European Journal of Neuroscience, Vol. 9, pp. 1514–1523, 1997

Metabotropic Glutamate Receptors in the Rat Nucleus Accumbens

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Keywords: excitatory synaptic transmission, glutamate, metabotropic glutamate receptors, nucleus accumbens, presynaptic inhibition,

Abstract

The effects of glutamate metabotropic receptors (mGluRs) on excitatory transmission in the nucleus accumbens were investigated using electrophysiological techniques in rat nucleus accumbens slices. The broad-spectrum mGluR agonist (1*S*,3*R*)-1-aminocyclopentyl-1,3-dicarboxylate, the mGluR group 2 selective agonists (*S*)-4-carboxy-3-hydroxyphenylglycine, (1*S*,3*S*)-ACPD) and (2*S*,1′*S*,2′*S*)-2-(2′-carboxycyclopropyl)glycine (L-CCG1), and the mGluR group 3 specific agonist L-2-amino-4-phosphonobutyrate (L-AP4) all reversibly inhibited evoked excitatory synaptic responses. The specific group 1 mGluR agonist (*R*,*S*)-3,5-dihydroxyphenylglycine [(*R*,*S*)-DHPG] did not depress transmission. Dose–response curves showed that the rank order of agonist potencies was: L-CCG1 > L-AP4 > (1*S*,3*S*)-ACPD. Group 2 and 3 mGluRs inhibited transmission via a presynaptic mechanism, as they increased paired-pulse facilitation, decreased the frequency of miniature excitatory postsynaptic currents and had no effect on their amplitude. The mGluRs did not inhibit transmitter release by reducing voltage-dependent Ca^{2+} currents through N- or P-type Ca^{2+} channels, as inhibition persisted in the presence of ω-conotoxin-GVIA or ω-Aga-IVA. The depression induced by mGluRs was not affected by specific antagonists of dopamine D1, GABA-B or adenosine A1 receptors, indicating direct effects. Finally, (*R*,*S*)-DHPG specifically blocked the postsynaptic afterhyperpolarization current (I_{AHP}). Our results represent the first direct demonstration of functional mGluRs in the nucleus accumbens of the rat.

Introduction

Because of its predominant role as an excitatory neurotransmitter (Headley and Grillner 1990), glutamate is of major importance to a variety of fundamental central nervous system phenomena. The diverse physiological functions of glutamate are mediated through both ionotropic receptors and G-protein-coupled metabotropic receptors (mGluRs). Several cDNAs encoding mGluRs have been cloned and classified into three families (Nakanishi, 1992; Watkins and Collingridge, 1994): group 1 consists of mGluR1 and mGluR5, both positively coupled to phospholipase C, stimulated by (1*S*,3*R*)-1-aminocyclopentyl-1,3-dicarboxylate [(1*S*,3*R*)-ACPD] and (*R*,*S*)-3,5-dihydroxyphenylglycine [(*R*,*S*)-DHPG] (Ito *et al.*, 1992; Schoepp *et al.*, 1994; Gereau and Conn, 1995b); group 2 receptors (mGluR2/3), negatively coupled to adenylate cyclase, are selectively activated by (2*S*,1′*S*,2′*S*)-2-(2′-carboxycyclopropyl)glycine (L-CCG1) and [(1*S*,3*S*)-ACPD] and specifically by (*S*)-4-carboxy-3-hydroxyphenylglycine [(S)-4C3HPG] (Hayashi *et al.*, 1994; Thomsen *et al.*, 1994); finally, group 3 mGluRs (mGluR4/6/7/8) are negatively coupled to adenylate cyclase and specifically activated by L-2-amino-4-phosphonobutyrate [L-AP4] (Hayashi *et al.*, 1994; Thomsen *et al.*, 1994; Saugstad *et al.*, 1997). The mGluRs were first described in primary cultured striatal neurons (Sladeczek *et al.*, 1985) and a role for these receptors in striatal synaptic transmission/plasticity has been proposed (Calabresi *et al.*, 1992; Lovinger and McCool, 1995). Little is known, however, about the physiological roles of mGluRs in other parts of the basal ganglia and in the nucleus accumbens, also known as the 'ventral striatum'.

The nucleus accumbens has a role in connecting the limbic system to the basal ganglia and to the extrapyramidal motor system. This structure has received considerable attention due to its involvement in functions such as locomotion (Pennartz *et al.*, 1994) and spatial memory (Pennartz *et al.*, 1994). Moreover, the nucleus accumbens plays a central role in the mechanisms at the origin of the rewarding properties of many drugs of abuse, such as cocaine, amphetamines, opiates and alcohol (Pettit *et al.*, 1984; Wise and Bozarth, 1987; Koob, 1992; Nestler, 1992; Samson and Harris, 1992; Woolverton and Johnson, 1992). Glutamate is the neurotransmitter at the synapses between prefrontal cortex afferents and neurons of the nucleus accumbens (Horne *et al.*, 1990), and recent work by Pennartz *et al.* (1993) and Kombian and Malenka (1994) have demonstrated the existence of NMDA-dependent synaptic plasticity. Several morphological studies have described various mGluR subtypes in the nucleus accumbens (Baude *et al.*, 1993; Ohishi *et al.*, 1993a, b, 1995; Shigemoto *et al.*, 1993), and *in vivo* studies have suggested a role for mGluRs in regulating dopamine release (Ohno and Watanabe, 1995; Taber and Fibiger, 1995).

Using rat brain slices (Pennartz *et al.*, 1990; Kombian and Malenka, 1994; Nicola *et al.*, 1996), we looked for mGluRs at the excitatory projections pathways to the nucleus accumbens.

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Received 27 November 1996, revised 10 February 1997, accepted 14 February 1997

Materials and methods

Nucleus accumbens slices

Parasagittal nucleus accumbens slices (500 μm thick) were prepared as described (Kombian and Malenka, 1994). Briefly, 4- to 5-week-old male Sprague–Dawley rats were deeply anaesthetized with pentobarbital and decapitated, the brains were rapidly transferred to ice-cold, oxygenated artificial cerebrospinal fluid. Parasagittal slices were cut in ice-cold oxygenated artificial cerebrospinal fluid using a motorized vibratome (Camden Instruments, UK). The artificial cerebrospinal fluid contained 126 mM NaCl, 2.5 mM KCl, 1.2 mM $MgCl_2$, 2.4 mM $CaCl_2$, 18 mM $NaHCO_3$, 1.2 mM NaH_2PO_4 and 11 mM glucose, and was equilibrated with 95% O_2/5% CO_2. After at least 1 h of incubation at room temperature to allow recovery, a slice was transferred to a superfusing chamber for recordings. Picrotoxin (100 μM) was present in all experiments to block $GABA_A$ synaptic responses. All experiments were carried out at room temperature (22–25°C).

Electrophysiological recordings and data analysis

Extracellular field potential recording or whole-cell voltage-clamp recording was used. To evoke synaptic potentials, stimuli (100 μs duration) were usually delivered at 0.33 Hz through bipolar stainless steel electrodes placed at the prefrontal–accumbens border (Kombian and Malenka, 1994). Field potentials were recorded with a glass pipette filled with 3 M NaCl. Recordings were made in the rostral–medial dorsal accumbens close to the anterior commissure. Whole-cell recordings were made using 'blind' whole-cell recording techniques (Coleman and Miller 1989) with patch pipettes filled with an internal solution containing 122.5 mM caesium gluconate, 17.5 mM CsCl, 10 mM HEPES, 0.2 mM EGTA, 8 mM NaCl, 2 mM MgATP, 0.2 mM cAMP and 0.3 mM Na_3GTP (pH 7.2, 290–300 mosm). When filled with this solution, patch pipettes with resistance 8–10 Ω and series resistance up to 30 Ω were accepted and monitored throughout the experiments. Paired-pulse facilitation was elicited by stimulating the input twice with a 50 ms interval. For I_{AHP} experiments 0.5 μM tetrodotoxin and 5 mM tetraethylammonium acetate were also added to the perfusion medium. The pipettes were filled with a solution containing 150 mM K^+-methyl sulphate, 10 mM HEPES, 2 mM MgATP and 0.3 mM Na_3GTP (pH 7.2). The I_{AHP} was induced as previously described (Manzoni *et al.*, 1994b). The cells were voltage-clamped at ~–50mV to optimize the I_{AHP}, and a 100 ms depolarizing pulse to +20 mV was given every 30 s to induce the Ca^{2+} current necessary to generate the I_{AHP}. During the miniature excitatory postsynaptic currents (mEPSC) recordings, 1 μM tetrodotoxin was added to the perfusion medium and the input and access resistances were monitored.

An Axopatch-1D (Axon Instruments) was used to record the data, which were filtered at 2 kHz, digitized at 10 kHz on a DigiData 1200 interface (Axon Instruments) and collected on a Pentium PC using a home-written acquisition/analysis program using ACQUIS 1 software (DIPSI, France). In field potential recordings, both the slope of the excitatory postsynaptic potential (EPSP; calculated by a least-squares method) and its amplitude [the second potential of the biphasic response was due to synaptic transmission (Nicola *et al.*, 1996)] were measured. In whole-cell recordings, the amplitude of excitatory postsynaptic currents (EPSCs) was measured by averaging a 5 ms window around the peak and subtracting the average value obtained during a 5 ms window immediately before the stimulus. The mEPSCs were detected off-line using software (written with ACQUIS1) that selected putative mEPSCs according to their fast rise time and slower decay time. Events between 4 and 80 pA were considered as genuine mEPSCs, and average mEPSC amplitude and frequency were computed in 1 min bins.

All values are given as mean ± SEM. The fitting curves were calculated according to the equation

$$y = \{y_{max} - y_{min}/1 + (x/EC_{50})^n\} + y_{min} \quad (1)$$

where y_{max} is the response in the absence of agonist, y_{min} is the response remaining in the presence of maximal agonist concentration, x is the concentration of the agonist, EC_{50} is the concentration of agonist producing 50% of the maximal response, and n is the slope. Kaleidagraph software (Abelbeck Software, USA) was used for the calculation.

Drugs

Drugs used were dopamine-HCl, L-AP4, picrotoxin, tetrodotoxin and tetraethylammonium acetate from Sigma; (1*S*,3*R*)-ACPD, L-CCG1,(1*S*,3*S*)-ACPD, (S)-4C3HPG, (*R*,*S*)-DHPG, alpha-methyl-AP4 and alpha-methyl-LCCGI from Tocris Neuramin; and (+)-SCH-23390 hydrochloride and 8-CPT from RBI. CGP-38345 was a gift of Dr A. Sedlaceck (Ciba-Geigy, Switzerland). ω-Conotoxin-GVIA was from Alomone Laboratories (Jerusalem, Israel) and ω-Aga-IVA was a gift from Dr Saccomano (Pfizer). Dopamine solutions (75 mM stock, 75 μM final) were made up fresh before each experiment in 50 mM sodium metabisulphite (50 μM final) and 10–20 mM stock solutions were made up daily. (1*S*,3*R*)-ACPD, (1*S*,3*S*)-ACPD, L-CCG1, (*S*)-4C3HPG, L-AP4 and (*R*,*S*)-DHPG were made up as 100 mM (10 mM for L-AP4) stock solutions in 100 mM NaOH and stored at –20°C. Other chemicals were from Sigma.

Results

Synaptic transmission in nucleus accumbens slices is depressed by specific mGluR agonists

Figure 1 depicts a typical experiment in which we first applied L-CCG1 [5 min at 5 μM, a concentration that specifically activates both mGluR2 and mGluR3 (Hayashi *et al.*, 1994; Thomsen *et al.*, 1994)]; similar results were obtained in six other slices prepared from different animals (not shown). L-CCG1 reversibly decreased evoked excitatory field potentials (fEPSP) to 40% of the control value. To evaluate the possible role of group 3 mGluRs, after washout of L-CCG1 we applied the specific mGluR4/6/7/8 agonist L-AP4 (100 μM, 5 min) (Tanabe *et al.*, 1993; Hayashi *et al.*, 1994; Thomsen *et al.*, 1994; Saugstad *et al.*, 1997). L-AP4 caused a clear and reversible reduction in the size of the fEPSP (Fig. 1), suggesting that a group 3 mGluRs also depresses transmission at these synapses. We then tested the effect of (*R*,*S*)-DHPG, a specific mGluR1/5 agonist (Ito *et al.*, 1992; Schoepp *et al.*, 1994; Manzoni and Bockaert, 1995). (*R*,*S*)-DHPG (100 μM) only slightly depressed fEPSPs (by 10%). The broad-spectrum agonist (1*S*,3*R*)-ACPD induced reversible depression of the fEPSPs (Fig. 1; compare sample traces 7).

To identify the subtypes of mGluRs implicated in the inhibition of transmission at these synapses, we constructed complete dose–response curves for (1*S*,3*S*)-ACPD, L-CCG1 and L-AP4. Figure 1 summarizes these experiments. The EC_{50} was determined in each individual experiment by fitting the data according to the equation 1 (see Materials and methods). We found that the rank order of agonist potency was: L-CCG1 > L-AP4 > (1*S*,*SR*)-ACPD, with EC_{50} values of 0.8 ± 0.2 (n = 4), 5.3 ± 2.8 (n = 3) and 25 ± 7.7 μM (n = 3) respectively.

To determine the origin of the depression of synaptic transmission

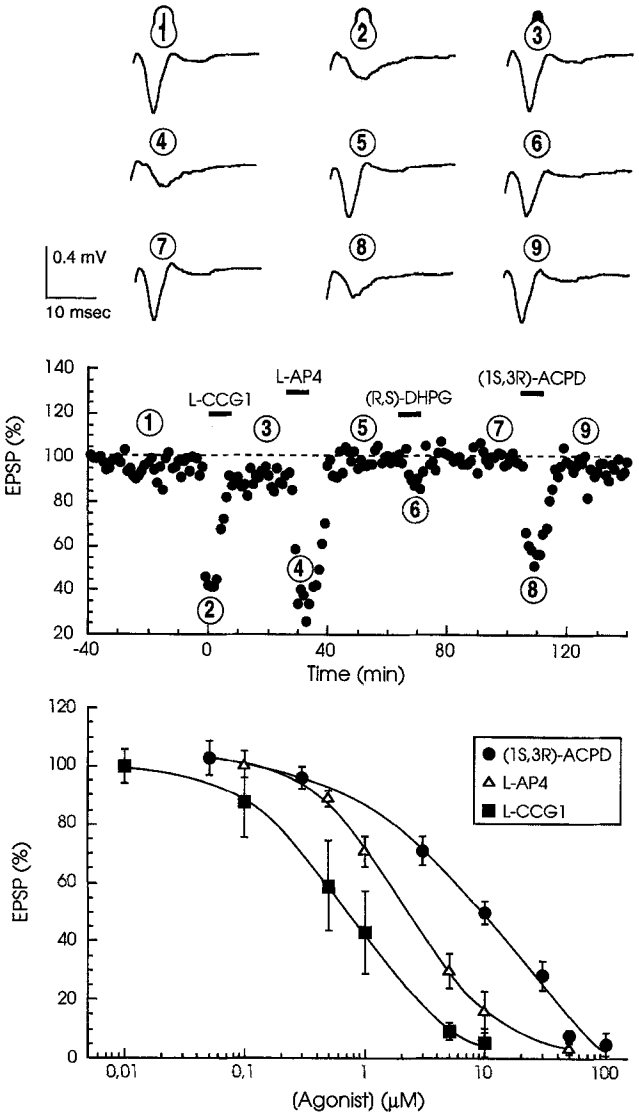

FIG. 1. (Top) Time course of the inhibitory effect of mGluR agonists on excitatory synaptic transmission in the nucleus accumbens. The metabotropic glutamate agonists L-CCG1 (5 μM, 5 min), L-AP4 (100 μM, 5 min) and (1*S*,3*R*)-ACPD (50 μM, 5 min) clearly diminished the field EPSP. The group 1 specific agonist (*R*,*S*)-DHPG (75 μM, 5 min) did not cause significant inhibition of the field EPSP. The effects of these agonists were completely reversible within 10–20 min of washing (similar results were observed in six other slices). Traces represent averages of ten consecutive EPSPs obtained at the times marked by the numbers on the graphs. (Bottom) Dose–response relationship of L-CCG1, (1*S*,3*S*)-ACPD and L-AP4 on excitatory synaptic transmission in the nucleus accumbens. Each point represents the mean ± SEM of three or four independent measurements and is expressed as the percentage of control response in the absence of agonist.

induced by mGluR activation, we measured the variation in paired-pulse facilitation of excitatory transmission, a presynaptic phenomenon thought to reflect changes in transmitter release and presynaptic manipulation (Manabe *et al.*, 1993). We first tested the effects of the broad-spectrum mGluR agonist (1*S*,3*R*)-ACPD (Manzoni *et al.*, 1992) on evoked EPSCs. Application of (1*S*,3*R*)-ACPD (100 μM, 10 min) induced reversible depression of the EPSC, always accompanied by a clear increase in the ratio of facilitation. At the peak of the depression induced by (1*S*,3*R*)-ACPD the EPSC was reduced to 51 ± 8% of its control value while the paired-pulse facilitation was increased to 168 ± 16% of its control value (n = 7).

To test for the presence of presynaptic group 2 mGluRs we applied the selective agonists L-CCG1 and (*S*)-4C3HPG. (*S*)-4C3HPG acts both as an antagonist of the mGluR1/5 family and a specific agonist at mGluR2/3 receptors. Figure 2A summarizes our experiments. At the peak of the depression (Fig. 2A, sample traces 2) the EPSC was reduced to 54 ± 11% (n = 5) of the control value (Fig. 2A, top) while the paired-pulse facilitation was increased to 158 ± 10% of control (Fig. 2A, bottom). During application of 10 μM L-CCG1 the EPSC was reduced to 42 ± 8% of its control value while the paired-pulse facilitation was increased to 197 ± 37% (n = 5). The L-AP4-induced depression was also concomitant with an increase in paired-pulse facilitation (Fig. 2B): the EPSC was reduced to 39.8 ± 7.4% of its control value while the paired-pulse facilitation was increased to 174 ± 30% of control (n = 8). We finally examined whether group 1 mGluRs contribute to the depression of EPSC. Figure 2C summarizes the whole-cell recordings where 75–100 μM (*R*,*S*)-DHPG was bath-applied. The maximal EPSC inhibition was 21 ± 5% (n = 6) and was never accompanied by an increase in paired-pulse facilitation (Fig. 2C, bottom). We verified in three of these six experiments that application of an mGluR2/3 agonist led to a clear increase in paired-pulse facilitation (not shown), reinforcing the idea that the absence of an increase in paired-pulse facilitation during (*R*,*S*)-DHPG application was genuinely due to a lack of presynaptic effect of the mGluR1/5 agonist.

Presynaptic mGluRs are responsible for inhibition

Activation of group 2 and 3 mGluRs induced an increase in paired-pulse facilitation, suggesting that mGluRs can decrease presynaptic neurotransmitter release. We recorded spontaneous mEPSCs in the presence of 1 μM tetrodotoxin in the whole-cell patch-clamp configuration (holding potential –80 mV). A decrease in the frequency of the mEPSC is interpreted to be a result of a presynaptic action (e.g. a reduction in transmitter release) whereas a decrease in mEPSC amplitude classically reveals a postsynaptic site of action (Katz, 1966). We found a high frequency of mEPSCs in the nucleus accumbens (7–15 Hz) and the amplitude ranged from 4 to 80 pA; in agreement with Scanziani *et al.* (1992), a high concentration of the Ca^{2+} channel blocker cadmium (100 μM) did not affect mEPSC frequency or amplitude, showing their independence of external Ca^{2+} entry (not shown). A typical experiment is shown in Figure 3A, and it is clear that mEPSC frequency was reversibly depressed by L-AP4 while the amplitude was unaffected. Accordingly, the distribution of mEPSC amplitude was not modified by L-AP4 (Fig. 3B) whereas the time interval distribution curve was shifted to the right (Fig. 3C). This experiment was repeated in a total of five cells, and the average time courses of mEPSC frequency and amplitude are shown in Figure 3D. The mEPSC frequency was reduced to 52 ± 2.7% of the baseline value (n = 8) whereas the amplitude remained unchanged (97.2 ± 1.9% of basal). Similar experiments were performed in eight neurons using L-CCG1 (10 μM), which reduced mEPSC frequency to 64.4 ± 9.2% of baseline value, whereas the amplitude remained at 98.2 ± 1.9% (Fig. 3E).

Effects of MAP4 and MCCG1 on L-CCG1- and L-AP4-induced depressions

Recently MCCG1 and MAP4, putative selective antagonists of group 2 and 3 mGluRs, have been described in several preparations (Jane *et al.*, 1994; Bushell *et al.*, 1995; Manzoni *et al.*, 1995). After the depression of fEPSP induced by agonist application [L-CCG1 5 μM (Fig. 4A) or L-AP4 30 μM (Fig. 4B)] had stabilized, the ability of the antagonists to reverse the inhibition was tested. MCCG1 (300

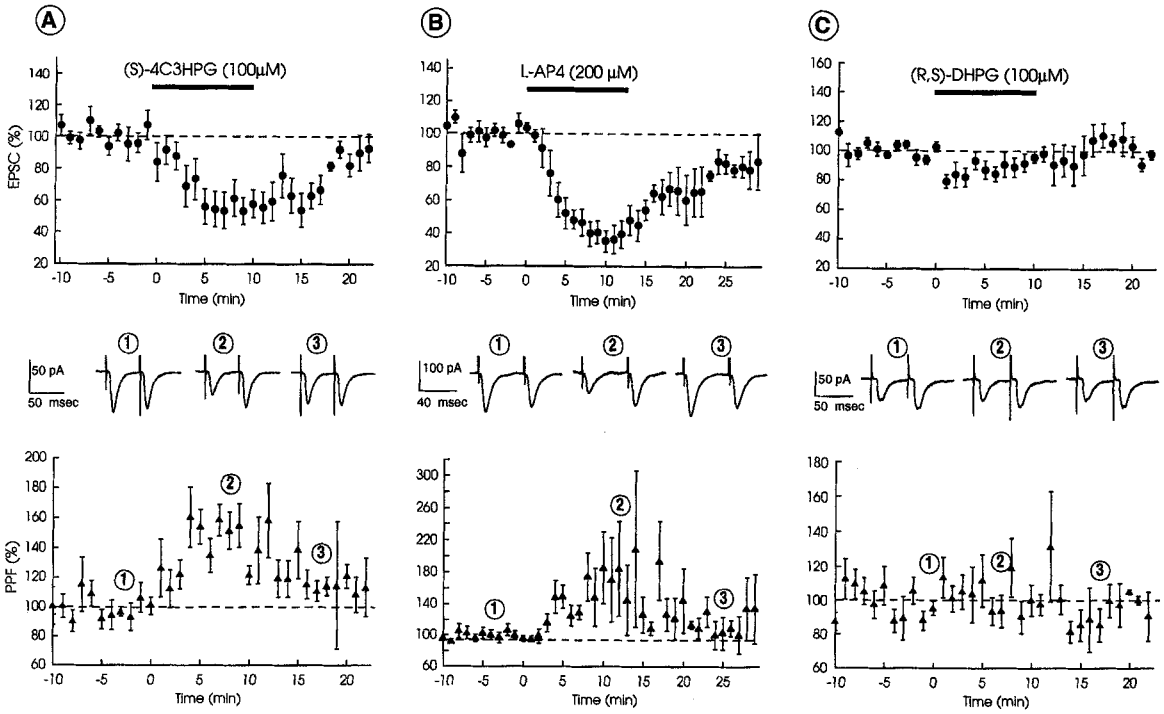

FIG. 2. Selective mGluR agonists induce presynaptic depression of excitatory synaptic transmission in the nucleus accumbens. (A) Top panel summarizes the time course of the depressing action of bath-applied (*S*)-4C3HPG (100 μM for 10 min) in the five cells tested. Bottom panel shows the time course of paired-pulse facilitation in all the cells to which (*S*)-4C3HPG was applied (same cells as in C; $n = 5$). (B) Top panel shows time course of the depressing action of bath-applied L-AP4 (200 μM for 10 min) in the eight cells recorded. Bottom panel summarizes the time course of paired-pulse facilitation in all cells to which L-AP4 was applied (same cells as in A; $n = 8$). (C) Top panel shows time course of the depressing action of bath-applied (*R,S*)-DHPG (100 μM for 10 min) in the six cells tested. Bottom panel shows time course of paired-pulse facilitation in all cells to which (*R,S*)-DHPG was applied (same cells as in A; $n = 6$). Sample traces are from a typical experiment included in the averaged data. Traces represent averages of ten consecutive EPSCs obtained at the times marked by the numbers on the graph.

μM) reversed the synaptic depression evoked by 5 μM L-CCG1 (Fig. 4A). The maximum depression induced by L-CCG1 was 49 ± 5% of control and MCCG1 restored 76 ± 3% ($n = 3$) of this response. Figure 4B describes similar experiments performed using L-AP4 and MAP4, and shows that the maximum L-AP4-induced inhibition (55 ± 18% of control, $n = 3$) could be reversed by MAP4 (78 ± 13%). In another set of three experiments we tested the specificity of these antagonists, and found that MAP4 did not significantly affect the L-CCG1-induced depression [47 ± 6 and 37 ± 7% of basal transmission in the absence and the presence of MAP4 ($n = 3$) respectively] and that MCCG1 was not an antagonist of the L-AP4-induced inhibition [49 ± 7 and 55 ± 5% of basal transmission in the absence and the presence of MCCG1 ($n = 3$) respectively]. This suggested that the depression induced by L-CCG1 and L-AP4 was mediated through two pharmacologically distinct receptors.

Role of dopamine/GABA-B/adenosine release

A recent paper by Nicola *et al.* (1996) has demonstrated that presynaptic D1-like dopamine receptors can depress excitatory synaptic transmission in nucleus accumbens neurons. We tested the possibility that mGluR activation leads to the release of dopamine, which would in turn presynaptically depress excitatory transmission. Table 1 summarizes our experiments, in which we applied for 10 min dopamine (75 μM), (1*S*,3*R*)-ACPD (30 μM), (1*S*,3*S*)-ACPD (20 μM) or L-AP4 (100μM) in the control condition or after treatment (20 min) with the D1 receptor antagonist SCH-23390 (20 μM). SCH-23390 was able to antagonize dopamine-induced inhibition but not mGluR-induced depression. Other candidates for mediating this inhibitory action were GABA acting on presynaptic $GABA_B$ receptors (Isaacson *et al.*, 1993) and adenosine acting on A1 receptors (Manzoni *et al.*, 1994a), but a mixture of the selective $GABA_B$ receptor antagonist CGP 35348 (200 mM) and the A1 adenosine antagonist 8-cyclopentyl theophylline (20 μM) had no effect on the mGluR-induced depression (inhibition induced by L-AP4 and L-CCG1 was 46 ± 12 and 55 ± 15% respectively in the control condition and 55 ± 13 and 55 ± 19% in the presence of the antagonists; $n = 3$).

Inhibition by mGluR is not affected by increasing the cAMP level

Group 2 and 3 mGluRs are negatively coupled to the adenylate cyclase in a variety of models (Nakanishi 1992). In order to determine if the adenylate cyclase/cAMP pathway interacted with the mGluR-induced synaptic depression, we examined the effects of forskolin, a powerful activator of adenylate cyclase. Bath application of forskolin (10 μM) induced a reversible increase in transmission (of ~30%; not shown). Bath application of L-CCG1 and L-AP4 before and during the forskolin perfusion induced similar levels of depression (inhibition induced by L-AP4 and L-CCG1 was 40 ± 14 and 67 ± 12% respectively in control conditions and 51 ± 14 and 74 ± 12% in the presence of the antagonists; $n = 3$).

Inhibition by mGluR does not require calcium channels sensitive to ω-conotoxin-GVIA or ω-Aga-IVA

The fact that mGluR activation could inhibit the Ca^{2+}-independent spontaneous release of glutamate in the nucleus accumbens (Fig. 3) did

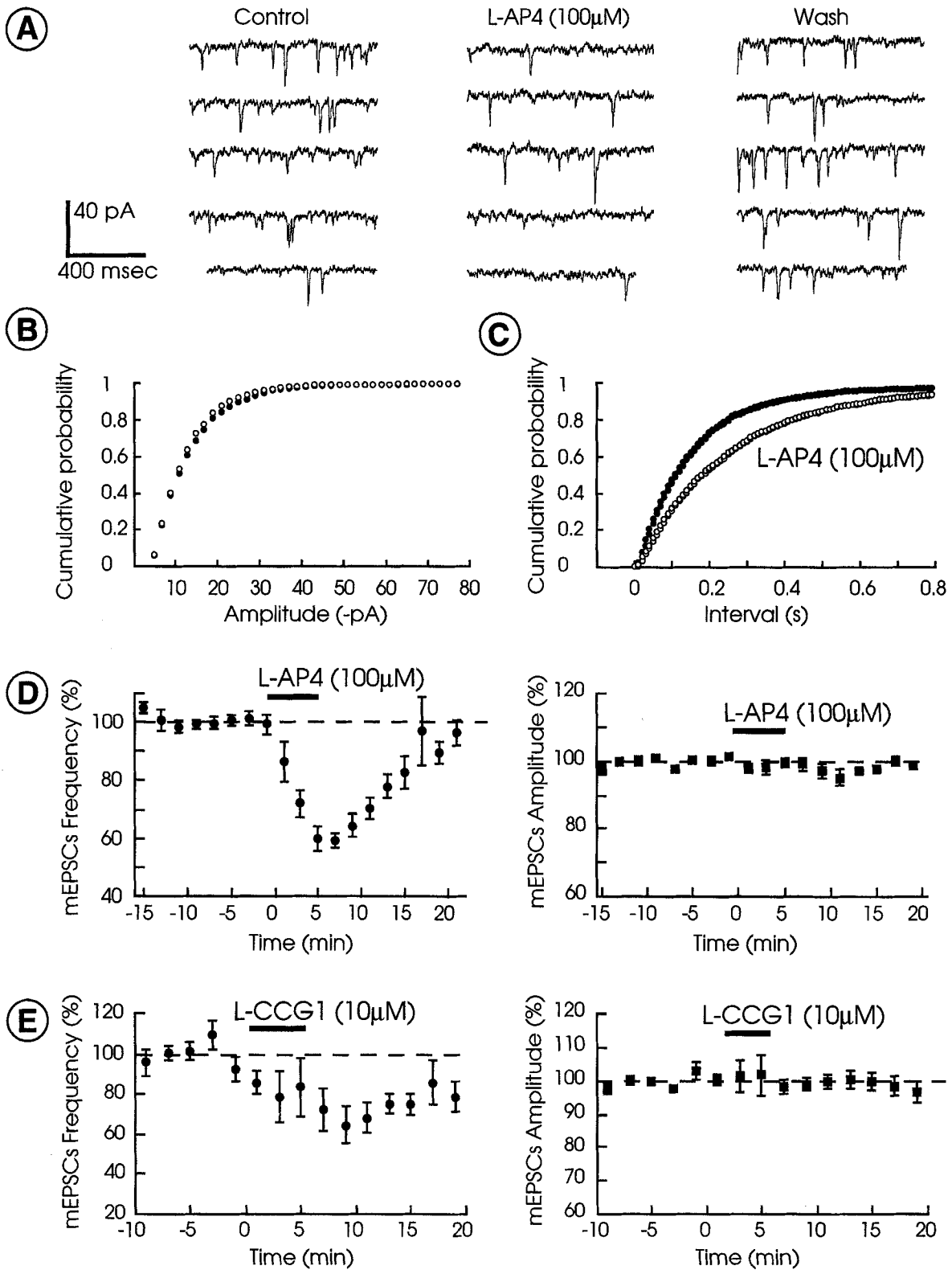

FIG. 3. L-AP4 and L-CCG1 reduce mEPSC frequency but not amplitude. (A) Representative consecutive 1 s current sweeps from a cell (holding potential −80 mV) in which spontaneous mEPSCs were recorded in the absence or presence of 100 μM L-AP4. (B) Distribution of mEPSC amplitude before and during application of L-AP4 in the cell shown in A was unchanged by the group 3 mGluR agonist. (C) Distribution of the time intervals between successive mEPSCs in the neuron shown in A and B reveals that mEPSC frequency was reduced during L-AP4 application. (D) Average mEPSC frequency (left panel) but not amplitude (right panel) was reduced by L-AP4 (100 μM) in the five cells recorded (1 min bins). (E) Average mEPSC frequency (left panel) but not amplitude (right panel) was reduced by L-CCG1 (10 mM) in the eight cells recorded.

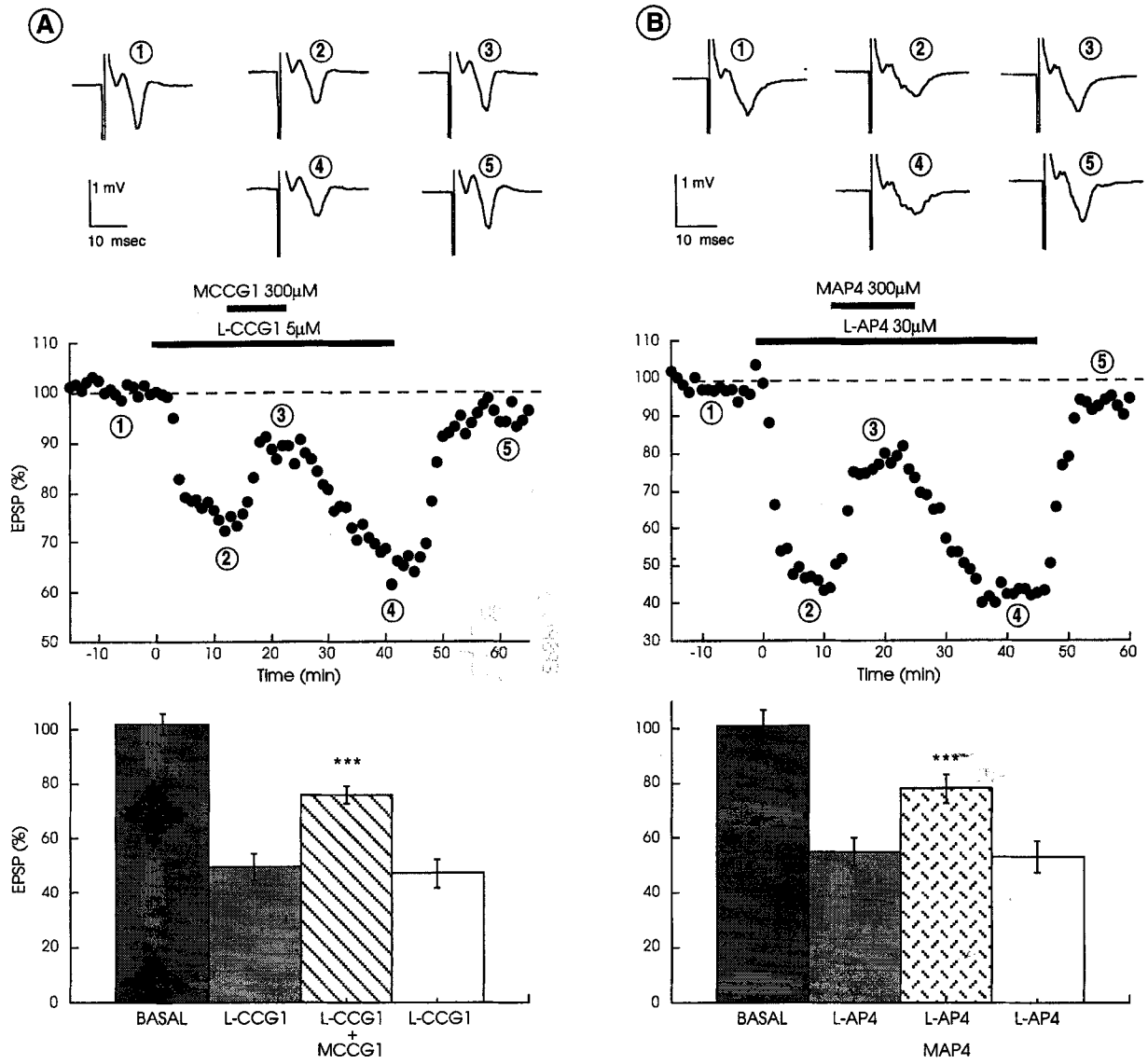

FIG. 4. Effects of MCCG1 and MAP4 on the depressant actions of L-CCG1 and L-AP4. (A) Typical experiment (similar results were obtained in two other independent experiments) in which the action of 300 μM MCCG1 was tested during inhibition of excitatory transmission by 5 μM L-CCG1. Traces represent averages of ten consecutive EPSPs obtained at the times marked by the numbers on the graph. The lower graph summarizes the three experiments performed as described above. (B) Typical experiment (similar results were obtained in two other independent experiments) in which the action of 300 μM MAP4 was tested during blockade of excitatory transmission in the nucleus accumbens by 30 μM L-AP4. Traces represent averages of ten consecutive EPSPs obtained at the times marked by the numbers on the graph.

TABLE 1. Lack of effect of a specific D1 receptor antagonist

fEPSP	Control (%)	SCH-23390
Dopamine	54 ± 16	86 ± 13
(1*S*,3*S*)-ACPD	69 ± 12	66 ± 12
L-AP4	53 ± 4	51 ± 14
(1*S*,3*R*)-ACPD	62 ± 2	65 ± 5

The table summarizes all the experiments where the inhibitory effects induced by dopamine (75 μM), (1*S*,3*R*)-ACPD (30 μM), (1*S*,3*S*)-ACPD (20 μM) and L-AP4 (100 μM) were tested in control conditions or after a 20 min treatment with the D1 receptor antagonist SCH-23390 (20 μM) (n = 3). The values were taken at the peak of the corresponding effects.

not exclude additional interaction with the Ca^{2+} channels underlying evoked synaptic release (Luebke *et al.*, 1993; Castillo *et al.*, 1994; Wheeler *et al.*, 1994). For instance, adenosine has been shown to be able to inhibit spontaneous transmitter release downstream of Ca^{2+} channels, but also to inhibit evoked transmitter release mediated by N-type Ca^{2+} channels (Scanziani *et al.*, 1992; Wu and Saggau, 1994). We therefore decided to alter presynaptic Ca^{2+} entry by blocking N- or P-type Ca^{2+} channels with ω-conotoxin-GVIA or ω-Aga-IVA respectively. Figure 5A shows a representative experiment where it is clear that ω-conotoxin-GVIA-sensitive Ca^{2+} channels (presumably of the N type) are responsible for most of the evoked-transmission. In the presence of ω-conotoxin-GVIA (1 μM), both L-AP4 and L-CCG1 still induced depression. In agreement with a previous study (Horne and Kemp, 1991), ω-conotoxin-GVIA reduced synaptic transmission to 34 ± 14% of its basal value (n = 6). All the experiments

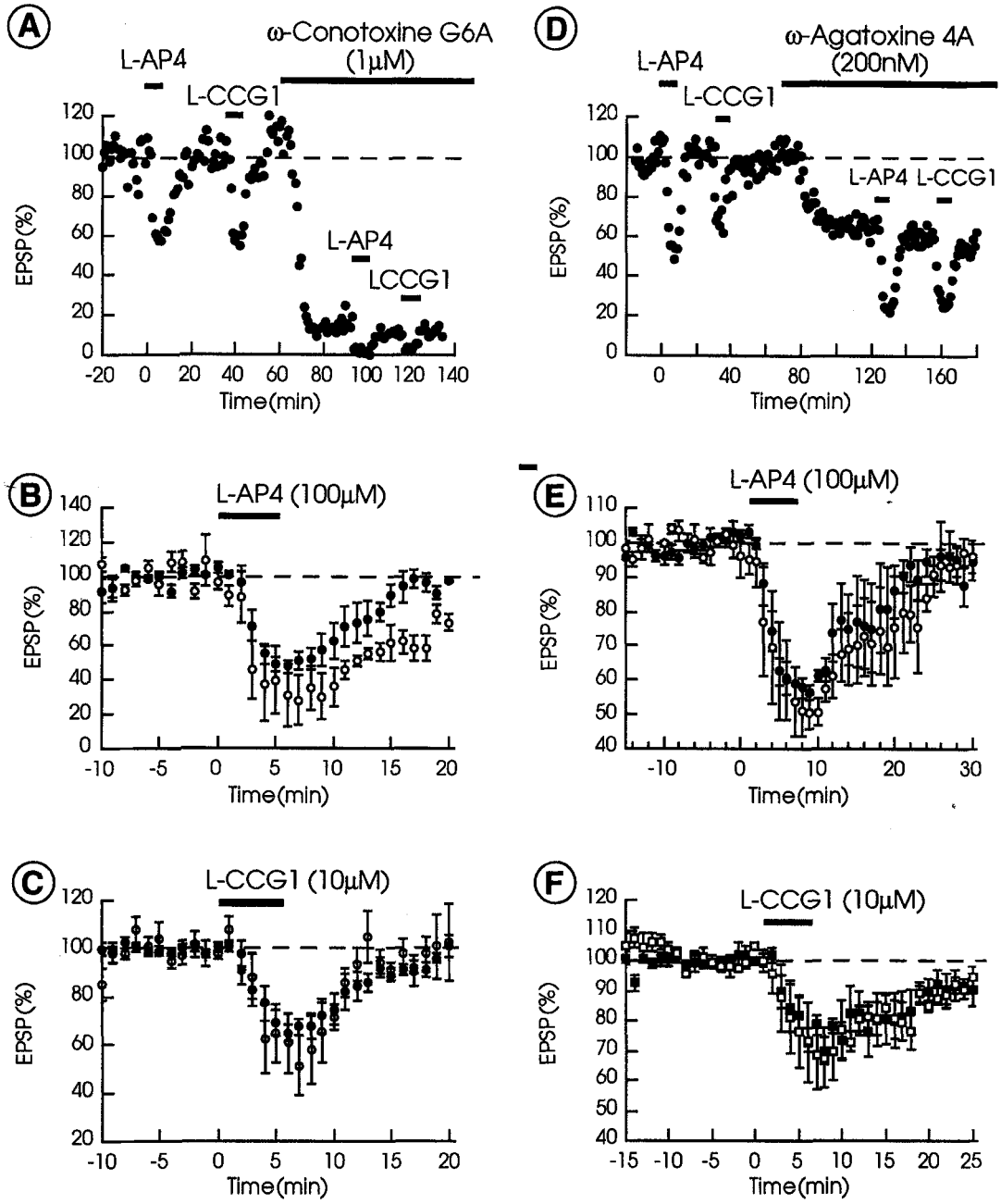

FIG. 5. The mGluR-induced inhibition neither requires N- nor P-type channel modulation. (A) Typical experiment in which, after verifying the depressing effects of L-CCG1 (10 μM) and L-AP4 (100 μM), the slice was perfused with 1 μM ω-conotoxin-GVIA, which almost completely blocked the EPSPs. The fraction of synaptic transmission insensitive to ω-conotoxin-GVIA was blocked by the mGluR agonists. (B) Summary of all experiments performed as above with 100 μM L-AP4 (n = 6). (C) Summary of all experiments performed as in B with 10 μM L-CCG1 (n = 6). (D) Typical experiment in which, after verifying the depressing effects of L-CCG1 (10 μM) and L-AP4 (100 μM), the slice was perfused with 200 nM ω-Aga-IVA. The fraction of synaptic transmission insensitive to ω-Aga-IVA was blocked by the mGluR agonists. (E) Summary of all experiments performed as in D with 100 μM L-AP4 (n = 4). (F) Summary of all experiments performed as in E with 10 μM L-CCG1 (n = 4).

performed with ω-conotoxin-GVIA (1 μM, 15–20 min) gave similar results and are summarized in Figure 5 (B and C). These experiments indicate that depression induced by mGluRs of groups 2 and 3 does not require the reduction of presynaptic Ca^{2+} entry through N-type channels. Figure 5D shows a typical experiment in which bath-perfusion with 200 nM ω-Aga-IVA reduced synaptic transmission by ~30% but did not affect the inhibitory actions of the mGluR agonists. A summary of all the experiments is shown in Figure 5 (E and F); it suggests that P-type channels, selectively blocked by bath-perfusion of ω-Aga-IVA (200 nM, 15–20 min), are unlikely to be targets for presynaptic mGluRs.

Group 1 but not group 2/3 mGluRs inhibit I_{AHP}

The reduction of the Ca^{2+}-dependent K^+ current underlying the slow afterhyperpolarization has proved to be a reliable indicator of the presence of postsynaptic mGluRs (Baskys and Malenka, 1991; Manzoni *et al.*, 1994b). Figure 6A describes a typical experiment in which I_{AHP} was completely inhibited by bath-perfusion of the specific group

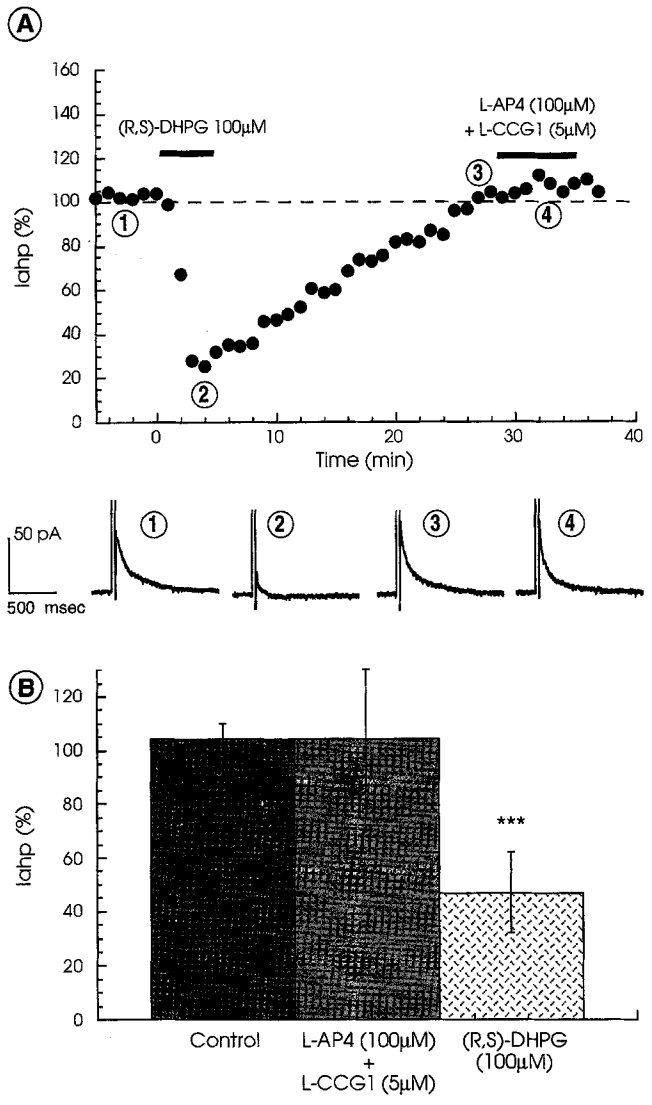

FIG. 6. (*R,S*)-DHPG inhibited I_{AHP}, but neither L-CCG1 nor L-AP4 did so. (A) Typical experiment in which stable I_{AHP}s were recorded in the whole-cell patch-clamp configuration. I_{AHP} was reversibly blocked by perfusion with (*R,S*)-DHPG (compare traces 1 and 2) whereas a mixture of L-CCG1 (5 μM) and L-AP4 (100 μM) had no effect (compare traces 3 and 4). (B) Summary of all experiments performed as above. At the peak of its effect, (*R,S*)-DHPG decreased I_{AHP} to 47 ± 15% of its control value (n = 5), whereas a mixture of L-AP4 and L-CCG1 was without effect. The inhibitory effect of (*R,S*)-DHPG on I_{AHP} was significant as determined with Student's t-test (***P < 0.001; n = 5).

1 agonist (*R,S*)-DHPG. After washout of the (*R,S*)-DHPG-induced inhibition, we applied a cocktail of L-CCG1 (5 μM) and L-AP4 (100 μM) which did not depress I_{AHP}. Figure 6B summarizes all the experiments performed this way (n = 5).

Discussion

Both immunohistochemical (Baude *et al.*, 1993; Shigemoto *et al.*, 1993) and mRNA *in situ* hybridization studies (Ohishi *et al.*, 1993a, b, 1995; Testa *et al.*, 1994) have suggested the expression of mGluRs in the nucleus accumbens. The nucleus accumbens is believed to be a critical substrate for drug reinforcement and drug-dependence (Koob, 1992; Nestler, 1992). Recent *in vivo* experiments have proposed a role for mGluR in regulating the dopamine level within the nucleus accumbens (Ohno and Watanabe, 1995; Taber and Fibiger, 1995), reinforcing the idea that mGluR expression might be of functional importance to nucleus accumbens physiology.

We therefore searched for possible mGluRs at the excitatory synapses between prefrontal cortex excitatory afferents and nucleus accumbens neurons. The main finding of this work is that group 2 mGluRs [activated by L-CCG1, (1*S*,3*S*)-ACPD and (*S*)-4C3HPG] and group 3 mGluRs (sensitive to L-AP4) but not group 1 mGluRs [specifically activated by (*R,S*)-DHPG] can induce synaptic inhibition. This result is in agreement with a number of convergent studies showing that mGluR activation modulates both excitatory and inhibitory transmission in a wide variety of models (Baskys and Malenka, 1991; Glaum and Miller, 1993; Lovinger and McCool, 1995; Manzoni and Bockaert, 1995; Manzoni *et al.*, 1995). Moreover, paired-pulse facilitation was increased and mEPSC frequency was decreased, suggesting that a presynaptic mechanism is responsible for the actions of the mGluR agonists. We also observed that group 1 mGluRs activated by (*R,S*)-DHPG could specifically inhibit I_{AHP}, in agreement with previous studies that have demonstrated a similar role for mGluRs (Manzoni *et al.*, 1994b; Gereau and Conn, 1995a). The postsynaptic actions of the group 1-specific agonist (*R,S*)-DHPG might be due to mGluR5 activation, as suggested by *in situ* hybridization data (Baude *et al.*, 1993; Shigemoto *et al.*, 1993).

An important issue is the specificity of the pharmacological agents used in this study. The postsynaptic effects of (*R,S*)-DHPG are certainly due to the specific activation of group 1 mGluRs (Schoepp *et al.*, 1994; Gereau and Conn, 1995b) and the L-AP4 effects probably result from specific activation of group 3 mGluRs (Hayashi *et al.*, 1994; Thomsen *et al.*, 1994; Saugstad *et al.*, 1997). In the majority of our experiments we used L-CCG1 at a concentration thought to be selective for group 2 mGluRs (Hayashi *et al.*, 1992). However, the latest addition to the group 3 family, mGluR8, shows a high affinity for L-CCG1 and is sensitive to MAP4 (IC_{50} < 50 μM) (Saugstad *et al.*, 1997). In the nucleus accumbens, we found that L-CCG1-induced synaptic depression was sensitive to MCCG1 but not to MAP4 [300 μM, a concentration that would be sufficient to block mGluR8 (Saugstad *et al.*, 1997)], strongly suggesting that the effects of L-CCG1 in the nucleus accumbens were due to the selective activation of group 2 mGluRs.

The nucleus accumbens receives a major innervation from the dopaminergic mesolimbic system, and recent papers have shown on the one hand that mGluR activation modulates the release of dopamine in the nucleus accumbens *in vivo* (Ohno and Watanabe, 1995; Taber and Fibiger, 1995) and on the other hand that presynaptic D1 receptors inhibit excitatory transmission to the nucleus accumbens *in vitro* (Nicola *et al.*, 1996). Since both glutamatergic and dopaminergic terminals can be closely located on medium spiny nucleus accumbens neurons (Smith and Bolam, 1990), it was tempting to postulate that the inhibitory effects were due to mGluR-induced release of dopamine that would subsequently bind to D1 presynaptic inhibitory receptors. We found that the specific D1 antagonist SCH-23390 did not affect mGluR-induced depression, excluding a role for D1 receptors. Taber and Fibiger (1995) have shown that mGluR activation has inhibitory effects on basal and stimulated dopamine release, Nicola *et al.* (1996) have found that 'psychostimulants depress excitatory synaptic transmission in the nucleus accumbens via presynaptic D1-like dopamine receptors', and we now show that mGluRs can inhibit glutamate release. Taken together, these results suggest subtle cross-talk between dopaminergic and glutamatergic innervations at their synaptic contacts with medium spiny neurons.

In the hippocampus, activation of ionotropic glutamate receptors can release GABA or adenosine (Isaacson *et al.*, 1993; Manzoni

et al., 1994a). Group 2/3 mGluR activation could also lead to release of GABA or adenosine that would inhibit glutamate release via either $GABA_B$ (Isaacson *et al.*, 1993) or A1 adenosine receptors (Manzoni *et al.*, 1994a). We found that both $GABA_B$ and A1 antagonists were unable to reduce mGluR actions, reinforcing the idea that the mGluR inhibition of transmitter release was the result of direct presynaptic modulation.

The observations that the mGluR-induced inhibition remained in the presence of N- and P-type calcium channels blockers and that mGluRs could decrease the frequency of mEPSCs suggest that mGluRs can inhibit glutamate release downstream of the intracellular calcium influx.

More detailed experiments will be necessary to determine the exact process responsible for the presynaptic glutamate-mediated inhibition and to explain why two different subtypes of mGluR expressed at the same synapse mediate similar effects. One can speculate that these two subtypes of mGluR have different presynaptic sublocalizations (depending on their distance to the active zone of the synapse) which would provide the synaptic terminal with a refined way of modulating glutamate release in response to different levels of synaptic activity.

In addition to the predominant role of the mesolimbic dopaminergic system in the molecular mechanisms of psychostimulant drug addiction (for reviews see Koob, 1992; Nestler, 1992), evidence has accumulated pointing towards a role for glutamate in this phenomenon (Pulvirenti *et al.*, 1989; Nie *et al.*, 1994; Pierce *et al.*, 1996). The pre- and postsynaptic actions of mGluRs combined with their interactions with dopaminergic systems might be of crucial importance in the physiological regulation of nucleus accumbens neurons and the molecular processes responsible for addiction to psychostimulant drugs.

Acknowledgements

This research was supported by grants from the CNRS, CEE (BIO2-CT93–0243 and BMH4-CT96–0228), DRET (95/075), Bayer Company (Germany). O. M. was supported by INSERM and is grateful to Drs R. Malenka, S. Nicola and S. Kombian for their help with the nucleus accumbens preparation and for the communication of unpublished results. O. M. also thanks Drs P. Castillo and L. Fagni for critical reading of the manuscript, M. Passama for the artwork and Dr D. Pujalte for his help with Ca^{2+} channel toxin experiments.

Abbreviations

(1*S*,3*R*)-	(1*S*,3*R*)-1-aminocyclopentyl-1,3-dicarboxylate
(*R*,*S*)-DHPG	(*R*,*S*)-3,5-dihydroxyphenylglycine
4C3HPG	(*S*)-4-carboxy-3-hydroxyphenylglycine
EPSC	excitatory postsynaptic current
EPSP	excitatory postsynaptic potential
I_{AHP}	afterhyperpolarization current
L-AP4	L-2-amino-4-phosphonobutyrate
L-CCG1	(2*S*,1′*S*,2′*S*)-2-(2′-carboxy-cyclopropyl)glycine
mEPSC	miniature excitatory postsynaptic current
mGluR	metabotropic glutamate receptor

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