animals receiving a combined vehicle infusion (PB 0.2 M followed by NaCl 0.9% into the N. Acc.; \( P > .05 \), Newman–Keuls test). When tested during the nocturnal cycle of the rats, when the basal level of locomotor activity is enhanced, MCCG at the dose of 50 nmol did not produce any detectable change, indicating that this dose did not lead to nonspecific sedative effects. In contrast, animals treated with L-CCG-I at a dose of 50 nmol prior to vehicle infusion showed a level of locomotion that was significantly different from that observed in controls or in animals following infusions of MCCG \( P < .05 \), New-

Table 1

<table>
<thead>
<tr>
<th>Effect of intra-accumbens infusion of DHPG on locomotor activity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Doses</strong> (nmol/0.5 μl)</td>
</tr>
<tr>
<td>------------------------</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>10</td>
</tr>
</tbody>
</table>

Rats were given various low doses of DHPG (3, 5 and 10 nmol/0.5 μl) bilaterally into the N. Acc. and their locomotor activity was recorded for 180 min. Values are mean locomotor activity counts ± S.E.M. for the 180-min test period.

Fig. 2. Effects of intra-accumbens infusion of a Group I mGluR agonist (DHPG; A) or a Group II mGluR agonist L-CCG-I on locomotor activity (B). Immediately after the bilateral infusion of the compounds, rats were placed in the photocell activity cages and locomotor activity was recorded for 180 min. DHPG at the various doses tested (25, 50 and 100 nmol/0.5 μl; \( n = 10/dose \)) had no effect on locomotor behavior (A). L-CCG-I (25, 50 and 100 nmol/0.5 μl; \( n = 11/dose \)) induced a robust and long-lasting increase in locomotor activity at the highest doses (B). The ordinate gives the mean number of photocell counts in 10-min periods. The insert shows the total activity counts (± S.E.M.) during the 180-min test. * Significantly different from controls, \( P < .05 \), Newman–Keuls test after significant ANOVA.
man–Keuls test). Animals coadministered with MCCG and L-CCG-I in the N. Acc. showed a level of locomotion that was significantly lower than that observed in animals following L-CCG-I as a single treatment \((P < .05, \text{Newman–Keuls test})\).

3.3. Functional interactions between Group II mGluR and the dopaminergic system

3.3.1. Effects of combined \(\alpha\)-amphetamine and l-CCG-I administration on locomotor activity

Consistent with previous reports (Ouagazzal and Amalric, 1995; Attarian and Amalric, 1997) \(\alpha\)-amphetamine injection at a dose of 0.5 mg/kg induced a marked increase in locomotor activity (Fig. 4). Coadministration of \(\alpha\)-amphetamine (0.5 mg/kg) injected systemically and l-CCG-I (50 nmol) infused bilaterally into the N. Acc. produced a significant increase in locomotor activity when compared to levels observed in rats treated either with l-CCG-I or \(\alpha\)-amphetamine alone (Fig. 4). A two-factor ANOVA revealed significant main effects of group treatment \([F(3,36) = 16.57, P < .0001]\), time \([F(17,612) = 50.90, P < .0001]\) and a significant Group \(\times\) Time interaction \([F(51,612) = 9.139, P < .0001]\). Post hoc comparisons revealed that, as expected, \(\alpha\)-amphetamine injection or l-CCG-I infusion produced a robust and long-lasting locomotor response when compared with vehicle-injected rats (Newman–Keuls test, \(P < .05\)). Furthermore, the level of locomotor hyperactivity following coadministration of \(\alpha\)-amphetamine and l-CCG-I was significantly higher than that of rats treated either with l-CCG-I or \(\alpha\)-amphetamine alone \((P < .05; \text{Newman–Keuls test})\). However, no significant difference was revealed between these two latter groups, indicating a similar behavioral activation produced by each treatment over the 180-min testing period.

3.3.2. Effects of DA receptors blockade and l-CCG-I infusion on locomotor activity

The effects of DA receptors blockade on the locomotor response to l-CCG-I were further studied in a series of experiments using the mixed D1/D2 receptor antagonist haloperidol. Previous studies have shown that haloperidol at similar concentrations dose-dependently reduced the locomotor response to the nonselective mGluR agonist, 1S,3R-ACPD, without producing any sedative effects (Attarian and Amalric, 1997). As illustrated in Fig. 5, the motor response induced by l-CCG-I (50 nmol) was also significantly abolished by pretreatment with haloperidol (25 min before l-CCG-I infusion into the N. Acc.). The two-factor ANOVA revealed a significant main effect of group \([F(2,13) = 8.26, P < .05]\), a significant main effect of time \([F(17,221) = 9.49, P < .001]\) and a significant Drug \(\times\) Time interaction \([F(34,221) = 2.40, P < .001]\). Post hoc comparisons revealed that, as expected, l-CCG-I treated animals, \(P < .05\), Newman–Keuls test after significant ANOVA.

Fig. 3. Effects of the Group II mGluRs antagonist (MCCG) on the locomotor stimulating effects of L-CCG-I. One group of rats \((n = 10)\) received a bilateral infusion of l-CCG-I (50 nmol/0.5 \(\mu\)l) immediately prior to MCCG (50 nmol/0.5 \(\mu\)l) infusion in the N. Acc. Control experiments were conducted to test the effects of each compound infused on its own and coadministered along with the respective vehicle solution of the other compound to check for double intracerebral injection procedure. Group 2 \((n = 8)\) was administered Ve1 (PB 0.2 M, i.e., solvent of L-CCG-I) and Ve2 (NaCl 0.9%, i.e., solvent of MCCG). Group 3 received Ve1 and MCCG at a dose of 50 nmol (0.5 \(\mu\)l). Group 4 \((n = 8)\) received l-CCG-I at a dose of 50 nmol (0.5 \(\mu\)l) prior to Ve2 infusion. * Significantly different from controls and MCCG-treated animals, \(P < .05\), Newman–Keuls test after significant ANOVA. \(\bullet\) Significantly different from L-CCG-I treated animals, \(P < .05\), Newman–Keuls test after significant ANOVA.

Fig. 4. Effects of simultaneous administration of L-CCG-I infused in the N. Acc. and D-amphetamine (0.5 mg/kg ip) on locomotor activity. Rats received a microinfusion of vehicle (PB 0.2M, Ve1) or L-CCG-I (50 nmol/0.5 \(\mu\)l) into the N. Acc. immediately before D-amphetamine injection. Additional control groups were infused with vehicle or L-CCG-I (50 nmol/0.5 \(\mu\)l) as a single treatment. * Significantly different from controls, \(P < .05\), Newman–Keuls test after significant ANOVA. \(\bullet\) Significantly different from L-CCG-I or D-amphetamine as a single treatment, \(P < .05\), Newman–Keuls test after significant ANOVA.
hoc comparison revealed that rats pretreated with haloperidol exhibited a significant lower locomotor response to L-CCG-I when compared with L-CCG-I treatment ($P < .05$, Newman–Keuls test). The level of locomotor activity was not any different from that of vehicle-injected animals ($P > .05$, Newman–Keuls test).

### 3.3.3. Effects of selective D1 or D2 receptors blockade on L-CCG-I induced locomotor activity

In order to specify the DA receptor subtype involved in this interaction, a selective blockade of D1 or D2 DA receptors was further tested with the SCH 23390 or raclopride compounds, respectively (Fig. 6A and B). The motor response induced by L-CCG-I (50 nmol) was reduced by a pretreatment with SCH 23390 injected intraperitoneally. The decreased activity produced by L-CCG-I administration was observed at all the doses of SCH 23390 tested (0.005, 0.01 and 0.02 mg/kg). No significant effect of the order of injection was found [$F(3,10) = 0.42$, $P > .05$]. The following overall ANOVA demonstrated a significant main effect of dose [$F(3,39) = 5.15$, $P < .05$] and a significant main effect of time [$F(17,221) = 10.57$, $P < .001$]. In addition, a significant Drug × Time interaction [$F(51,663) = 4.046$, $P < .001$] was measured. Post hoc comparisons showed that locomotor activity for rats infused with L-CCG-I and pre-treated with SCH 23390 were significantly decreased compared with rats which received L-CCG-I and the SCH 23390 vehicle ($P < .05$, Newman–Keuls test).

Furthermore, the motor response induced by L-CCG-I (50 nmol) was totally blocked by the DA D2 receptor antagonist raclopride. The ANOVA testing the effects of the order of injection [$F(3,8) = 4.216$, $P < .05$] showed a significant blockade of activity when the highest dose of raclopride was injected first in comparison to the other orders of injection. The following ANOVA demonstrated a significant effect of Doses × Time interaction [$F(51,561) = 7.33$, $P < .001$]. All doses of raclopride tested (0.05, 0.1 and 0.2 mg/kg) potently abolished the locomotor response to L-CCG-I infusion in a similar way ($P < .05$, Newman–Keuls test).
4. Discussion

Recent studies revealed that mGluRs in the basal ganglia contribute importantly to the generation of motor behaviors (Sacaan et al., 1992; Kronthaler and Schmidt, 1996; Feeley Kearney et al., 1997; Vezina and Kim, 1999; Kim et al., 2000; Wang and Mao, 2000; Swanson and Kalivas, 2000; David and Abraini, 2001a,b). While experiments conducted with the nonselective Group I/II agonist 1S,3R-ACPD in the N. Acc. reported a general increase in motor functions (Attaran and Amalric, 1997; Kim and Vezina, 1997, 1998; Vezina and Kim, 1999), recent experiments using more selective pharmacological ligands for the Group I, Group II or Group III mGluRs reported apparently conflicting results (Swanson and Kalivas, 2000; Kronthaler and Schmidt, 2000; David and Abraini, 2001a,b). The present results demonstrate that the Group II mGluRs agonist L-CCG-I infused bilaterally in the N. Acc. produced a motor stimulant response whereas the stimulation of the Group I receptors with (S)-3,5-DHPG had no effect on locomotor activity. The specificity of L-CCG-I actions was assessed by coinfusion of the Group II mGluRs antagonist, MCCG, in the same site. MCCG did not modify the spontaneous locomotor activity but prevented the locomotor response to L-CCG-I. The locomotor hyperactivity induced by L-CCG-I appeared to be DA dependent since the blockade demonstrated that the Group II mGluRs agonist L-CCG-I on the other hand induced a robust increase in locomotor activity at doses of 50 and 100 nmol. Furthermore, the blockade of L-CCG-I-induced locomotor hyperactivity by the Group II mGluR antagonist MCCG confirms the involvement of Group II receptors in these effects. Although L-CCG-I is only 10–100 fold more selective for Group II over Group I mGluRs (Hayashi et al., 1992; Lombardi et al., 1993), the activation of Group I with the selective Group I agonist DHPG did not modify locomotor activity. The present finding is in line with a recent experiment demonstrating that intra-accumbal infusion of the agonist Group II mGluRs DCG-IV produced a short-duration increase of locomotor activity in rats (Swanson and Kalivas, 2000). It should be noted however that other studies reported no effect of other Group II receptor agonists on spontaneous locomotor activity when administered intra-cerebrally (Kronthaler and Schmidt, 2000; David and Abraini, 2001a) or systemically (Helton et al., 1998; Moghaddam and Adams, 1998). Such a discrepancy could be due to the different pharmacological profile of the drugs used and, as previously mentioned for Group I mGluRs, to the different site of action of these compounds in the brain. For example, L-CCG-I depressed spontaneous motor behavior when infused in the dorsal striatum at doses similar to those used in the present study (Kronthaler and Schmidt, 2000). Consistent with previous reports indicating that mGluRs antagonists such as (R,S)-MCPG, EGLU and AIDA had no effect on spontaneous activity (Attaran and Amalric, 1997; Kim and Vezina, 1998; Kim et al., 2000), MCCG by itself had no effect on locomotor activity, suggesting that Group II mGluRs localized in the N. Acc. are not involved in the tonic regulation of motor behavior. L-CCG-I-induced hyperactivity was partially reversed by MCCG pretreatment at a single dose of 50 nmol. While higher concentrations of MCCG may have totally reversed it, it might also be suggested that L-CCG-I is still acting on Group III as it also exhibits appreciable affinities for mGluR4 (Hayashi et al., 1992; Brabet et al., 1998; Schoepp et al., 1999 for review).

Furthermore, it is well known that the specificity of pharmacological compounds is highly dependent upon the concentrations used and those chosen in the present study are well below those reported to produce behavioral modifications or to generate side effects such as convulsion-like reactions or sedation (Van der Staa et al., 1995; Kearney et al., 1997; Kronthaler and Schmidt, 1998). For all the experiments involving L-CCG-I, the lowest dose of 50 nmol of our site of injection mainly distributed in the core region. In line with these findings, 80 nmol DHPG infused into the shell produced a long-lasting increased locomotor activity in rats (Wang and Mao, 2000). The location of the brain site for DHPG to produce its stimulant effect appears thus to be critical since it was even found to induce a moderate reduction of locomotor activity when injected intraventricularly (Kronthaler and Schmidt, 1998).

Activation of Group II mGluRs in the N. Acc. with the agonist L-CCG-I on the other hand induced a robust increase in locomotor activity at doses of 50 and 100 nmol. Furthermore, the blockade of L-CCG-I-induced locomotor hyperactivity by the Group II mGluR antagonist MCCG confirms the involvement of Group II receptors in these effects. Although L-CCG-I is only 10–100 fold more selective for Group II over Group I mGluRs (Hayashi et al., 1992; Lombardi et al., 1993), the activation of Group I with the selective Group I agonist DHPG did not modify locomotor activity. The present finding is in line with a recent experiment demonstrating that intra-accumbal infusion of the agonist Group II mGluRs DCG-IV produced a short-duration increase of locomotor activity in rats (Swanson and Kalivas, 2000). It should be noted however that other studies reported no effect of other Group II receptor agonists on spontaneous locomotor activity when administered intra-cerebrally (Kronthaler and Schmidt, 2000; David and Abraini, 2001a) or systemically (Helton et al., 1998; Moghaddam and Adams, 1998). Such a discrepancy could be due to the different pharmacological profile of the drugs used and, as previously mentioned for Group I mGluRs, to the different site of action of these compounds in the brain. For example, L-CCG-I depressed spontaneous motor behavior when infused in the dorsal striatum at doses similar to those used in the present study (Kronthaler and Schmidt, 2000). Consistent with previous reports indicating that mGluRs antagonists such as (R,S)-MCPG, EGLU and AIDA had no effect on spontaneous activity (Attaran and Amalric, 1997; Kim and Vezina, 1998; Kim et al., 2000), MCCG by itself had no effect on locomotor activity, suggesting that Group II mGluRs localized in the N. Acc. are not involved in the tonic regulation of motor behavior. L-CCG-I-induced hyperactivity was partially reversed by MCCG pretreatment at a single dose of 50 nmol. While higher concentrations of MCCG may have totally reversed it, it might also be suggested that L-CCG-I is still acting on Group III as it also exhibits appreciable affinities for mGluR4 (Hayashi et al., 1992; Brabet et al., 1998; Schoepp et al., 1999 for review).

Furthermore, it is well known that the specificity of pharmacological compounds is highly dependent upon the concentrations used and those chosen in the present study are well below those reported to produce behavioral modifications or to generate side effects such as convulsion-like reactions or sedation (Van der Staa et al., 1995; Kearney et al., 1997; Kronthaler and Schmidt, 1998). For all the experiments involving L-CCG-I, the lowest dose of 50 nmol
able to induce significant locomotor activity was chosen in order to avoid nonspecific activity of the compound. Intracerebral infusion of L-CCG-I or MCCG was not associated with excitotoxicity and there was no evidence of gliosis, suggesting tissue lesion, except from mechanical injury caused by the cannula (data in line with those of Kwak et al., 1996).

Neuroanatomical studies revealed that the N. Acc. expresses abundant mRNA encoding for both mGluR2 and mGluR3 subtypes (Testa et al., 1994; Shigemoto and Mizuno, 2000). In the N. Acc. mGluR3 is known to be expressed in high levels in comparison to mGluR2, suggesting a preferential action of L-CCG-I on this subtype, the exact location of this receptor in this brain area is, however, not yet well established (Testa et al., 1994, 1998). Converging evidence has supported the idea that Group II agonists presynaptically inhibit glutamate synaptic potentials in the N. Acc. (Manzioni et al., 1997). It is therefore tempting to suggest that L-CCG-I, by acting on mGluR2/3 receptors, may reduce the glutamate tone which in turn results in a hyperlocomotor response such as that previously seen with blockade of glutamate activity at the NMDA receptors with MK 801 or APV injected into the N. Acc. (Schmidt et al., 1992; Ouagazzal and Amalric, 1995). However, Group II mGluRs have been found to be located both presynaptically and postsynaptically in other brain regions such as the hippocampus (Neki et al., 1996; for review, Cartmell and Schoepp, 2000; Shigemoto and Mizuno, 2000). Therefore, L-CCG-I may alternatively activate postsynaptic receptors on the DA nerve terminals producing enhanced DA levels in this area or act on intrinsic neurons (Vezina and Kim, 1999). Despite the high level of expression of mGluR5 subtypes in the N. Acc., there was no effect of their activation with the DHPG compound in the present study. It might be proposed that these receptors may only be involved when the basal level of glutamate activity is enhanced as suggested by Verma and Moghaddam (1998). Interestingly, and consistent either with the lack of effect of DHPG on spontaneous locomotor activity or the unaltered extracellular levels of DA following DHPG injection (Hu et al., 1999), the Group I selective antagonist AIDA was without effect on the amphetamine-induced hyperactivity (Kim et al., 2000).

4.2. Functional interactions between mGluR and the dopaminergic system

Behavioral and neurochemical studies have evidenced functional interactions in the N. Acc. between mGluRs and DA transmission (Ohno and Watanabe, 1995; Taber and Fibiger, 1995; Attarian and Amalric, 1997; Kim and Vezina, 1997; Kim et al., 2000; David and Abraini, 2001a,b). Moreover, ultrastructural studies of the N. Acc. indicate that terminals of the descending excitatory amino acid projections from the cortex and those of ascending mesencephalic projections not only come on close apposition to each other but also form synaptic contacts with the same intrinsic N. Acc. neurons as well (Sesack and Pickel, 1990, 1992). Blocking mGluRs in the rat N. Acc. indeed prevents the generation of locomotion induced by d-amphetamine or apomorphine (Attarian and Amalric, 1997; Kim and Vezina, 1997, 1998). In addition, DA depletion in the N. Acc. after local injection of the DA neurotoxin 6-hydroxydopamine prevented the locomotor hyperactivity induced by 1S,3R-ACPD, indicating that the dopaminergic transmission is necessary for the functional expression of Group II mGluRs on motor behaviors (Meeker et al., 1998). In this study, the locomotor response to L-CCG-I and d-amphetamine coadministration reached a higher level than those obtained when the two agonists were injected separately. Comparative effects were ob-served with an intra-accumbens coadministration of another selective Group II agonist APDC and the selective D1-like receptor agonist SKF 38393 (David and Abraini, 2001a). Furthermore, L-CCG-I-induced motor hyperactivity was totally abolished by the mixed D1/D2 receptors antagonist haloperidol, and by the selective D1 or D2 receptor antagonists SCH 23390 and raclopride, respectively injected systemically. SCH 23390 injected alone at the same concentrations were found to be ineffective in a reaction time task sensitive to a neuroleptic treatment (Amalric et al., 1993) while a mild sedative effect was observed with raclopride treatment at concentrations above 0.2 mg/kg (Amalric et al., 1993; Marrow et al., 1993). Clearly, the doses of SCH 23390 and raclopride that induce measurable catalepsy are much higher than doses used here (Hauber et al., 1998) suggesting that the blockade of L-CCG-I effect at the lower range of doses is specific of the DA receptor antagonism. L-CCG-I might produce its locomotor effect by activating mGluRs on intrinsic neurons in the N. Acc. and thereby regulating DA transmission via intracel- lular second-messengers pathways. This interaction would require the activation of both D1 and D2 receptors. Whether this interaction involves a specific action on D1 or D2 receptors located in different neuronal output pathways as observed in the dorsal striatum (Gerfen et al., 1990) or is a final common output still remains to be investigated. Alternatively, the Group II agonist could modulate DA release by acting at mGluRs located presynaptically on dopaminergic nerve terminals as suggested by others (Verma and Moghaddam, 1998; Kim and Vezina, 1998) and reinforced by neuroanatomical studies reporting the high level of mGluR2/3 protein expression in the ventral tegmental area (Romano et al., 1995; Petralia et al., 1996). These glutamate/DA interactions are complex since the activation of the DA system with amphetamine is also blocked by the Group II antagonist EGLU in rats (Kim et al., 2000). These results suggest that similarly to the effects observed with NMDA receptors modulation, the glutamate and DA systems act in a reciprocal way to regulate motor function at the level of the N. Acc. (see Schmidt et al., 1992).
lished and the exact mechanisms underlying these behavioral effects remain to be clarified, the present findings demonstrate that Group II but not Group I mGluRs are involved in the generation of locomotor activity into the N. Acc. and do so in a DA-dependent manner via D1 and D2 receptors.

Acknowledgments

This study was supported by the CNRS, grants from DGA (convention no. 9434124004707501) and by the Fondation France Parkinson (Programme grant to MA). NB was supported by DGA (direction générale des armées) and CR by a fellowship of the Conseil Régional PACA. We are grateful to D. Terramorsi for taking care of the animals.

References

Attarian S, Amalric M. Microinfusion of the metabotropic glutamate receptor agonist 1S,3R-1-aminoacycloptene-1,3-dicarboxylic acid into the nucleus accumbens induces dopamine-dependent locomotor activation in the rat. Eur J Neurosci 1997;9:809 –16.


David HN, Abraini JH. The group I metabotropic glutamate receptor antagonist S-4-CPG modulates the locomotor response produced by the activation of D1-like, but not D2-like, dopamine receptors in the rat nucleus accumbens. Eur J Neurosci 2001b;13:2157–64.


Kronthaler UO, Schmidt WJ. 1S,3R-ACPD has cataleptogenic effects and reverses MK-801-, and less pronounced, ,3-aminobutyric acid-induced locomotion. Eur J Pharmacol 1996;316:129–36.

Kronthaler UO, Schmidt WJ. The mGluRs group II agonist (2S,3S,4S)-alpha-carboxycyclopolypropyl-glycine induces catalepsy in the rat, which is pronouncedly antagonised by dizocilpine and 3,4-amphetamine. Neurosci Lett 1998;253:25–8.


Petralia RS, Wang YX, Niedzielski AS, Wenthold RJ. The metabotropic glutamate receptors, mGluR2 and mGluR3, show unique postsynaptic, presynaptic and glial localizations. Neuroscience 1996;71:949–76.


Sesack SR, Pickel VM. In the rat medial nucleus accumbens, hippocampal and catecholaminergic terminals converge on spiny neurons and are in apposition to each other. Brain Res 1990;527:266–79.

Sesack SR, Pickel VM. Prefrontal cortical efferents in the rat synapse on unlabeled neuronal targets of catecholamine terminals in the nucleus accumbens septi and on dopamine neurons in the ventral tegmental area. J Comp Neurol 1992;320:145–60.


Testa CM, Standaert DG, Young AB, Penney JB. Metabotropic glutamate receptor mRNA expression in the basal ganglia of the rat. J Neurosci 1994;14:3005–18.


Testa CM, Friberg IK, Weiss SW, Standaert DG. Immunohistochemical localization of metabotropic glutamate receptors mGluR1a and mGluR2/3 in the rat basal ganglia. J Comp Neurol 1998;390:5–19.


