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Pharmacological characterization of metabotropic glutamate receptors potentiating NMDA responses in mouse cortical wedge preparations

Guido Mannaioni, Vincenzo Carlà & [1]Flavio Moroni

Dipartimento di Farmacologia, Preclinica e Clinica, Università di Firenze, Viale Morgagni 65, 50134 Firenze, Italy

1 Mouse cortical wedge preparations were used in order to study the effects of metabotropic glutamate receptor (mGluR) agonists and antagonists on the depolarization induced by N-methyl-D-aspartate (NMDA) or by (S)-α-amino-4-bromo-3-hydroxy-5-isoxazolepropionic acid (AMPA).

2 (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid (1S,3R-ACPD) (30-300 μM) significantly potentiated the depolarizations induced by NMDA, leaving unchanged those mediated by AMPA. This potentiation developed slowly and lasted for up to 60 min provided that the slices were continuously perfused with the mGluR agonist.

3 Concentration-response curves to NMDA in the absence and in the presence of 1S,3R-ACPD (100 μM) indicated that the potentiation was due to increased affinity of the NMDA receptor complex for its agonist. The maximal responses to NMDA were not potentiated.

4 Selective agonists of group 1 mGluR such as quisqualate (Quis) (30 μM) or (RS)-3,5-dihydroxyphenylglycine (DHPG) (300 μM) did not potentiate NMDA responses. Similarly, selective agonists of group 2 mGluRs, such as (2S,3S,4S)-α-carboxycyclopropyl-glycine (L-CCG-I) (3-30 μM), and of group 3, such as L-2-amino-4-phosphonobutyric acid (L-AP4) (100 μM) were inactive in our test. A number of other putative mGluR agents having partial agonist activity on mGluRs in brain slices and in expression systems, such as 1R,3S-ACPD (500 μM), DL-2-amino-3-phosphonopropionic acid (DL-AP3) (300 μM) and (S)-4-carboxy-3-hydroxyphenylglycine (S-4C3HPG; 500 μM), when placed in the experimental protocol we used, did not change NMDA responses.

5 Available mGluR antagonists, such as DL-AP3 (1 mM), (+)-α-methyl-4-carboxyphenylglycine (MCPG) (500 μM), S-4-carboxyphenylglycine (4CPG; 500 μM) and S-4-carboxy-3-hydroxyphenylglycine (S-4C3HPG; 500 μM), did not reduce 1S,3R-ACPD potentiation of NMDA responses.

6 It is concluded that the potentiation of NMDA currents induced by the mGluR agonist 1S,3R-ACPD, in mouse cortical wedges, has a pharmacological profile which is different from that of the three mGluR groups so far described in expression systems.

Keywords: (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid (1S,3R-ACPD); metabotropic glutamate receptor (mGluR); N-methyl-D-aspartate (NMDA); (+)-α-methyl-4-carboxyphenylglycine ((+)MCPG); DL-2-amino-3-phosphonopropionic acid (DL-AP3); L-2-amino-4-phosphonobutyric acid (L-AP4); (2S,3S,4S)-α-carboxycyclopropyl-glycine (L-CCG-I); (S)-4-carboxy-3-hydroxyphenylglycine (S-4C3HPG)

Introduction

It has been repeatedly proposed that N-methyl-D-aspartate (NMDA) receptors are involved in a wide range of processes such as neuronal development (Tsumoto *et al.*, 1987), synaptic plasticity (Collingridge & Lester, 1989; Ben-Ari *et al.*, 1992), epileptogenesis (Dingledine *et al.*, 1986) and excitotoxicity (Rothman & Olney, 1987). Modulation of these receptors could therefore be an interesting pharmacological target.

Several laboratories have clearly shown that the responses evoked by NMDA may be significantly modified by selective metabotropic glutamate receptor (mGluR) agonists such as (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid (1S,3R-ACPD) (Schoepp & Conn, 1993; Nakanishi, 1994). This compound potentiates NMDA-induced depolarization of *Xenopus* oocytes injected with rat brain mRNA (Kelso *et al.*, 1992) and of hippocampal or spinal cord neurones (Aniksztein *et al.*, 1992; Cerne & Randic, 1992; Bleakman *et al.*, 1992; O'Connor *et al.*, 1994; Harvey & Collingridge, 1993; Bond & Lodge, 1995; Jones & Headley, 1995). In striatal neurones, in contrast, 1S,3R-ACPD inhibits the depolarization induced by NMDA (Colwell & Levine, 1994) and a similar inhibition has been recently described in mesencephalic neurones in culture (Ambrosini *et al.*, 1995) thus indicating that NMDA responses may be either potentiated or inhibited by mGluR agonists.

It is now widely accepted that 1S,3R-ACPD interacts with at least eight different mGluR subtypes which have been so far described using molecular biology approaches (see for reviews: Nakanishi, 1992; Schoepp & Conn, 1993; Pin & Duvoisin, 1995). These eight mGluRs can be classified into three groups because of their amino acid sequence, pharmacological properties and second messenger cascades. The first comprises mGluR1 and mGluR5 and is associated with the stimulation of phospholipase C, the second comprises mGluR2 and mGluR3 and is negatively linked to the adenylyl cyclase cascade and the third group, comprising mGluR4, mGluR6, mGluR7 and mGluR8 is also negatively linked to the adenylyl cyclase, but can be distinguished from the second group as it can be stimulated by L-2-amino-4-phosphonobutyric acid (L-AP4).

During a series of experiments performed in mice cortical wedge preparations (Harrison & Simmonds, 1985; Burton *et al.*, 1988) with the aim of characterizing glutamate analogues acting as agonists of different NMDA receptor subtypes (Mannaioni *et al.*, 1994; Moroni *et al.*, 1995), we noticed that 1S,3R-ACPD potentiated the NMDA-induced depolarization of the slices. Since this modulation of NMDA responses could be an interesting target for pharmacological agents, we at-

[1] Author for correspondence.

tempted to characterize the mGluR type involved by utilizing currently available agonists and antagonists (Ishida *et al.*, 1995; Roberts, 1995). In this study we obtained results suggesting that the observed potentiation of NMDA responses induced by 1S,3R-ACPD has pharmacological properties that do not allow its classification into one of the three mGluR groups so far described (Nakanishi, 1992; Schoepp & Conn, 1993; Pin & Duvoisin, 1995).

Method

Mouse cortical wedge preparation

The cortical wedge preparation described by Harrison & Simmonds (1985) and modified by Burton *et al.* (1988) was used as previously described (Moroni *et al.*, 1991). Briefly, wedges obtained from white Swiss mice (male 15/25 g) were placed in a two-compartment bath and silicone grease was placed between the two portions of the bath. The wedges were incubated at room temperature and perfused with Krebs solution (mM; NaCl 135, $CaCl_2$ 2.4, KH_2PO_4 1.3, $MgCl_2$ 1.2, $NaHCO_3$ 16.2 and glucose 7.7), gassed with 95% O_2 and 5% CO_2 at a flow rate of 2 ml min^{-1}. After stabilization, the grey matter was perfused with a Mg^{2+} free medium. NMDA and AMPA were repeatedly applied for 2 min every 15 min, whereas the other compounds were applied continuously. The NMDA-induced variations of the d.c. potentials between the two compartments were monitored via Ag/AgCl electrodes and displayed on a chart recorder. The preparations were initially stabilized by repeated application of 10 μM NMDA, a concentration which gave sub-maximal responses but which did not significantly reduce the response of subsequent applications of the agonist (Mannaioni *et al.*, 1994; Moroni *et al.*, 1995). After stabilization, NMDA was applied at 5 μM, a concentration which gave a mean response of $28 \pm 9\%$ of that evoked by 10 μM NMDA (mean $\pm$ s.e.mean of 25 experiments). Stable responses to 5 μM NMDA in each experimental preparation were to be considered 100% and changes in this response were evaluated as described in the Results section.

Drugs

NMDA and tetrodotoxin were obtained from Sigma Chemical Co (St Louis Mo, USA). (1S,3R)-ACPD; AMPA; (1R,3S)-ACPD; L-quisqualic acid (Quis); (RS)-3,5-dihydroxyphenylglycine (DHPG); (S)-4-carboxy-3-hydroxyphenyl-glycine (S-4C3HPG); (2S,3S,4S)-α-carboxycyclo-propyl-glycine (L-CCG-I); DL-2-amino-3-phosphonopropionic acid (DL-AP3); (S)-4-carboxyphenylglycine (S-4CPG) and (+)-α-methyl-4-carboxyphenylglycine ((+)-MCPG) were obtained from Tocris Cookson (Bristol, U.K.). 6-Nitro-7-sulphamyl-benzo(f)-quinoxaline-2,3-dione (NBQX) was kindly provided by Dr Sheardown, Novo-Nordisk (Malov, Denmark).

Results

Characterization of 1S,3R-ACPD-induced potentiation of NMDA responses

Figure 1 shows that the responses to NMDA (5 μM) were strongly potentiated by the addition of 1S,3R-ACPD (100 μM) to the perfusion medium. This potentiating effect was stereoselective since 1S,3R-ACPD (500 μM) was inactive (see Table 1) and required two NMDA applications to reach its maximum. In the experimental protocol we used, maximal responses were obtained 30 min after the beginning of perfusion of the slices with 1S,3R-ACPD-containing solution (see Figures 1 and 2a). These responses, obtained at the second application of NMDA after perfusion with the mGluR agonist, were used for quantitative studies. The potentiating action of

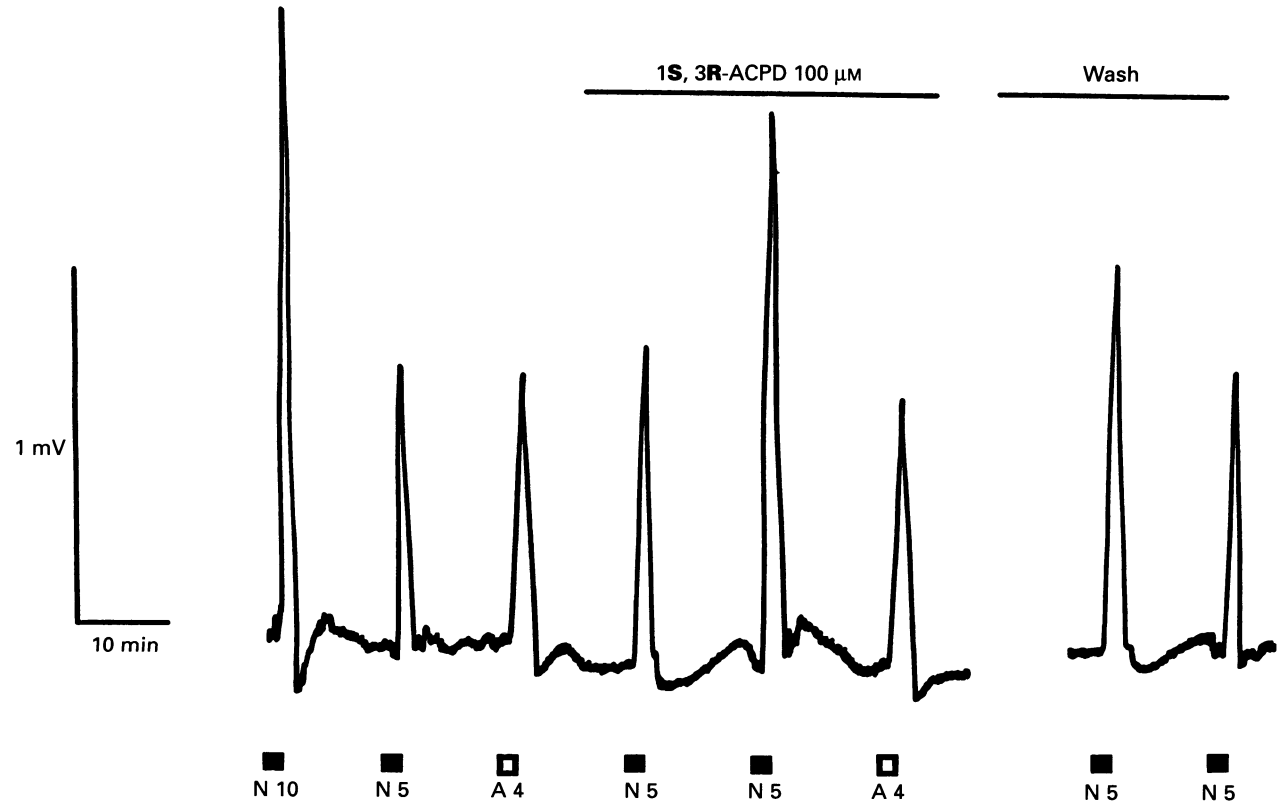

Figure 1 Records of a typical experiment showing the effects of (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid (1S,3R-ACPD) (100 μM) on N-methyl-D-aspartate (NMDA; N) and (S)-α-amino-4-bromo-3-hydroxy-5-isoxalepropionic acid (AMPA; A) responses. The ionotropic glutamate receptor agonists were applied for 2 min every 15 min at the concentrations shown (μM). 1S,3R-ACPD was continuously applied for 45 min; the slices were then washed with Mg^{2+}-free Krebs medium for 30 min.

NMDA lasted for the duration of 1S,3R-ACPD application (up to 60 min). When 1S,3R-ACPD was washed out (see Figure 1), the effect slowly reversed. Concentration-response studies (see Figure 2b) showed that the effect of 1S,3R-ACPD reached a maximum at 100 μM, a concentration able to cause an increase in the NMDA (5 μM) response of $77 \pm 10\%$ ($n=25$). When different concentrations of NMDA were tested in the presence of 1S,3R-ACPD (100 μM), the results shown in Figure 3 were obtained. The figure shows that 1S,3R-ACPD does not increase maximal responses to NMDA; however, it does significantly increase the responses to lower NMDA concentrations, suggesting that 1S,3R-ACPD increases the affinity of the NMDA receptor for the agonist.

To determine whether this potentiation was dependent upon the presence of conducted action potentials, we added tetrodotoxin (1 μM) to the perfusion fluid. The results obtained indicated that tetrodotoxin did not change the effects of 1S,3R-ACPD on NMDA-induced depolarization ($77 \pm 10\%$ in control slices $n=25$ *vs* 73 ± 11 $n=5$ in tetrodotoxin-treated slices).

Neither the first, nor the subsequent AMPA (4–10 μM) responses were modified when 1S,3R-ACPD (30–300 μM) was added to the perfusion medium.

Effects of selective mGluR agonists on NMDA-induced depolarization

In order to attempt a characterization of the mGluR subtype involved in the potentiation of NMDA responses, we used: (a) DHPG (Schoepp *et al.*, 1994; Ito *et al.*, 1995) or Quis in the presence of NBQX (10 μM) as prototype mGluR agonists of the first group. Appropriate controls ruled out a direct effect of this concentration of NBQX on NMDA responses; (b) L-CCG-I as a prototype mGluR agonist of the second group (Hayashi *et al.*, 1992; Lombardi *et al.*, 1993) and (c) L-AP4 as a selective mGluR agonist of the third group (Nakanishi, 1992; Tanabe *et al.*, 1992; Nakajima *et al.*, 1993; Kristensen *et al.*, 1993; Schoepp, 1994; Pin & Duvoisin, 1995; Saugstad *et al.*, 1995).

Table 1 shows that none of the above-mentioned prototype agonists, applied at concentrations fully active on the respective receptor sybtype, potentiated the depolarization induced by NMDA (5 μM). Table 1 also shows that other putative agonists, such as DL-AP3, a compound which has been considered as either an agonist or antagonist at mGluRs (Schoepp & Johnson, 1989; Lonart *et al.*, 1992; Novelli *et al.*, 1994) or (S)-4C3HPG which has been shown to act as an antagonist at mGluR1 and as an agonist at mGluR2 and at mGluR5 (Thomsen *et al.*, 1994b; Joly *et al.*, 1995; Brabet *et al.*, 1995) did not potentiate NMDA responses.

Table 1 Effects of metabotropic glutamate receptor (mGluR) agonists on the depolarization of mouse cortical wedges induced by N-methyl-D-aspartate (NMDA; 5 μM)

	% of potentiation of NMDA response
Controls (NMDA 5 μM)	100
+ 1S,3R-ACPD 100 μM	177 ± 10**
+ 1R,3S-ACPD 500 μM	120 ± 9
Group 1 agonists	
+ Quis 30 μM + NBQX 10μM	113 ± 7
+ DHPG 100 μM	100 ± 2
+ DHPG 300 μM	102 ± 5
+ DHPG 500 μM	90 ± 7
Group 2 agonist	
+ L-CCG-I 3 μM	103 ± 5
+ L-CCG-I 30 μM	120 ± 12
Group 3 agonist	
+ L-AP4 100 μM	111 ± 7
+ L-AP4 300 μM	115 ± 9
Others	
DL-AP3 0.5 mM	98 ± 6
+ DL-AP3 1 mM	101 ± 13
+ S-4C3HPG 100 μM	100 ± 4
+ S-4C3HPG 500 μM	105 ± 7

The depolarization induced by 5 μM NMDA was considered to be 100%. One of the metabotropic agonists was then added to the superfusion medium and changes in the responses to further applications of 5 μM NMDA were evaluated. Each molecule was tested in at least 4 different experiments similar to those shown in Figure 1. The data are means ± s.e. and were obtained by measuring the second NMDA induced depolarization after the treatment with each of the mGluR agonists. For key to abbreviations used see Methods section.

Effects of mGluR antagonists on 1S,3R-ACPD-induced potentiation of NMDA responses

(+)-MCPG has been shown to antagonize 1S,3R-ACPD effects in rat neonatal motoneurones or in guinea-pig brain slices in which either phosphatidyl-inositol hydrolysis or forskolin stimulated adenosine 3′:5′-cyclic monophosphate (cyclic AMP)

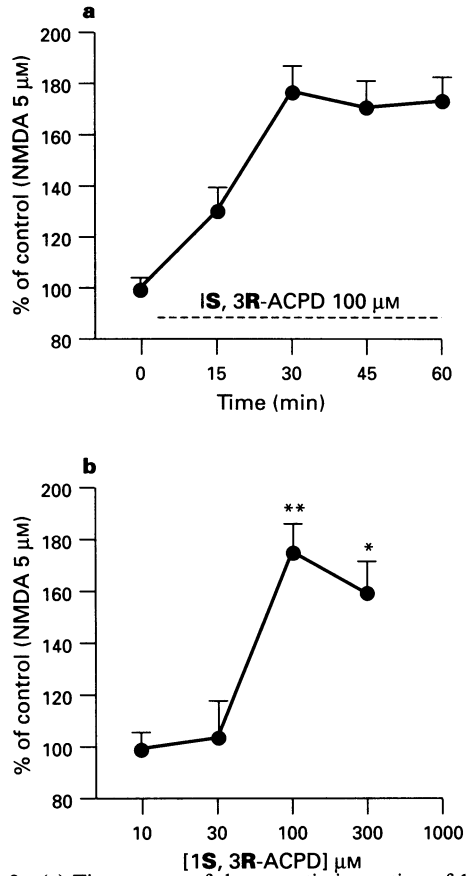

Figure 2 (a) Time course of the potentiating action of 1S,3R-ACPD on NMDA-induced depolarization of mouse cortical wedges. Each point represents the mean % changes of NMDA (5 μM) responses obtained in at least 5 separate experiments. Such increased responses reached a maximum 30 min after beginning perfusion with 1S,3R-ACPD and continued for the duration of perfusion with the mGluR agonist (up to 60 min). (b) Effects of different concentrations of 1S,3R-ACPD on NMDA (5 μM)-induced depolarization of mouse cortical wedges. Standard responses to NMDA (5 μM) were obtained at the beginning of the experiments. The effects of 1S,3R-ACPD were calculated on the basis of the second NMDA application after the beginning of the perfusion with the mGluR agonist (see Figure 1). In each experiment, a single concentration of 1S,3R-ACPD was used. Each point is the mean value obtained in at least 6 experiments; vertical lines show s.e. Statistical analysis was performed by analysis of variance and Tuckey-Kramer test for multiple comparison; $^{**}P<0.01$, $^{*}P<0.05$ *versus* controls. For key to abbreviations used see Figure 1.

production was evaluated (Watkins & Collingridge, 1994). Furthermore, in transfected cells, it has been demonstrated that (+)-MCPG is able to interact as an antagonist at the mGluR1, mGluR2 and mGluR5 level, with an IC_{50} in the range of 100–300 μM (Hayashi *et al.*, 1994; Thomsen *et al.*, 1994a; Brabet *et al.*, 1995; Kingston *et al.*, 1995). Figure 4 shows that (+)-MCPG (0.5 mM) did not modify the effects of 1S,3R-ACPD in our system. Similarly, other antagonists such as DL-AP3 (1 mM), S-4C3HPG (0.5 mM) or S-4CPG (0.5 mM) (Brabet *et al.*, 1995; Kingston *et al.*, 1995; Roberts, 1995) failed to reduce significantly 1S,3R-ACPD effects when added to the perfusion medium together with 1S,3R-ACPD (100 μM).

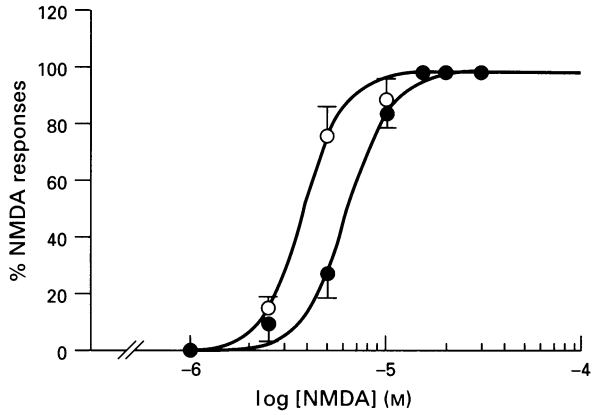

Figure 3 Effect of 1S,3R-ACPD (100 μM) on the depolarization induced by different NMDA concentrations. In preliminary experiments, we noticed that the submaximal responses to NMDA occurred at a concentration of 10 μM (see: Mannaioni *et al.*, 1994 and Moroni *et al.*, 1995 for details). A concentration-response curve to NMDA was performed (●), then perfusion with 1S,3R-ACPD was begun and responses to one concentration of NMDA were obtained in the same slice (○), according to the protocol shown in Figure 1. The potentiation was calculated on the basis of the second response (after beginning perfusion with the mGluR agonist) to each concentration of NMDA. Each point in the 1S,3R-ACPD curve (○) represents the mean ± s.e. of at least 6 experiments; in the control curve (●) the mean ± s.e. of at least 18 experiments. For key to abbreviations used see Figure 1.

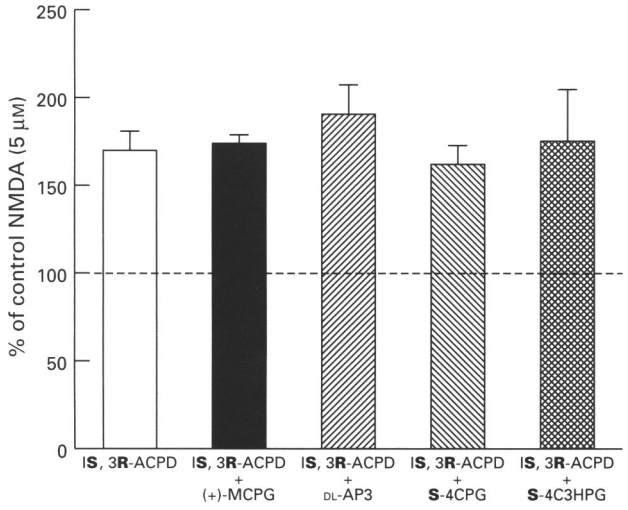

Figure 4 Lack of effect of mGluR antagonists on 1S,3R-ACPD (100 μM) potentiation of NMDA responses. The columns represent the % potentiation of 1S,3R-ACPD obtained in the presence of the antagonist. Vertical bars show s.e.; the concentration of (+)-α-methyl-4-carboxyphenylglycine ((+)-MCPG), (S)-4-carboxyphenylglycine (S-4CPG) and (S)-4-carboxy-3-hydroxyphenylglycine (S)-4C3HPG) was 0.5 mM, that of DL-2-amino-3-phosphonopropionic acid (DL-AP3) was 1 mM.

Discussion

Our results show that, in cortical wedge preparations (Harrison & Simmonds, 1985; Burton *et al.*, 1988), 1S,3R-ACPD (30–300 μM) potentiates the depolarizing effects of NMDA but does not modify the effects of AMPA. The concentration of 1S,3R-ACPD used in the present experiments is widely considered to be selective for mGluRs (Conn & Desai, 1991; Schoepp & Conn, 1993) and therefore we can reasonably suggest that the stimulation of mGluRs increases NMDA responses. Similar data have been previously obtained in rat hippocampal slices in which the effects of 1S,3R-ACPD were evaluated on NMDA responses by using either intracellular (Aniksztein *et al.*, 1992) or grease-up recording techniques (Harvey & Collingridge, 1993). It has also been demonstrated, by use of whole cell recording in granule cells of the rat dentate gyrus, that 1S,3R-ACPD potentiates NMDA currents and that this effect could be the basis for the induction of long term potentiation (O'Connor *et al.*, 1994). Furthermore, in rat spinal neurones, the application of 1S,3R-ACPD significantly potentiated the effects of prototype agonists of ionotropic glutamate receptors (Jones & Headley, 1995; Bond & Lodge, 1995). However, in granule cells of the dentate gyrus and in neurones of the spinal cord, 1S,3R-ACPD effects were antagonized by DL-AP3 or by (+)-MCPG at lower concentrations than those used in the present experiments (see Figure 4), thus suggesting the involvement of a mGluR with different pharmacological properties. In fact, in cortical wedges, large concentrations of DL-AP3, (+)-MCPG, 4CPG and 4C3HPG were totally inactive in modifying the mGluR modulation of NMDA responses (Figure 4).

In our experiments, 1S,3R-ACPD selectively potentiated NMDA responses leaving AMPA responses unaffected (see Figure 1). In other preparations, such as the spinal cord or the cerebellum, 1S,3R-ACPD has also been shown to potentiate the responses to AMPA and kainate (Cerne & Randic, 1992; Bleakman *et al.*, 1992; Glaum *et al.*, 1992; Jones & Headley, 1995; Bond & Lodge, 1995). Opposite results have also been observed: for instance, 1S,3R-ACPD has been shown to reduce NMDA responses in the caudate nucleus or in mesencephalic neurones (Colwell & Levine, 1994; Ambrosini *et al.*, 1995).

The effects of 1S,3R-ACPD on NMDA receptors could be ascribed to possible direct interactions of the metabotropic agonist with the ion channel or with the modulatory sites present on the NMDA receptor complex (see: Collingridge & Lester, 1989 for a review). This is unlikely because at the concentration used, 1S,3R-ACPD is not able to interact directly with the ionotropic glutamate receptors (Conn & Desai, 1991) and the effect we describe develops quite slowly. Another more reasonable interpretation of these data is based on the knowledge that 1S,3R-ACPD interacts with several types of mGluRs, and our goal was the identification of the mGluR type involved. We soon realised that the pharmacology of the potentiation of NMDA responses could not be ascribed to any of the three groups in which the eight types of mGluRs so far identified and studied in expression systems have been classified.

The involvement of group 1 mGluRs was ruled out by the following observations: (1) Quis, an agonist of both mGluR1 and mGluR5 type (Abe *et al.*, 1992) in expression systems and one of the most potent agonists of phospholipase C coupled receptors in brain slices (Nicoletti *et al.*, 1986; Nakanishi, 1992), was unable to potentiate NMDA responses. (2) DHPG, a selective agonist of mGluRs linked to phospholipase C (Schoepp *et al.*, 1994; Ito *et al.*, 1995), failed to potentiate NMDA responses. (3) (+)-MCPG and 4CPG, antagonists of mGluR1 and mGluR5 (Hayashi *et al.*, 1994; Thomsen *et al.*, 1994b; Kingston *et al.*, 1995; Roberts, 1995), were unable to affect 1S,3R-ACPD effects in our system.

The involvement of group 2 mGluRs was excluded by the observation that L-CCG-I, an agonist of both mGluR2 and mGluR3, was not able to potentiate NMDA-induced depolarization when used in concentrations shown to exhibit

maximal responses on these receptor subtypes both in expression systems and brain slices (Ishida *et al.*, 1990; Hayashi *et al.*, 1992; Lombardi *et al.*, 1993). Furthermore, (+)-MCPG, an antagonist of mGluR2 (Hayashi *et al.*, 1994; Thomsene *et al.*, 1994a), was completely unable to reduce the 1S,3R-ACPD effects here described.

Finally, the involvement of group 3 mGluRs was ruled out by the lack of activity of L-AP4, the prototypic agonist of mGluR belonging to this group (Kristensen *et al.*, 1993; Saugstad *et al.*, 1995). Other described agonists of mGluRs, such as ibotenic acid, cysteinsulphinic acid or L-CCG-IV (Nicoletti *et al.*, 1986; Wilsch *et al.*, 1994; Boss *et al.*, 1994), could not be used because they do not discriminate between mGluR and NMDA receptors.

The pharmacology of the effect we observed was therefore quite unusual and suggests that 1S,3R-ACPD interacts with metabotropic glutamate sites that are different from the eight mGluRs so far described.

One of the proposed mechanisms of the 1S,3R-ACPD-mediated potentiation of the NMDA responses involves the activation of the phospholipase C cascade and subsequent phosphorylation of the NMDA receptor complex mediated by one of the protein kinase C isoenzymes (Aniksztein *et al.*, 1992; Kelso *et al.*, 1992). This concept is supported by direct evidence obtained in mammalian expression systems showing that the NR1 subunit of the NMDA receptor complex is directly phosphorylated at different sites by protein kinase C. Furthermore, alternative splicing of the NR1 gene may regulate this phosphorylation (Tingley *et al.*, 1993) as well as the receptor function (Zukin & Bennett, 1995). It has also been proposed that an increased activity of protein kinase C may potentiate NMDA currents by reducing the Mg^{2+} block of the ion channel complex (Nowak *et al.*, 1984; Chen & Huang, 1992). The cortical wedges used in our experimental protocol were perfused in a nominally Mg^{2+}-free medium for a few hours, thus ruling out this possibility as the mechanism of 1S,3R-ACPD-induced potentiation of NMDA responses. However, we have not investigated whether or not a protein kinase mediated phosphorylation of the NMDA receptor ion channel complex may increase the affinity of the receptor protein for the agonist. This is certainly a reasonable basic mechanism which may explain our results. However, the approach we used is certainly not suitable for revealing other effects that 1S,3R-ACPD may have on membrane properties.

Numerous transduction systems may be associated with mGluR stimulation (Schoepp & Conn, 1993; Schoepp, 1994; Pin & Duvoisin, 1995) and recently our and other laboratories have clearly demonstrated the presence of new mGluRs linked to activation of phospholipase D in the mammalian brain (Boss & Conn, 1992; Holler *et al.*, 1993; Pellegrini-Giampietro *et al.*, 1994). The possibility that this transduction system (Loffelholz, 1989; Billah & Anthes, 1990; Thompson *et al.*, 1991) is in some way involved in mGluR modulation of the NMDA responses is currently under investigation in our laboratory.

In conclusion, we have shown that the mGluR agonist 1S,3R-ACPD selectively potentiates the depolarizing effects of NMDA by interacting with recognition sites having pharmacological properties which are different from those of the three groups of mGluRs so far cloned. The elucidation of the pharmacology of these recognition sites requires the development of new selective agonists and antagonists together with the molecular cloning of new mGluRs. As pointed out in the Introduction, it can be easily predicted that the availability of tools able to stimulate or to antagonize the mGluRs here described may be important in a wide range of processes such as neuronal development (Tsumoto *et al.*, 1987), synaptic plasticity (Collingridge & Lester, 1989; Ben-Ari *et al.*, 1992), epileptogenesis (Dingledine *et al.*, 1986) and excitotoxicity (Rothman & Olney, 1987).

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