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Enhancement of NMDA responses by group I metabotropic glutamate receptor activation in striatal neurones

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1 The interactions between N-methyl-D-aspartate (NMDA) and metabotropic glutamate receptors (mGluRs) were investigated in striatal slices, by utilizing intracellular recordings, both in current- and voltage-clamp mode.

2 Bath-application (50 μM) or focal application of NMDA induced a transient membrane depolarization, while in the voltage-clamp mode, NMDA (50 μM) caused a transient inward current. Following bath-application of the non-selective mGluR agonist 1**S**,3**R**-aminocyclopentane-1,3-dicarboxylic acid (1**S**,3**R**-ACPD, 10 μM), NMDA responses were reversibly potentiated both in current ($197 \pm 15\%$ of control) and voltage-clamp experiments ($200 \pm 18\%$ of control).

3 Bath-application of the group I mGluR agonist (**RS**)-3,5-dihydroxyphenylglycine (3,5-DHPG, 10–300 μM) resulted in a dose-dependent potentiation of NMDA-induced membrane depolarization (up to $400 \pm 33\%$ of control). This potentiation was either prevented by preincubation with (**RS**)-α-methyl-4-carboxyphenylglycine (**RS**-α-MCPG, 300 μM), or blocked when applied immediately after 3,5-DHPG wash-out.

4 Neither (2**S**,1′**S**,2′**S**)2-(2′-carboxycyclopropyl)glycine (L-CCG I, up to 100 μM) nor (2**S**,1′**R**,2′**R**,3′**R**)-2-(2,3-dicarboxycyclopropyl)-glycine (DCG-IV, 1 μM), agonists for group II mGluRs caused any change in NMDA responses. Likewise, L-serine-O-phosphate (L-SOP, 30 μM), agonist for group III mGluRs, did not affect the NMDA-induced depolarization.

5 The enhancement of the NMDA responses was mimicked by phorbol-12,13-diacetate (PDAc, 1 μM) which activates protein kinase C (PKC). The 3,5-DHPG-mediated potentiation of the NMDA-induced depolarization was prevented by preincubation with staurosporine (100 nM) or calphostin C (1 μM), antagonists of PKC.

6 Electrophysiological responses to α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptor activation were not affected by agonists for the three-classes of mGluRs.

7 The present data suggest that group I mGluRs exert a positive modulatory action on NMDA responses, probably through activation of PKC. This functional interaction in the striatum appears of crucial importance in the understanding of physiological and pathological events, such as synaptic plasticity and neuronal death, respectively.

Keywords: Electrophysiology; striatum; N-methyl-D-aspartate (NMDA); metabotropic glutamate receptors (mGluRs); phenylglycines; plasticity; excitotoxicity; neurodegenerative disorders

Introduction

Glutamate is widely accepted as the main excitatory transmitter in the CNS. It acts through two distinct categories of receptors: ionotropic and metabotropic glutamate receptors (mGluRs). Ionotropic receptors are coupled to ligand-gated ion channels and include N-methyl-D-aspartate (NMDA), kainate and α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) receptors (Monaghan *et al*., 1989; Nakanishi, 1992). At least eight members compose the family of G-protein-coupled mGluRs, termed mGluR1-mGluR8, which have been grouped into three classes (Schoepp & Conn, 1993; Nakanishi, 1994; Pin & Duvoisin, 1995). Group I comprises mGluR 1 and 5 which promote polyphosphoinositides (PI) hydrolysis. Group II (mGluR 2, 3) and group III (mGluR 4, 6, 7, 8) inhibit forskolin-stimulated accumulation of intracellular adenosine 3′ : 5′-cyclic monophosphate (cyclic AMP). New pharmacological tools bearing a cyclopropylglycine motif have enabled discrimination between the different subclasses of mGluRs (Birse *et al*., 1993; Thomsen *et al*., 1994). (**RS**)-3,5-dihydroxyphenylglycine (3,5-DHPG) has been shown to be a selective and potent agonist of group I, with negligible effects on cyclic AMP-coupled mGluRs and on ionotropic glutamate receptors (Schoepp *et al*., 1994). On the basis of pharmacological selectivity, group II is preferentially activated by (2**S**,1′**S**,2′**S**)2-(2′-carboxycyclo-propyl)-glycine (L-CCG I) and (2**S**,1′**R**,2′**R**,3′**R**)-2-(2,3-dicarboxy-cyclopropyl)-glycine (DCG-IV), whilst L-serine-O-phosphate (L-SOP) and (L-2-amino-4-phosphonobutyrate (L-AP4) are selective agonists for group III (Pin & Duvoisin, 1995). The characterization of the functional cross-talk between NMDA receptors and mGluRs in the striatum appears of primary importance for several reasons. First, ligand binding and *in situ* hybridization studies have provided evidence for the presence of both NMDA receptors (Monaghan *et al*., 1989; Albin & Greenamyre, 1992; Landwehrmeyer *et al*., 1995) and mGluRs in the striatum (Shigemoto *et al*., 1993; Testa *et al*., 1994; 1995). mGluR5, a group I, PI-linked mGluR, was particularly prominent in medium spiny neurones (Testa *et al*., 1994; 1995). Second, both NMDA receptors and mGluRs have been involved in synaptic plasticity processes in the striatum as well as in other brain structures (Calabresi *et al*., 1992a,b,c; McGuiness *et al*., 1991; Bear & Malenka, 1994). A subthreshold tetanic stimulation combined with administration of aminocyclopentane-1,3-dicarboxylic acid (ACPD) (Aniksztejn *et al*., 1992) or the co-application of NMDA and ACPD (Musgrave *et al*., 1993) induces long-term potentiation (LTP) in hippocampal slices. Third, NMDA receptors have been demonstrated to mediate excitotoxic effects (Rothman & Olney, 1987; Choi, 1988). Recently, evidence has begun to

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accumulate in favour of an involvement of mGluR group I in NMDA-mediated excitotoxicity (Bruno *et al*., 1995b; Buisson & Choi, 1995; Orlando *et al*., 1995). Moreover, antagonists at mGluRs group I protect against NMDA-induced damage (Buisson & Choi, 1995; Orlando *et al*., 1995). The present study represents the first attempt to identify and characterize the role of the different mGluR subtypes in the modulation of NMDA-mediated electrophysiological responses in neostriatal neurones, by utilizing the new selective compounds available.

The pharmacological modulation of excitatory glutamatergic transmission within the basal ganglia might be a target for new possible therapeutic approaches to movement disorders.

Methods

Preparation and maintenance of the slices

Adult male Wistar rats (200–250 g) were used for all the experiments. Details on the preparation and maintenance of the slices have been described previously (Calabresi *et al*., 1992a,b,c). Briefly, rats were killed by a heavy blow to the chest under ether anaesthesia, which severed major blood vessels. The brain was quickly removed and coronal slices (200–300 μm thick) including cortex and neostriatum were prepared from tissue blocks with the use of a vibratome. Slices were maintained at 34°C in an oxygenated solution (see composition below) for at least 1 h before the experiment. A single slice was then transferred to a submerged recording chamber and continuously perfused with a solution (33–34°C, 2–3 ml min^{-1}) containing (in mM): NaCl 126, KCl 2.5, NaH_2PO_4 1.2, $MgCl_2$ 1.2, $CaCl_2$ 2.4, glucose 10 and $NaHCO_3$ 26; the solution was gassed with a mixture of 95% O_2 and 5% CO_2.

Electrophysiological recordings and data analysis

Intracellular recording electrodes were filled either with 2 M KCl or with 2 M K-acetate (30–60 mΩ). An Axoclamp 2A amplifier was used for both current- and voltage-clamp recordings. During voltage-clamp experiments headstage voltage was monitored continuously. Traces were displayed on an oscilloscope and stored on a digital system. Quantitative data on changes of membrane depolarization and inward current were usually expressed as a percentage of the control value. The experiments in which membrane depolarization or inward current induced by the application of the drugs were not followed by a complete return to the baseline, were discarded from the statistics. Values given in the text and in the figures are expressed as mean $\pm$ s.e.mean. The statistical significance of the experiments was evaluated by use of Student's *t* test. Statistics were performed on 'Kaleidagraph 3.0' running on a Power Macintosh.

Drug source and application

Drugs were bath-applied by switching the superfusing solution to one containing known concentrations of drugs. Drug solutions entered the recording chamber within 40 s after a three-ways tap had been turned on. In some experiments NMDA was applied by ejecting (pressure ejection, Picospritzer) a few nanolitres of a 500 μM solution from the tip of a blunt pipette beneath the surface of the superfusing solution and just above the tissue slice.

All the compounds utilized were supplied by Tocris Cookson (Bristol, U.K.) with the exception of NMDA, phorbol-12,13-diacetate (PDAc), tetrodotoxin (TTX) and staurosporine (from Sigma, St. Louis, U.S.A.). In some experiments performed in current-clamp mode, and in all the ones recorded in the voltage-clamp mode, 1 M TTX was applied throughout the recording session.

Results

Electrophysiological characterization of the recorded cells

Conventional sharp-microelectrode intracellular recordings were obtained from 124 electrophysiologically identified 'principal' striatal cells. Experiments were performed either in the current- or in the voltage-clamp mode. The main characteristics of these cells have been described in detail previously both *in vivo* and *in vitro* (Calabresi *et al*., 1990a,b; 1996). All striatal neurones recorded showed high resting membrane potential (RMP, -85 ± 5 mV), absence of spontaneous action potential discharge, tonic firing activity during current-induced membrane depolarization. Morphological studies have demonstrated that these cells are medium-sized spiny neurones which represent the largest cell population in the striatum (Groves, 1983; Kawaguchi *et al*., 1989). All measurements were performed at the original RMP of the recorded neurone.

*Effects of 1**S**,3**R**-ACPD on NMDA responses*

Bath-applied NMDA (50 μM, 20 s) induced a transient and reversible membrane depolarization of the neurones recorded in the current-clamp mode (9 ± 3 mV; $n = 11$; Figure 1A, a1). Before the slice was incubated with 1**S**,3**R**-ACPD, the NMDA-induced response was repeated at least once more, at the same time interval (5 min), in order to have reproducible control responses. Bath-application of 1**S**,3**R**-ACPD (10 μM, 5 min) did not cause, *per se*, changes in the membrane properties of the recorded cell. However, after incubation with 10 μM 1**S**,3**R**-ACPD, the amplitude of the NMDA-induced membrane depolarization was significantly potentiated ($197 \pm 15\%$; $n = 11$; $P < 0.005$; Figure 1A, a2). After 10 min wash-out of 1**S**,3**R**-ACPD, NMDA responses were similar to the control ones (Figure 1A, a3). As previously shown (Calabresi *et al*., 1992b), higher doses of 1**S**,3**R**-ACPD (30–100 μM), in most of the recorded cells, produced, by themselves, a membrane depolarization. Thus, to avoid interference of the 1**S**,3**R**-ACPD-induced response with the NMDA-mediated response, only a submaximal dose (10 μM) of 1**S**,3**R**-ACPD was used. Experiments were also performed with focal administration of NMDA by pressure ejection (see Methods for details). Figure 1B shows the effect of focal application of NMDA which induced a transient membrane depolarization (10 ± 4 mV; $n = 8$; Figure 1B, b1). In the presence of 10 μM 1**S**,3**R**-ACPD, the amplitude of the depolarization was significantly increased ($160 \pm 18\%$; $n = 8$; $P < 0.005$; Figure 1B, b2). NMDA resposes returned to the control values after wash-out of 1**S**,3**R**-ACPD (10 min, Figure 1B, b3). The effect of 1**S**,3**R**-ACPD on the NMDA response was also investigated by means of the single microelectrode voltage-clamp technique. Bath-applied NMDA (50 μM, 20 s) generated a transient inward current (110 ± 20 pA; $n = 9$; Figure 1C, c1). Application of 1**S**,3**R**-ACPD (10 μM, 5 min) did not induce any effect *per se*. However, the amplitude of the NMDA-induced inward current, was significantly increased after incubation with 1**S**,3**R**-ACPD ($200 \pm 18\%$; $n = 9$; $P < 0.005$; Figure 1C, c2). This effect was fully reversed after washing-out 1**S**,3**R**-ACPD (10 min, Figure 1C, c3). Some experiments were performed in order to show that NMDA responses, recorded both in current- and voltage-clamp mode were prevented by D-2-amino-5-phosphonovalerate (D-APV) (50 μM; $n = 5$; not shown).

Actions of 3,5-DHPG on NMDA-induced membrane depolarization

To define further the mGluR subtype involved in the enhancement of NMDA responses by 1**S**,3**R**-ACPD, we utilized 3,5-DHPG, a novel compound which is considered a selective agonist for group I mGluRs. Bath-applied NMDA (50 μM, 20 s) induced a small and transient membrane depolarization (9 ± 3 mV; $n = 18$; Figure 2A, a1). Bath-application of 300 μM 3,5-DHPG (5 min) caused no modifications in the membrane

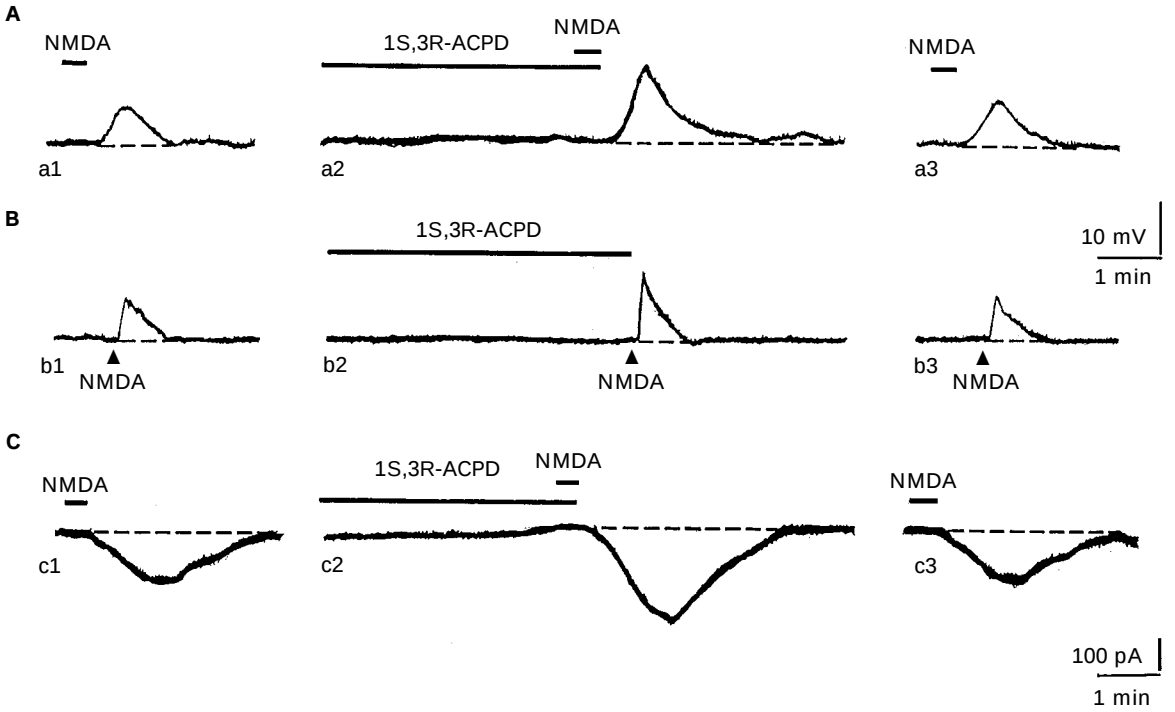

Figure 1 Effects of 1**S**,3**R**-ACPD on NMDA responses. (A) The membrane depolarization induced by bath-applied NMDA (50 μM, 20 s) in control condition (A, a1) was increased after 5 min incubation with 10 μM 1**S**,3**R**-ACPD (A, a2). After 10 min wash-out of 1**S**,3**R**-ACPD, the NMDA response recovered (A, a3). (B) Focally-applied (see Methods) NMDA induced a membrane depolarization (B, b1) which was increased in the presence of 10 μM 1**S**,3**R**-ACPD (B, b2). After 1**S**,3**R**-ACPD wash-out (10 min), NMDA-induced depolarization was similar to the control values (B, b3). Calibration applies for both (A) and (B). (C) The inward current induced by NMDA (50 μM, 20 s) recorded in the voltage-clamp mode (C, c1) was increased after 5 min preincubation with 10 μM 1**S**,3**R**-ACPD (C, c2) and returned to basal values after 1**S**,3**R** wash-out (10 min, C, c3). Holding potential was -82 mV.

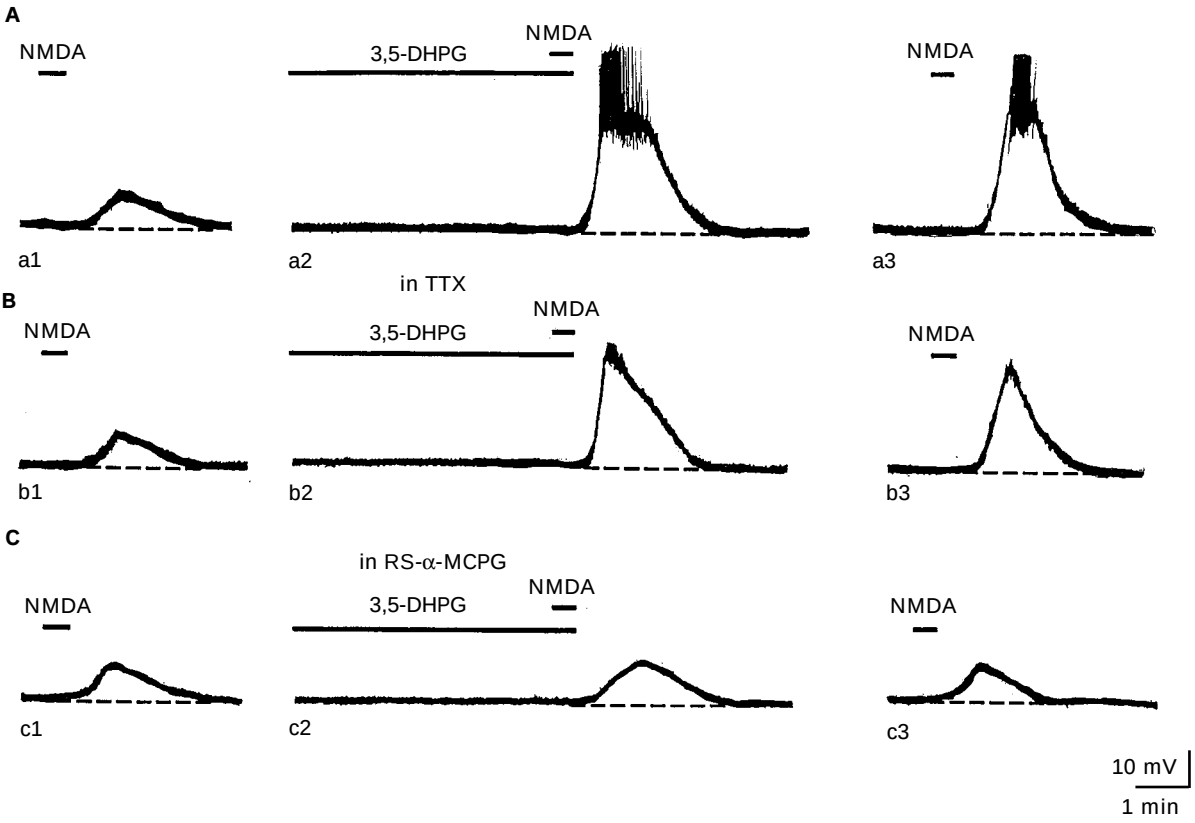

Figure 2 Group I mGluRs mediate enhancement of NMDA responses. (A) The NMDA-induced membrane depolarization (A, a1) was greatly enhanced after 5 min incubation with 300 μM 3,5-DHPG (A, a2). Note that the potentiation led the cell to a sustained firing discharge. After 15 min wash-out, the NMDA-induced membrane depolarization was still enhanced (A, a3). (B) In the presence of 1 μM TTX the NMDA-mediated membrane depolarization (B, b1) was potentiated to the same extent as in (A) (B, b2). Likewise, the potentiation persisted after 15 min wash-out of 3,5-DHPG (B, b3). (C) In the presence of 300 μM **RS**-α-MCPG, the NMDA-induced membrane depolarization (C, c1) was not potentiated by 3,5-DHPG (300 μM, C, c2). After wash-out, the NMDA-mediated membrane depolarization was similar to the control one (C, c3).

properties of the recorded cell, but produced a marked increase in the amplitude of the NMDA-mediated membrane depolarization (400±33%; $n=18$; $P<0.001$; Figure 2A, a2), which caused a sustained firing discharge. Moreover, the time-to-peak of the NMDA response was substantially decreased. Interestingly, after 3,5-DHPG wash-out, the potentiation of the NMDA-mediated membrane depolarization persisted (Figure 2A, a3). As shown in Figure 3a, the 3,5-DHPG-mediated potentiation lasted for at least 30 min. This enhancement of NMDA-induced membrane depolarization occurred also with lower doses of 3,5-DHPG (10–100 μM, $n=10$, not shown). Moreover, Figure 4 shows that the enhancement by 3,5-DHPG of NMDA responses occurred in a dose-dependent fashion. The minimal dose producing a potentiation of NMDA response was 10 μM 3,5-DHPG; the maximal effect was reached at 300 μM. The extrapolated EC_{50} value was 66.322 μM. In order to avoid the possible contribution to the observed enhancement by a TTX-sensitive release of endogenous transmitters, experiments were also performed in TTX. In the presence of 1 μM TTX in the perfusing solution, the NMDA response in control condition ($n=7$; Figure 2B, b1) as well as the potentiation in the presence of 3,5-DHPG were unaffected ($n=7$; Figure 2B, b2). Also in the presence of TTX, the enhancement persisted after wash-out of 3,5-DHPG (Figure 2B, b3). The effect of (**RS**)-α-methyl-4-carboxyphenylglycine (**RS**-α-MCPG), an antagonist for both group I and II, was tested on the 3,5-DHPG-mediated potentiation of NMDA responses. Figure 2C shows that in control condition NMDA induced a transient membrane depolarization (8±3 mV; $n=7$; Figure 2C, c1). Preincubation of the slice with **RS**-α-MCPG (300 μM, 10 min) prevented the potentiation of NMDA response by a saturating dose of 3,5-DHPG (300 μM; $n=7$; Figure 2C, c2). In order to test the possibility that the long-lasting enhancement of NMDA responses might be due to a slow wash-out of 3,5-DHPG, in some experiments **RS**-α-MCPG (300 μM) was added to the bathing solution immediately after the onset of the wash-out of 3,5-DHPG. As shown in the Figure 3b, 5 min perfusion with 300 μM 3,5-DHPG potentiated the NMDA-induced membrane depolarization. However, incubation with 300 μM **RS**-α-MCPG immediately after the onset of 3,5-DHPG wash-out blocked the long-term effect of 3,5-DHPG (Figure 3b, $n=4$).

The role of protein kinase C in the mGluR-mediated enhancement of NMDA responses

In order to analyse the possible role of protein kinase C (PKC) in the potentiation of NMDA responses by mGluR activation, we tested the effect of (PDAc), an irreversible activator of PKC on NMDA-induced currents recorded in the voltage-clamp mode. In control condition, bath-applied NMDA (50 μM, 20 s) produced a transient inward current (90±21 pA; $n=11$; Figure 5A, a1). After 5 min incubation with 1 μM PDAc, the amplitude of the NMDA-induced inward current was significantly enhanced (185±31%; $n=11$; $P<0.005$; Figure 5A, a2). The NMDA response was still potentiated after 10–12 min PDAc wash-out (Figure 5A, a3). To confirm these results we performed another set of experiments looking at the effect of staurosporine and calphostin C, two PKC inhibitors, on the potentiation of NMDA response mediated by 3,5-DHPG. In control condition NMDA (50 μM, 20 s) produced a transient membrane depolarization (8±3 mV; $n=6$; Figure 5B, b1). In order to be sure that the NMDA response, in control condition, was not reduced by calphostin C, the NMDA application was repeated in the presence of 1 μM calphostin C, added before applying 3,5-DHPG. Preincubation of the slice in calphostin C did not affect the NMDA response, *per se*. However, the 3,5-DHPG-mediated enhancement in amplitude of the membrane depolarization caused by NMDA was significantly attenuated (110±15%; $n=6$; Figures 3a and 5B, b2). After drugs wash-out the NMDA response was similar to that recorded in control condition (Figure 5B, b3). In the presence of 100 nM staurosporine the 3,5-DHPG-mediated potentiation was prevented ($n=5$, Figure 3a).

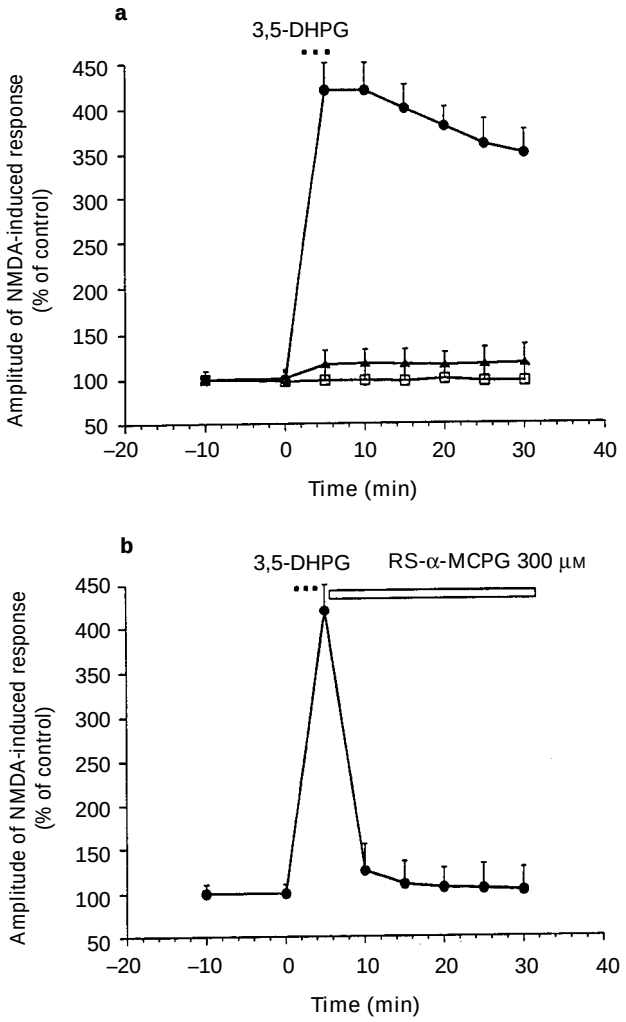

Figure 3 Time-course of the 3,5-DHPG-mediated enhancement of NMDA response, in control condition, in the presence of PKC blockers, and short-term potentiation after perfusion with 300 μM RS-α-MCPG. (a) The graph shows that 5 min after application of 300 μM 3,5-DHPG, in control condition, there was an enduring enhancement of the amplitude of the response to 50 μM NMDA (●). In the presence of 100 nM staurosporine (□) the enhancement was prevented, whilst preincubation with 1 μM calphostin C (▲), markedly reduced the potentiation. Each point represents the mean of at least four experiments; vertical lines show s.e. mean. (b) The graph shows that a 5 min application of 300 μM 3,5-DHPG potentiated the NMDA-induced membrane depolarization. Bath-application of 300 μM **RS**-α-MCPG immediately after the onset of the wash-out phase of 3,5-DHPG, prevented the long-lasting enhancementt of the NMDA response.

Agonists for group II and III mGluRs do not affect NMDA responses

In order to investigate further the modulation of NMDA responses by mGluR subgroups, experiments were performed with selective agonists for mGluR group II and III. Bath-application of DCG-IV (1 μM) and L-CCG I (20–100 μM), agonists for group II (mGluR2 and mGluR3) affected neither membrane properties nor caused any change in the NMDA-induced membrane depolarization ($n=7$, data not shown). In addition, bath-applied L-SOP (10–30 μM), a selective agonist for group III mGluRs, also failed to affect NMDA responses, and did not modify intrinsic membrane properties of the recorded cells ($n=5$, data not shown). Electrophysiological responses to ionotropic AMPA (300 nM) receptor activation were not affected by either the group I mGluR agonist 3,5-

DHPG (100–300 μM, $n=5$, not shown) or agonists of both group II and III (DCG-IV, 1 μM; $n=3$; L-SOP, 10 μM; $n=3$; data not shown).

Discussion

The present study provides evidence in favour of a positive modulatory role of group I mGluRs on ionotropic NMDA receptor activation in striatal neurones. 1**S**,3**R**-ACPD, a non-selective agonist for these receptors, reversibly enhanced NMDA responses. Interestingly, the selective agonist for mGluRs coupled to phosphoinositide (PI) hydrolysis, 3,5-DHPG, produced a potentiation of NMDA responses in the recorded cells. This enhancement was dose-dependent, and was prevented or blocked by **RS**-α-MCPG. The finding that the 3,5-DHPG-mediated enhancement of the NMDA response was blocked by application of **RS**-α-MCPG after the onset of wash-out of 3,5-DHPG, suggests that this effect observed in the presence of 3,5-DHPG might be due to a slow wash-out of this drug. Alternatively, it might be hypothesized that **RS**-α-MCPG blocks the 'expression' phase of this potentiation. The positive modulation appeared to be mediated by PKC, as demonstrated by the finding that PDAc mimicked the potentiation induced by 3,5-DHPG, and that staurosporine and calphostin C, PKC inhibitors, prevented this potentiation. Moreover, we also showed that (i) agonists at group II and III mGluRs did not alter NMDA responses; (ii) AMPA responses were not affected by agonists for the three classes of mGluRs. In striatal neurones, it has been observed that iontophoretic application of 1**S**,3**R**-ACPD reduces NMDA responses (Colwell & Levine, 1994). Our findings appear to be in contrast with the work by Colwell and Levine (1994). Differences in the experimental approach utilized by these authors (iontophoretic application) might be the reason for this discrepancy.

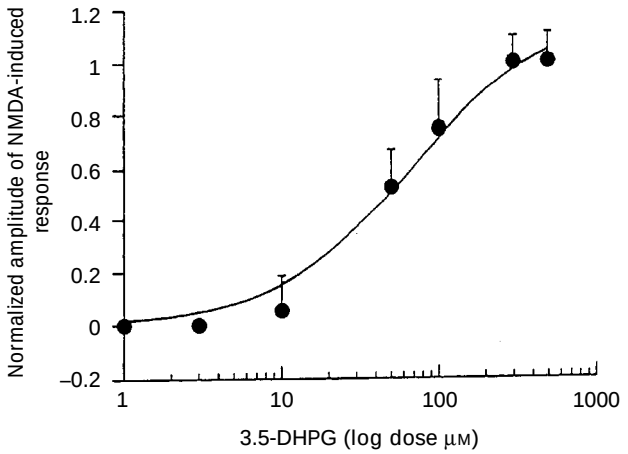

Figure 4 Dose-dependency of the 3,5-DHPG-mediated enhancement of NMDA response. The graph shows the dose-response curve for the 3,5-DHPG-induced potentiation of NMDA response. Each point represents the mean of at least four experiments; vertical lines show s.e.mean. Normalized data points were fitted according to the equation: $y=ml\times m0/(m2+m0)$, where y is NMDA response in the presence of differential doses of 3,5-DHPG divided by NMDA maximal response in the presence of a saturating dose of 3,5-DHPG (300 μM; *m2 defines the* EC_{50} *value (66.322* μM).

A facilitatory role of mGluR agonists on NMDA responses has been described in *Xenopus* oocytes expressing rat brain mRNA (Kelso *et al.*, 1992), hippocampal CA1 neurones (Harvey *et al.*, 1991; Aniksztejn *et al.*, 1992; Harvey & Collingridge, 1993; Fitzjohn *et al.*, 1996), olfactory cortex (Collins, 1993), dorsal horn neurones (Bleakman *et al.*, 1992), spinal motoneurones (Birse *et al.*, 1993), turtle cerebellar granule cells (Kinney & Slater, 1993), neocortical neurones (Rahman & Neuman, 1996). This facilitatory action is selective for NMDA receptors in hippocampal neurones (Aniksztejn *et al.*, 1992), whilst in dorsal horn neurones and cerebellar Purkinje cells, mGluR activation has also been shown to potentiate AMPA and kainate responses (Bleakman *et al.*, 1992; Glaum *et al.*, 1992). However, the mechanism underlying the potentiation of NMDA responses is not fully understood. In *Xenopus* oocytes the potentiation of NMDA response was dependent on PKC activation (Kelso *et al.*, 1992), whilst in hippocampal neurones

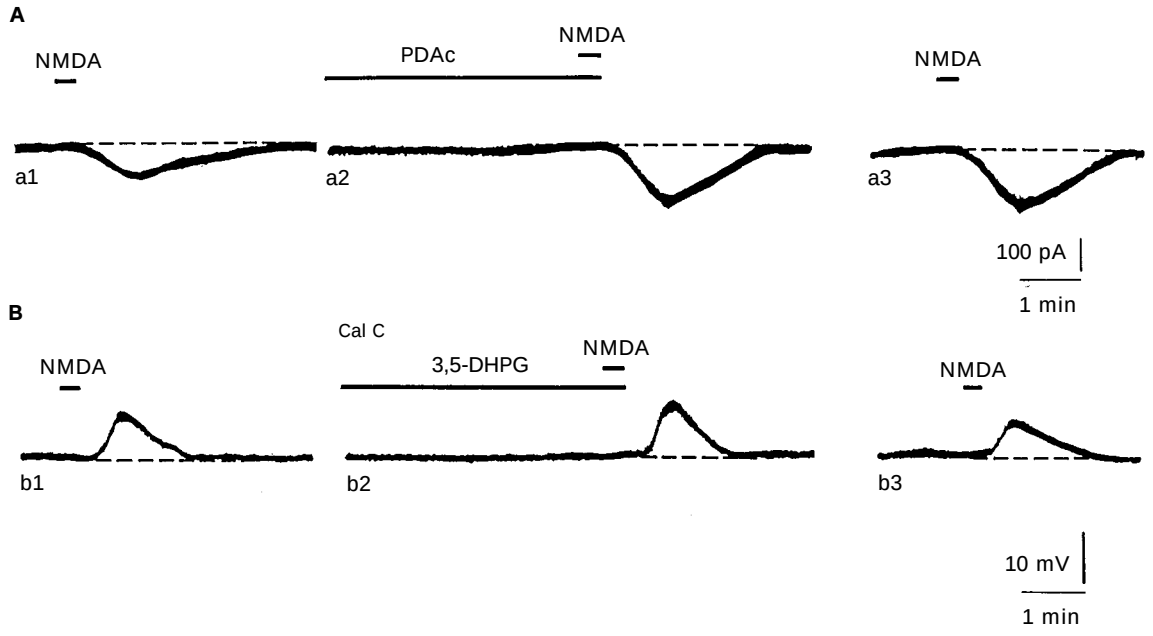

Figure 5 Role of PKC in the potentiation of NMDA response. (A) In a voltage-clamped neurone, application of 50 μM NMDA (20 s) produced an inward current (A, a1) which was increased in the presence of PDAc (1 μM, 5 min, A, a2). This potentiation persisted after PDAc wash-out (10 min, A, a3). Holding potential was −84 mV. (B) NMDA (50 μM, 20 s) caused a membrane depolarization of the recorded neurone (B, b1). In the presence of calphostin C (Cal C, 1 μM), the enhancement of the membrane depolarization caused by 50 μM NMDA after 5 min incubation with 300 μM 3,5-DHPG was markedly reduced (B, b2). After wash-out, the NMDA response returned to the control values (B, b3).

a role of PKC is still controversial (Anikszteljn *et al*., 1992; Harvey & Collingridge, 1993). MGluR activation has also been shown to potentiate NMDA-mediated synaptic transmission, by exerting a facilitatory role on the induction of hippocampal long-term potentiation (LTP) (McGuiness *et al*., 1991; Anikszteljn *et al*., 1992; Ben-Ari *et al*., 1992; O'Connor *et al*., 1994). Recently, it has been suggested that, with respect to hippocampal LTP, mGluRs enable a 'conditioning' switch to be set, involving mGluR-mediated activation of PKC and subsequent phosphorylation processes (Bortolotto *et al*., 1994). Accordingly, PKC is known to potentiate NMDA responses by reducing the voltage-dependent magnesium blockade of the NMDA receptor-channel complex (Chen & Huang, 1992).

Understanding the functional roles of mGluRs in the brain and their complex interplay with ionotropic glutamate receptors has been hindered in the past by the lack of specific compounds for mGluRs. Thus, whilst the role of NMDA glutamate receptors in excitotoxic neuronal death has been extensively studied and defined (Rothman & Olney, 1987; Choi, 1988), the effect of mGluR activation is less well understood. A protective action by *trans*-ACPD as well as by 1**S**,3**R**-ACPD, its active isomer, against excitotoxic damage has been described in cultured cortical (Koh *et al*., 1991; Bruno *et al*., 1994), retinal (Siliprandi *et al*., 1992) and cerebellar cells (Pizzi *et al*., 1993). However, trans-ACPD potentiated NMDA-induced death of cultured cerebellar granule cells (Aleppo *et al*., 1992). The recent advent of cyclopropylglycine derivatives in the study of the pharmacology of mGluRs clarified some functional aspects of the role played by these receptors in neuronal activity. A role for group I mGluRs in the enhancement of NMDA responses has already been described in the rat hippocampus (Fitzjohn *et al*., 1996). Much effort has been produced in the characterization of the function of mGluRs in excitotoxic damage. Experimental evidence supports the hypothesis that group I mGluRs amplify NMDA-induced neurotoxicity (Bruno *et al*., 1995b; Buisson & Choi, 1995; Orlando *et al*., 1995). Interestingly, Bruno *et al*. (1995b) showed that the amplification of NMDA-induced damage in cultured cortical cells by 3,5-DHPG was mimicked by phorbol esters and prevented by PKC inhibitors. This is in good agreement with our data showing that 3,5-DHPG-dependent potentiation of NMDA responses is mimicked by PKC activation and prevented by staurosporine and calphostin C. A neuroprotective effect against NMDA-mediated damage has been observed either by blocking group I mGluRs (Buisson & Choi, 1995; Orlando *et al*., 1995), or by activating group II and group III mGluRs (Bruno *et al*., 1994; 1995a; Ambrosini *et al*., 1995; Buisson *et al*., 1996). By utilizing the antagonist of group I mGluR, (**S**)-4-carboxy-3-hydroxyphenylglycine (**S**-4C3HPG), both Buisson and Choi (1995) and Orlando *et al*. (1995) showed that NMDA-induced toxicity was greatly attenuated.

Corticostriatal fibres are glutamatergic (Reubi & Cuenod, 1979). Medium spiny striatal neurones bear both NMDA receptors (Monaghan *et al*., 1989; Landwehrmeyer *et al*., 1995) and mGluRs (Testa *et al*., 1994; 1995), and degenerate in the course of Huntington's disease (Kowall *et al*., 1987). It is interesting to note that the NMDA receptor agonist, quinolinic acid, when injected into the striatum causes lesions which resemble the neuropathological features of Huntington's disease (Beal *et al*., 1986). This effect is prevented by decortication, a procedure which removes corticostriatal glutamatergic afferents (Beal *et al*., 1993). However, excitotoxicity is restored by coadministration of *trans*-ACPD (Beal *et al*., 1993). This effect has been thought to be due to a permissive role by mGluRs in the toxic damage produced by NMDA. Furthermore, it has been demonstrated that in the quinolinic acid model of Huntington's disease, antagonists at group I mGluR exert a protective action (Orlando *et al*., 1995). Interestingly, the degree of protection is similar to that produced by NMDA receptor antagonists (Orlando *et al*., 1995). Increased glutamatergic transmission within the basal ganglia has been involved in the pathophysiology of neurodegenerative disorders, such as Huntington's disease and Parkinson's disease (Beal, 1992; Turski & Turski, 1993). Impairment of energy metabolism has been shown to increase neuronal vulnerability to glutamate, via the attenuation of the voltage-dependent block of NMDA receptors operated by magnesium (Novelli *et al*., 1988; Zeevalk & Nicklas, 1991). Thus, activation of PKC and an impaired energy metabolism might act synergistically enabling endogenous glutamate to become excitotoxic through NMDA receptor activation.

These findings support a permissive role exerted by group I mGluRs in the NMDA-induced response. The specificity of the interplay occurring between NMDA receptors and mGluRs suggests that the latter group of glutamate receptors might represent an interesting target for therapeutic approaches to neurodegenerative disorders.

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