British Journal of Pharmacology (1996) 117, 1457–1462

Pharmacological antagonism of the actions of group II and III mGluR agonists in the lateral perforant path of rat hippocampal slices

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1 An understanding of the physiological and pathological roles of metabotropic glutamate receptors (mGluRs) is currently hampered by the lack of selective antagonists. Standard extracellular recording techniques were used to investigate the activity of recently reported mGluR antagonists on agonist-induced depressions of synaptic transmission in the lateral perforant path of hippocampal slices obtained from 12–16 day-old rats.

2 The group III specific mGluR agonist, (S)-2-amino-4-phosphonobutanoate (L-AP4) depressed basal synaptic transmission in a reversible and dose-dependent manner. The mean (±s.e.mean) depression obtained with 100 μM L-AP4 (the maximum concentration tested) was 74±3% and the IC_{50} value was 3±1 μM ($n=5$).

3 The selective group II mGluR agonists, (1S,3S)-1-aminocyclopentane-1,3-dicarboxylate ((1S,3S)-ACPD) and (2S,1'R,2'R,3'R)-2-(2',3'-dicarboxycyclopropyl)glycine (DCG-IV) also depressed basal synaptic transmission in a reversible and dose-dependent manner. The mean depression obtained with 200 μM (1S,3S)-ACPD was 83±8% and the IC_{50} value was 12±3 μM ($n=5$). The mean depression obtained with 1 μM DCG-IV was 73±7% and the IC_{50} value was 88±15 nM ($n=4$).

4 Synaptic depressions induced by the actions of 20 μM (1S,3S)-ACPD and 10 μM L-AP4 were antagonized by the mGluR antagonists, (+)-α-methyl-4-carboxyphenylglycine ((+)-MCPG), (S)-2-methyl-2-amino-4-phosphonobutanoate (MAP4), (2S,1'S,2'S)-2-methyl-2-(2'-carboxycyclopropyl)glycine (MCCG), (RS)-α-methyl-4-tetrazolylphenylglycine (MTPG), (RS)-α-methyl-4-sulphonophenylglycine (MSPG) and (RS)-α-methyl-4-phosphonophenylglycine (MPPG) (all tested at 500 μM).

5 (+)-MCPG was a weak antagonist of both L-AP4 and (1S,3S)-ACPD-induced depressions. MCCG was selective towards (1S,3S)-ACPD, but analysis of its effects were complicated by apparent partial agonist activity. MAP4 showed good selectivity for L-AP4-induced effects.

6 The most effective antagonist tested against 10 μM L-AP4 was MPPG (mean reversal 90±3%; $n=4$). In contrast, the most effective antagonist tested against 20 μM (1S,3S)-ACPD induced depressions was MTPG (mean reversal 64±4%; $n=4$). Both antagonists produced parallel shifts in agonist dose-response curves. Schild analysis yielded estimated K_D values of 11.7 μM and 27.5 μM, respectively. Neither antagonist had any effect on basal transmission or on depressions induced by the adenosine receptor agonist, 2-chloroadenosine (500 nM; $n=3$).

7 We conclude that both group II and group III mGluRs can mediate synaptic depressions induced by mGluR agonists in the lateral perforant path. The mGluR antagonists MTPG, MPPG and MAP4 should be useful in determining the roles of group II and III mGluRs in the central nervous system.

Keywords: Metabotropic glutamate receptor; lateral perforant path; (1S,3S)-ACPD; L-AP4; MTPG; MPPG; MSPG; MAP4; MCCG; (+)-MCPG

Introduction

Eight subtypes of metabotropic glutamate receptors (mGluRs) have been identified. These can be divided into three groups depending on sequence homology, and transduction mechanisms and pharmacology in expression systems. Group I (mGluRs 1 and 5) are linked to phosphoinositide hydrolysis, group II (mGluRs 2 and 3) and group III (mGluRs 4, 6, 7 and 8) inhibit adenylyl cyclase but can be distinguished by their sensitivity to L-AP4. Group III mGluRs are sensitive to L-AP4 whereas group II mGluRs are insensitive to this agonist (Nakanishi, 1992; Pin & Duvoisin, 1995).

The lateral perforant path of the rat hippocampus is particularly sensitive to the actions of L-AP4 (Koerner & Cotman, 1981) and thus is a convenient pathway for the investigation of the actions of mGluR antagonists at native mGluRs of the group III type (Bushell *et al.*, 1995; Johansen *et al.*, 1995). In the present study we have shown that this pathway is also highly sensitive to group II mGluR agonists. We have then compared the action of six mGluR antagonists (Figure 1) (Jane *et al.*, 1994; 1995; Kemp *et al.*, 1994b) on depressions induced by L-AP4 and the group II selective agonist (1S,3S)-ACPD (Pook *et al.*, 1992).

Methods

Transverse hippocampal slices, 400 μm thick, were prepared in ice cold medium by use of a vibroslice from neonatal (12–16 days) rats, as described previously (Bashir & Collingridge, 1992). The slices were then maintained in a submerged holding chamber and allowed to equilibrate for at least 1 h at room temperature. Slices were transferred to a submerged recording chamber which was continually perfused with medium (3–4 ml min^{-1}; 29–31°C) containing (in mM): NaCl 124, $NaHCO_3$ 26, NaH_2PO_4 1.25, KCl 3, $CaCl_2$ 2, $MgSO_4$ 1

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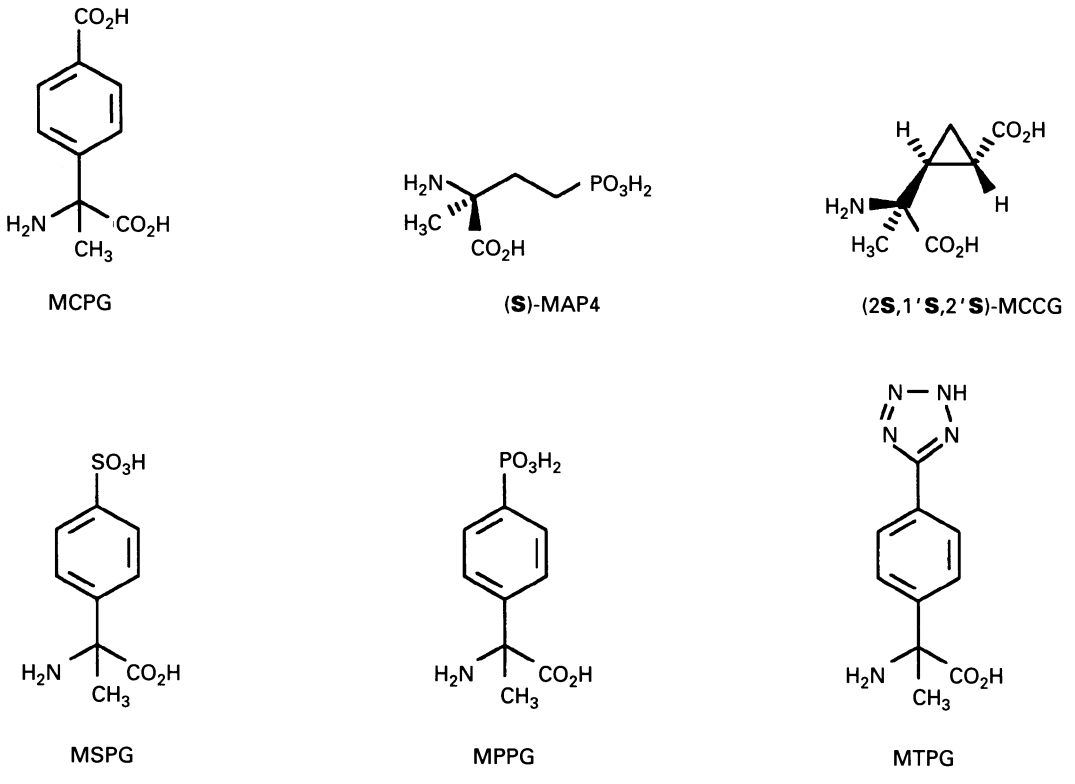

Figure 1 Structures of the six mGluR antagonists used.

and D-glucose 10 and bubbled continuously with 95% O_2/5% CO_2.

Bipolar metal stimulating and glass recording microelectrodes (containing 4 M NaCl) were placed in the lateral portion of the dorsal blade of the dentate gyrus (Collingridge *et al.*, 1984) and field excitatory postsynaptic potentials (f.e.p.s.ps) in the lateral perforant path were monitored by means of an acquisition and on-line analysis programme running on an IBM-compatible PC (software written by W.W. Anderson, Department of Anatomy, University of Bristol). The pathway was stimulated once every 30 s at an intensity which evoked f.e.p.s.p.s. of approximately 80% of the maximum value. Verification that the lateral perforant path was activated was obtained by use of a paired-pulse stimulation paradigm (inter pulse interval of 50 ms); this results in paired-pulse facilitation in the lateral and paired-pulse depression in the medial perforant path (McNaughton, 1980). Compounds were applied by addition to the perfusate.

(1S,3S)-1-aminocyclopentane-1,3-dicarboxylate ((1S,3S)-ACPD), (S)-2-amino-4-phosphonobutanoate (L-AP4), (2S,1'S,2'S)-2-methyl-2-(2'-carboxycyclopropyl)glycine (MCCG), (S)-2-methyl-2-amino-4-phosphonobutanoate (MAP4), (+)-α-methyl-4-carboxyphenylglycine ((+)-MCPG), (**RS**)-α-methyl-4-tetrazolylphenylglycine (MTPG), (**RS**)-α-methyl-4-sulphonophenylglycine (MSPG) and (**RS**)-α-methyl-4-phosphonophenylglycine (MPPG) were synthesized as described previously (Hayashi *et al.*, 1994; Jane *et al.*, 1994; 1995; Kemp *et al.*, 1994b). (2S,1'**R**,2'**R**,3'**R**)-2-(2',3'-dicarboxycyclopropyl)-glycine (DCG-IV) was kindly provided by Dr Y. Ohfune (Tokyo). All drugs were made up as stock solutions, dissolved in equimolar NaOH at a concentration of at least 100 times the final concentration and were stored in frozen aliquots.

Results

Effects of mGluR agonists

In agreement with previous studies (Koerner & Cotman, 1981; Monaghan *et al.*, 1989), L-AP4 depressed, in a dose-dependent and fully reversible manner, basal synaptic transmission with an IC_{50} of 3 ± 1 μM ($n = 5$; Figure 2a). The depression obtained with 100 μM L-AP4 (the maximum concentration tested) was $74 \pm 3\%$ ($n = 5$).

The group II mGluR agonists, (1S,3S)-ACPD and DCG-IV (Ishida *et al.*, 1993) also depressed basal synaptic transmission, in a fully reversible and a dose-dependent manner with IC_{50} values of 12 ± 3 μM ($n = 5$; Figure 2b) and 88 ± 15 nM ($n = 4$; Figure 2c), respectively. The depressions induced by the maximum agonist concentrations tested were $83 \pm 8\%$ for (1S,3S)-ACPD (200 μM) and $73 \pm 7\%$ for DCG-IV (1 μM).

Actions of mGluR antagonists

The mGluR antagonists were initially compared against fixed concentrations of mGluR agonists. L-AP4 was used as the prototypic agonist for group III mGluRs. (1S,3S)-ACPD was used as the group II mGluR agonist since this showed the greatest selectivity in previous studies with these antagonists in the spinal cord (Jane *et al.*, 1994). Agonist concentrations were selected to be submaximal but sufficient to induce a substantial depression of f.e.p.s.ps; we used 10 μM L-AP4 and 20 μM (1S,3S)-ACPD which induced depressions of $59 \pm 2\%$ ($n = 13$) and $63 \pm 3\%$ ($n = 14$), respectively. Agonists were applied continually for approximately 40 min and antagonists added after 10 min. Percentage reversals were calculated after a steady-state antagonism was obtained. Data were included only from preparations where the effects of both the antagonist and the agonist were reversible. Two or three different antagonists were examined per slice. Typical examples of the effects of some mGluR antagonists against L-AP4 and (1S,3S)-ACPD-induced depressions are shown in Figure 3.

Pooled data for the actions of each of the six antagonists *versus* L-AP4 and (1S,3S)-ACPD-induced depressions are presented in Figure 4. (+)-MCPG was similarly effective against both agonists whereas MAP4 was selective towards L-AP4-induced depressions. Interpretation of the actions of MCCG were complicated by an apparent partial agonist action. Thus, although MCCG reversed depressions induced by (1S,3S)-ACPD, it also depressed basal transmission (in 4 of 7

slices; data not shown). MCCG had little or no effect on large L-AP4-induced depressions (Figure 4) but produced further depressions when relatively small L-AP4-induced depressions were studied (data not shown). MPPG was the most effective L-AP4 antagonist but also strongly affected (1S,3S)-ACPD-induced depressions. Conversely, MTPG was the most effective (1S,3S)-ACPD antagonist but was not particularly selective. MSPG showed little selectivity between these two agonists.

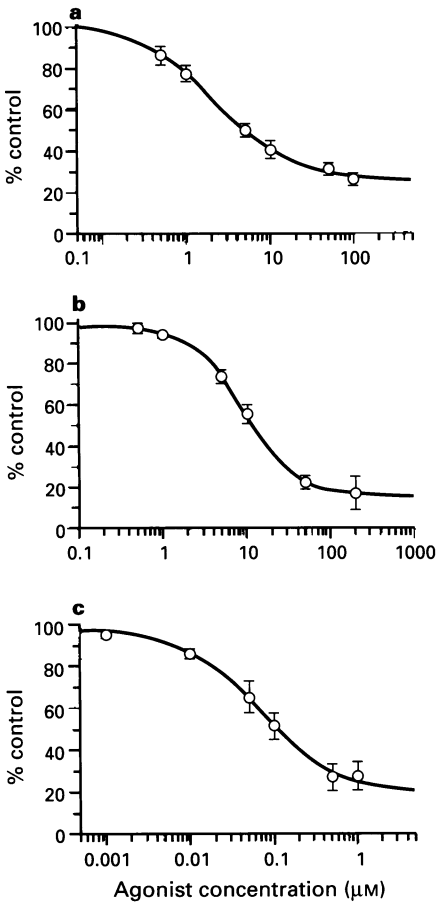

Figure 2 Log dose-response curves for the depressant actions of (a) L-AP4, (b) (1S,3S)-ACPD and (c) DCG-IV in the lateral perforant path. Each point plots the mean±s.e.mean values for 4–5 slices (each slice was tested with all six agonist concentrations). The curves were fitted by use of a four parameter logistic equation.

We studied the actions of MPPG and MTPG in more detail since these are the most effective L-AP4 and (1S,3S)-ACPD antagonists currently available. MPPG produced a parallel shift in the L-AP4 dose-response curve. Schild analysis gave a K_D of 11.7 μM with a slope of 0.81 (Figure 5). MTPG produced a parallel shift in the (1S,3S)-ACPD dose-response curve. Schild analysis gave a K_D of 27.5 μM with a slope of 0.86 (Figure 6). Neither MPPG nor MTPG, tested singly or in combination, had any effect on basal synaptic transmission (data not shown) or on synaptic responses depressed by 2-chloroadenosine ($n=3$), an agonist that acts independently of mGluRs (Figure 7).

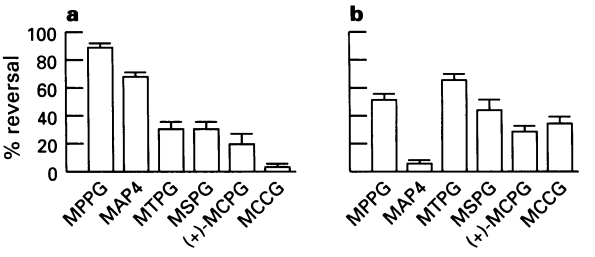

Figure 4 Summary of the effects of mGluR antagonists against depressions induced by (a) 10 μM L-AP4 and (b) 20 μM (1S,3S)-ACPD. Each column shows the mean±s.e.mean values for 4 slices.

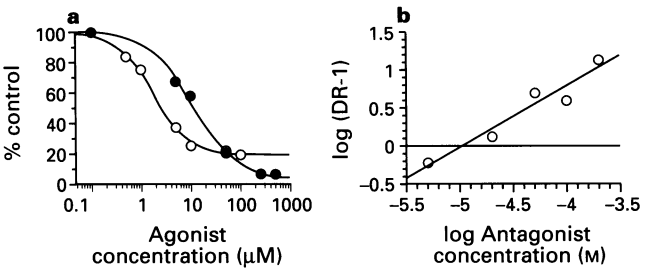

Figure 5 Antagonism by MPPG. (a) Representative log dose-response curve for L-AP4 in the absence (○) and presence (●) of 50 μM MPPG. (The change in maxima was not a consistent finding). (b) A Schild plot was constructed from data such as that presented in (a) for five antagonist concentrations (each one from a different slice). The line was fitted by linear regression and gave an interpolated estimated K_D of 11.7 μM with a slope of 0.81 with a correlation coefficient of 0.96.

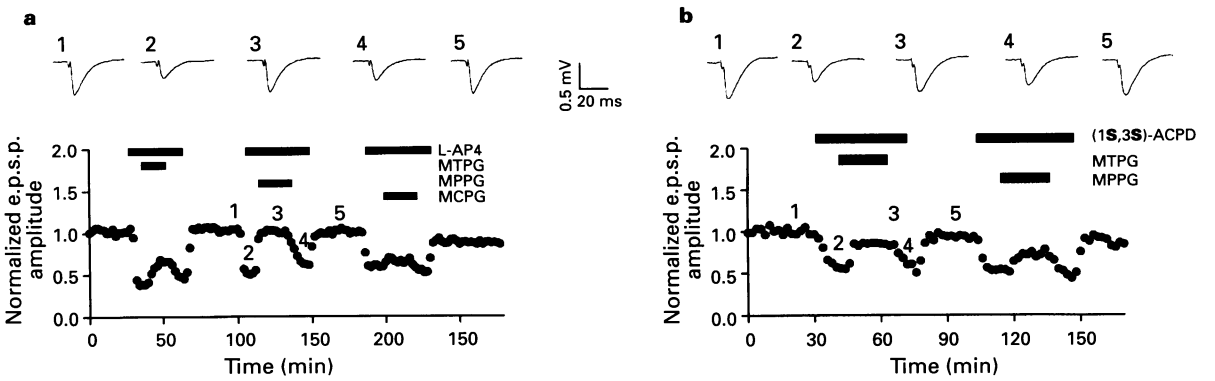

Figure 3 Examples of individual experiments for the antagonism of L-AP4 and (1S,3S)-ACPD-induced synaptic depressions. (a) Shows the effect of MTPG (500 μM), MPPG (500 μM) and MCPG (500 μM) against depressions of synaptic transmission induced by 10 μM L-AP4. (b) Shows the effects of MTPG (500 μM) and MPPG (500 μM) against depressions of synaptic transmission induced by 20 μM (1S,3S)-ACPD. Each point plots the average of the peak amplitude of 4 successive synaptic response. Representative synaptic records (each an average of four consecutive records) are shown for the corresponding times indicated on the graphs by 1-5.

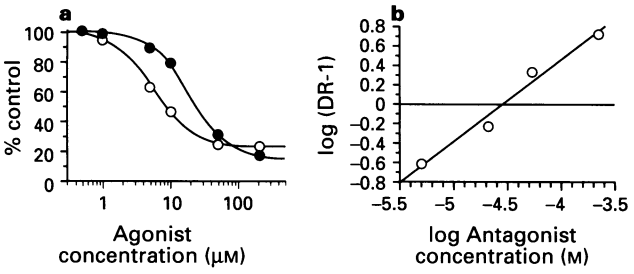

Figure 6 Antagonism by MTPG. (a) Representative log dose-response curve for (1S,3S)-ACPD in the absence (○) and presence (●) of 50 μM MTPG. (b) A Schild plot was constructed from data such as that presented in (a) for four antagonist concentrations (each one from a different slice). The line was fitted by linear regression and gave an interpolated estimated K_D of 27.5 μM and a slope of 0.86 with a correlation coefficient of 0.99.

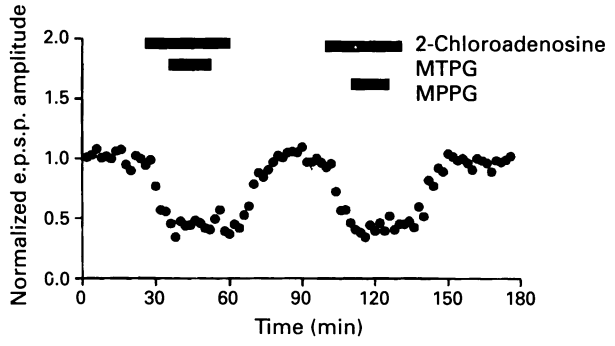

Figure 7 MTPG and MPPG act selectively at mGluRs. MTPG (500 μM) and MPPG (500 μM) have no effect on the depressions induced by the adenosine receptor agonist, 2-chloroadenosine (500 nM).

Discussion

The principal aim of this study was to compare the recently reported mGluR antagonists MPPG, MTPG and MSPG (Jane *et al.*, 1995) with the more established mGluR antagonists, MAP4, MCCG and (+)-MCPG (Jane *et al.*, 1994; Kemp *et al.*, 1994b) on agonist-induced depressions of synaptic transmission in the lateral perforant path of rat hippocampal slices. Initially, we confirmed that our preparation is particularly sensitive to the group III mGluR agonist, L-AP4 (Koerner & Cotman, 1981). We now demonstrate that the lateral perforant path is also highly sensitive to the group II mGluR selective agonists (1S,3S)-ACPD and DCG-IV. The presence of both group II and group III mGluRs, and the magnitude of synaptic depressions that result from the activation of these receptors, make the lateral perforant path a convenient hippocampal pathway to study the actions of mGluR antagonists.

The finding that (+)-MCPG weakly antagonizes L-AP4-induced depressions is consistent with a recent report for this pathway (Johansen *et al.*, 1995) and is also consistent with previous studies in the spinal cord (Kemp *et al.*, 1994b) and both the CA1 (Vignes *et al.*, 1995) and CA3 (Manzoni *et al.*, 1995) regions of the hippocampus. The finding that (+)-MCPG is also a weak antagonist of (1S,3S)-ACPD-induced depressions is consistent with previous studies in the spinal cord (Kemp *et al.*, 1994b) and the CA1 region of the hippocampus (Vignes *et al.*, 1995). Since (+)-MCPG also inhibits the postsynaptic excitatory effects of mGluR agonists in the spinal cord and CA1 region of the hippocampus (Watkins & Collingridge, 1994), (+)-MCPG is shown to be a broad spectrum antagonist against native mGluRs. The ability of MAP4 to antagonize L-AP4-induced depression of synaptic transmission in the lateral perforant path extends our initial report (Bushell *et al.*, 1995). Its high degree of selectivity for L-AP4 compared with (1S,3S)-ACPD agrees with studies in the spinal cord (Jane *et al.*, 1994) and CA1 region of the hippocampus (Vignes *et al.*, 1995). We have reported previously that MAP4 neither affects basal transmission nor synaptic responses depressed by 2-chloroadenosine (Bushell *et al.*, 1995). It is therefore a useful selective L-AP4 antagonist. The ability of MCCG to antagonize (1S,3S)-ACPD-induced but not L-AP4-induced depressions is also consistent with studies in the spinal cord (Jane *et al.*, 1994) and CA1 region of the hippocampus (Vignes *et al.*, 1995). However, the present study revealed a partial agonist action of MCCG, a finding consistent with an earlier report (Kemp *et al.*, 1994a). Such an action may explain certain problems encountered previously (Jane *et al.*, 1994; Vignes *et al.*, 1995) and limits its usefulness as an mGluR antagonist.

In agreement with studies in the spinal cord (Jane *et al.*, 1994), the three new analogues of (+)-MCPG were all comparatively effective L-AP4 and (1S,3S)-ACPD antagonists. It is likely that the relative lack of selectivity for particular mGluR subtypes was an intrinsic property of the antagonists rather than a lack of selectivity of the two agonists used for the two types of receptor since MAP4 and MCCG were selective in their actions. MPPG and MTPG were examined in most detail since they were the most effective antagonists of L-AP4 and (1S,3S)-ACPD, respectively. The potency of MPPG (K_D of 11.7 μM) is very similar to that reported previously in the spinal cord (9.2 μM; Jane *et al.*, 1995) whilst MTPG was slightly more potent in the present study (27.5 μM) than in the spinal cord (77 μM). This may be due to a different subtype of mGluR mediating the depressions induced by (1S,3S)-ACPD in these two regions. The slopes of the Schild plots were less than unity which could mean that the interactions were not (entirely) competitive; however, shallow Schild slopes have also been observed with competitive antagonists, such as 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), in the hippocampus (Blake *et al.*, 1989) and so may be due to other reasons (Simmonds, 1990). Since neither MPPG nor MTPG affected basal synaptic transmission nor synaptic transmission that had been depressed by means other than activation of mGluRs (i.e. by 2-chloroadenosine) these antagonists should be useful providing selectivity between group II and group III mGluRs is not the main requirement. The lack of effect of these antagonists on basal synaptic transmission implies that L-glutamate does not tonically activate these subgroups of mGluRs.

The identity of the mGluR responsible for the depressant actions of L-AP4 in the lateral perforant path is not known. L-AP4 has been shown in expression systems to be an agonist at mGluRs 4, 6, 7 and 8 (Tanabe *et al.*, 1993; Nakajima *et al.*, 1993; Okamoto *et al.*, 1994; Duvoisin *et al.*, 1995) however mGluRs 6 and 8 seem to be largely restricted to the retina and olfactory bulb (Nakajima *et al.*, 1993; Duvoisin *et al.*, 1995). The entorhinal cortex, which provides the major afferent input to the hippocampus via the perforant path (Witter, 1993), expresses mRNA for both mGluRs 4 and 7 (Thomsen *et al.*, 1992; Saugstad *et al.*, 1994). The high sensitivity of the pathway to L-AP4 would suggest that the mGluR mediating this depressant effect is mGluR4, as in expression systems this subtype is more sensitive than is mGluR7 to the actions of L-AP4 (Okamoto *et al.*, 1994; Saugstad *et al.*, 1994). Consistent with this possibility, MAP4 has recently been shown to be an antagonist of rat mGluR4a (K_i of 190 μM) expressed in BHK570 cells (Johansen & Robinson, 1995) and it is a weak partial agonist at human mGluR4a (Knöpfel *et al.*, 1995). However whereas (+)-MCPG partially reverses the depressant actions of L-AP4 in the lateral perforant path it has no effect on the actions of L-AP4 in expression systems (Cavanni *et al.*, 1994; Johansen *et al.*, 1995; Hayashi *et al.*, 1994; Saugstad *et al.*, 1994) and so the nature of the L-AP4-sensitive mGluR in this pathway remains unclear. The depressant actions of (1S,3S)-ACPD in the lateral perforant path may be mediated by either mGluR2 or 3 since both their mRNAs have been shown to be present in the entorhinal cortex (Ohishi *et al.*,

1993; Fotuhi *et al.*, 1994). Of the two subtypes in expression systems, mGluR2 has been shown to be antagonized by (+)-MCPG (Hayashi *et al.*, 1994) and by MCCG (Knöpfel *et al.*, 1995) and the action of (1S,3S)-ACPD was partially reversed by both of these antagonists in this pathway. Information on the pharmacological profile of mGluR3 is not available at present and so the actual identity of the mGluR mediating these depressions remains unclear.

In conclusion, the present data show that mGluRs from both groups II and III have the capacity to provide a powerful regulation of synaptic transmission in the lateral perforant path of the rat. Of the mGluR antagonists described, MAP4 is a moderately effective but selective L-AP4 antagonist. The new antagonist MPPG and MTPG are respectively the most effective L-AP4 and (1S,3S)-ACPD antagonists currently available.

This work was funded by the BBSRC, the MRC and Wellcome Research Laboratories. We are extremely grateful to Dr Ohfune for the gift of DCG-IV and to Dr Ceri Davies for valuable advice.

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(Received September 12, 1995
Revised December 7, 1995
Accepted December 12, 1995)