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Actions of two new antagonists showing selectivity for different sub-types of metabotropic glutamate receptor in the neonatal rat spinal cord

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1 The presynaptic depressant action of L-2-amino-4-phosphonobutyrate (L-AP4) on the monosynaptic excitation of neonatal rat motoneurones has been differentiated from the similar effects produced by (1S,3R)-1-aminocyclopentane-1,3-dicarboxylate ((1S,3R)-ACPD), (1S,3S)-ACPD and (2S,3S,4S)-α-(carboxycyclopropyl)glycine (L-CCG-I), and from the postsynaptic motoneuronal depolarization produced by (1S,3R)-ACPD, by the actions of two new antagonists, α-methyl-L-AP4 (MAP4) and α-methyl-L-CCG-I (MCCG). Such selectivity was not seen with a previously reported antagonist, (+)-α-methyl-4-carboxyphenylglycine (MCPG).

2 MAP4 selectively and competitively antagonized the depression of monosynaptic excitation produced by L-AP4 (K_D 22 μM). At ten fold higher concentrations, MAP4 also antagonized synaptic depression produced by L-CCG-I but in an apparently non-competitive manner. MAP4 was virtually without effect on depression produced by (1S,3R)- or (1S,3S)-ACPD.

3 MCCG differentially antagonized the presynaptic depression produced by the range of agonists used. This antagonist had minimal effect on L-AP4-induced depression. The antagonism of the synaptic depression effected by (1S,3S)-ACPD and L-CCG-I was apparently competitive in each case but of varying effectiveness, with apparent K_D values for the interaction between MCCG and the receptors activated by the two depressants calculated as 103 and 259 μM, respectively. MCCG also antagonized the presynaptic depression produced by (1S,3R)-ACPD.

4 Neither MAP4 nor MCCG (200–500 μM) significantly affected motoneuronal depolarizations produced by (1S,3R)-ACPD. At the same concentrations the two antagonists produced only very weak and variable effects (slight antagonism or potentiation) on depolarizations produced by (S)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and N-methyl-D-aspartate (NMDA).

5 It is concluded that MAP4 is a potent and selective antagonist for those excitatory amino acid (EAA) receptors on neonatal rat primary afferent terminals that are preferentially activated by L-AP4, and that MCCG is a relatively selective antagonist for different presynaptic EAA receptors that are preferentially activated by (1S,3S)-ACPD and (perhaps less selectively) by L-CCG-I. These receptors probably comprise two sub-types of metabotropic glutamate receptors negatively linked to adenylyl cyclase activity.

Keywords: Excitatory amino acids; metabotropic glutamate receptors; antagonism; neonatal rat; spinal cord; α-methyl amino acids; L-AP4; (1S,3R)-ACPD; (1S,3S)-ACPD; L-CCG-I; MAP4; MCCG

Introduction

Investigation of the role of metabotropic glutamate receptors (mGluRs) in central nervous function requires the development of selective agonists and antagonists for the various sub-types of these receptors now known to exist (Tanabe *et al.*, 1992; 1993; Nakajima *et al.*, 1993; Okamoto *et al.*, 1994; for reviews, see Nakanishi, 1992; Schoepp & Conn, 1993). Such receptors appear to comprise two main families, those coupled to phosphoinositide (PI) hydrolysis (mGluR1 and 5) and those negatively coupled to adenylyl cyclase activity (mGluR2,3,4,6 and 7). Recently, members of a series of phenylglycine derivatives were shown to possess a range of neurochemical and electrophysiological properties compatible with differential activity at mGluR sub-types (Birse *et al.*, 1993; Eaton *et al.*, 1993b; Kemp *et al.*, 1994). Thus, certain phenolic glycines have agonist activity. For example, (S)-3-hydroxyphenylglycine (3HPG) stimulates PI hydrolysis in rat pup cerebrocortical slices (Birse *et al.*, 1993) and in mGluR1-expressing Chinese hamster ovary (CHO) cells (Hayashi *et al.*, 1994) while (RS)-3,5-dihydroxyphenylglycine (DHPG) has a similar (and more potent) action in mGluR1-expressing *Xenopus* oocytes (Ito *et al.*, 1992). In contrast, (S)-4-carboxyphenylglycine (4CPG) antagonizes (1S,3R)-1-amino-1,3-cyclopentane dicarboxylate (ACPD)-stimulated PI hydrolysis in rat pup cerebrocortical slices (Birse *et al.*, 1993) while both (S)-4CPG and (S)-4-carboxy-3-hydroxyphenylglycine (4C3-HPG) were found to be antagonists of L-glutamate-stimulated PI hydrolysis in CHO cells expressing mGluR1 (Hayashi *et al.*, 1994). In addition, (S)-4C3HPG and (S)-4CPG (less effectively) are agonists at mGluR2 receptors expressed in CHO cells, while a related substance, (+)-α-methyl-4-carboxyphenylglycine (MCPG), is an antagonist at both mGluR1 and mGluR2 receptor sub-types expressed in these cells (Hayashi *et al.*, 1994). MCPG is also an antagonist at guinea-pig cerebrocortical receptors activated by the agonist (S)-2-amino-4-phosphonobutyrate (L-AP4) and negatively linked to the cyclic AMP cascade (Kemp *et al.*, 1994). These L-AP4-activated receptors are currently unidentified but are not of the mGluR4 sub-type, which is one of three cloned metabotropic glutamate receptors that have been shown to be highly sensitive to this agonist (Nakanishi, 1992; Nakajima *et al.*, 1993; Okamoto *et al.*, 1994), since MCPG has no action at mGluR4 (Hayashi *et al.*, 1994).

In electrophysiological experiments (S)-4C3HPG, (S)-4CPG and (+)-MCPG antagonize a number of effects produced by the mGluR-selective agonist (1S,3R)-ACPD. Thus, each of these phenylglycine compounds blocks the (1S,3R)-ACPD-induced depolarization of neonatal rat motoneurones

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in vitro (Birse *et al.*, 1993; Eaton *et al.*, 1993b; Jane *et al.*, 1993) and the excitation of rat thalamic neurones *in vivo* (Eaton *et al.*, 1993a). Also, in neurones of the nucleus tractus solitarius (NTS) in the rat brain stem *in vitro*, these same three phenylglycines antagonize the depression of excitatory postsynaptic currents (e.p.s.cs), the depression of inhibitory postsynaptic currents (i.p.s.cs), the depression of muscimol-induced currents and the potentiation of (**RS**)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)-induced currents that is effected in each case by (1**S**,3**R**)-ACPD (Glaum *et al.*, 1993). These three phenylglycines selectively block nociceptive responses in rat thalamic neurones *in vivo*, relative to non-nociceptive sensory responses (Eaton *et al.*, 1993a; Salt *et al.*, 1993), and MCPG has been reported to block the induction of both NMDA-receptor dependent and NMDA receptor-independent long-term potentiation (LTP) in rat hippocampal slices (Bashir *et al.*, 1993).

While highly promising as lead compounds for the development of more specific antagonists, (S)-4C3HPG, (S)-4CPG and (+)-MCPG, which all show little or no activity at NMDA and non-NMDA ionotropic glutamate receptors (Birse *et al.*, 1993; Eaton *et al.*, 1993a,b; Jane *et al.*, 1993; Bashir *et al.*, 1993), present a spectrum of activity that may make it difficult in some cases to identify the precise mGluR sub-type(s) involved in particular synaptic phenomena. Thus (S)-4C3HPG and (S)-4CPG are agonists (Birse *et al.*, 1992; Pook *et al.*, 1993), and MCPG an antagonist (Pook *et al.*, 1993; Kemp *et al.*, 1994), at presynaptic receptors activated by (1S,3R)-ACPD to effect depression of monosynaptic excitation of neonatal rat motoneurones. Together with the neurochemical effects of these three phenylglycines at specific mGluR sub-types (Hayashi *et al.*, 1994) such results would point to mGluR2 being the presynaptic receptor on the terminals of neonatal rat primary afferent fibres. However, MCPG also antagonizes L-AP4-induced depression of monosynaptic excitation in neonatal rat motoneurones (Kemp *et al.*, 1994) but L-AP4 is not an agonist at mGluR2 receptors (Tanabe *et al.*, 1992) while none of the phenylglycines affect mGluR4 receptors (Hayashi *et al.*, 1994), which are potently activated by L-AP4 (Tanabe *et al.*, 1992; Nakanishi, 1992). The range of potencies observed for (+)-MCPG as an antagonist of the depression of monosynaptic excitation of neonatal rat motoneurones depending on the agonist used to produce the depression (Kemp *et al.*, 1994) suggested to us the possibility that more than a single mGluR sub-type was involved in such presynaptically-mediated effects. These and other considerations have led us to seek more sub-type-specific mGluR agonists and antagonists which would help us to identify receptor sub-types more definitively. To aid this search, we included the mGluR agonist (2S,3S,4S)-α-(car-

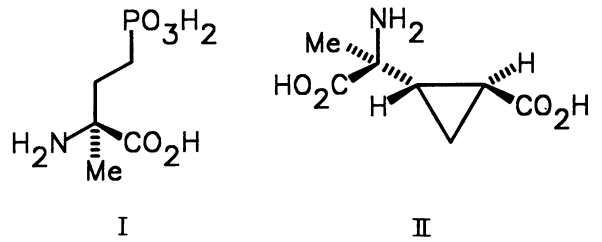

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Figure 1 Structures of MAP4 (I) and MCCG (II). For abbreviations, see text.

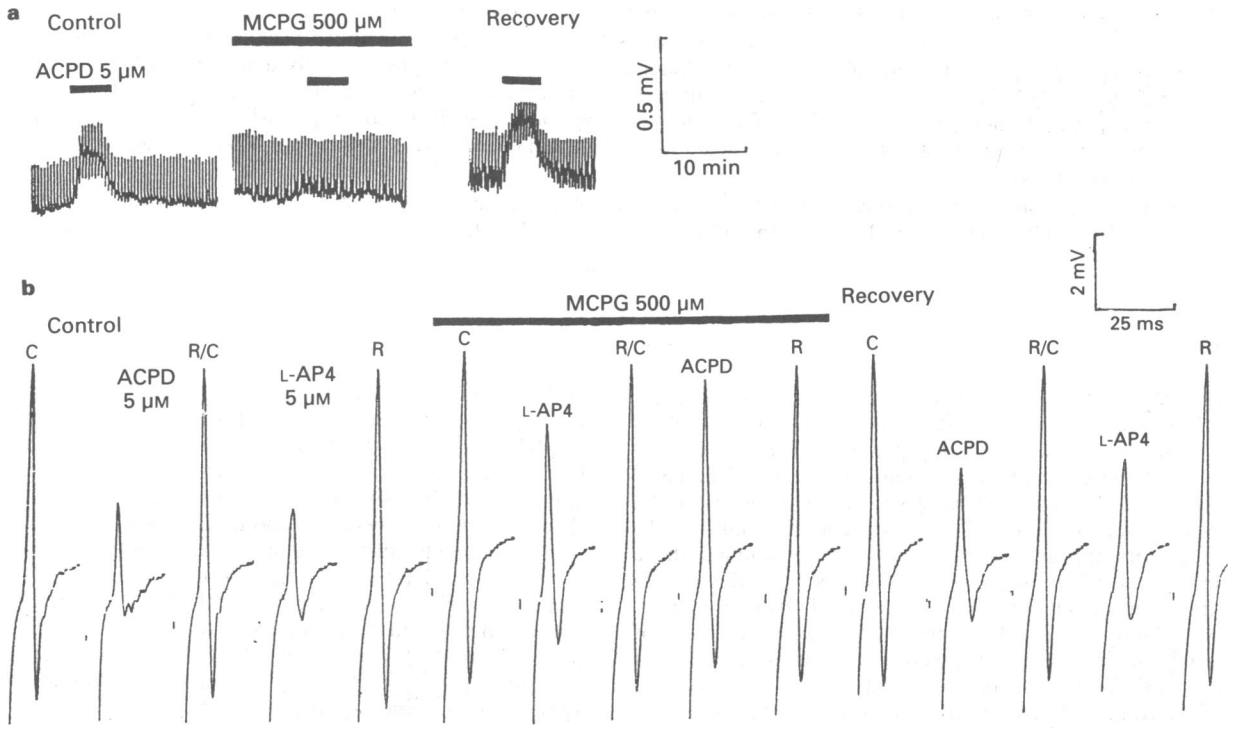

Figure 2 Non-selective antagonism of (1S,3R)-ACPD- and L-AP4-induced responses in neonatal rat motoneurones by (+)-α-methyl-4-carboxyphenylglycine (MCPG). (a) Shows chart recordings, in Mg^{2+}/D-AP5-containing medium, of slow components of the DR-VRP and depolarizing base line shift produced by 5 μM (1S,3R)-ACPD. L-AP4 (5 μM) produced no base-line shift (not shown; see Figure 2). Left, control response; centre, recorded during superfusion with medium containing 500 μM (+)-MCPG, showing abolition of the (1S,3R)-ACPD-induced depolarization; right, recovery, recorded 20 min after washout of antagonist. (b) In the same preparation as (a), both (1S,3R)-ACPD (5 μM) and L-AP4 (5 μM) produced a similar depression of the monosynaptic component of the DR-VRP. C denotes control responses recorded immediately prior to the addition of synaptic-depressant agonists; R denotes recovery responses, recorded 10 min after return to agonist-free medium; R/C, recovered response used as control for next agonist-induced depression. The centre panel of responses were recorded 30 min after the addition of (+)-MCPG (500 μM) to the superfusion medium; the depressant effects of both (1S,3R)-ACPD and L-AP4 were antagonized. The final sequence of responses, showing recovery from antagonism, was recorded 20 min after return to antagonist-free medium. For abbreviations, see text.

boxycyclopropyl)glycine (L-CCG-I) (Ishida *et al.*, 1993) among the substances we used to produce depression of monosynaptic excitation of neonatal rat motoneurones. Monosynaptic depression effected by L-CCG-I was less susceptible to antagonism by (+)-MCPG than that produced by L-AP4 (Kemp *et al.*, 1994) and may have involved a different receptor sub-type. We have also included (1S,3S)-ACPD which, like L-CCG-I and L-AP4, and unlike (1S,3R)-ACPD, causes presynaptic depression without causing postsynaptic depolarization (Pook *et al.*, 1992). We report here that two new mGluR antagonists, α-methyl-L-AP4 (MAP4) and α-methyl-L-CCG-I (MCCG) have selective actions at L-AP4-sensitive and (1S,3S)-ACPD/L-CCG-I-sensitive presynaptic receptors, respectively, on primary afferents to neonatal rat motoneurones. The structures of these compounds are shown in Figure 1.

Methods

MAP4 and MCCG were synthesized in our laboratory by methods that will be described in a subsequent publication. Hemisected isolated spinal cords from 1- to 5-day old rats were used (Evans *et al.*, 1982). Recordings were made from a ventral root of monosynaptic responses of motoneurones evoked by stimulation of the corresponding dorsal root (30 V, 2 pulses min^{-1}). The standard medium contained: (mM) NaCl 118, $NaHCO_3$ 25, KCl 3, $CaCl_2$ 2.5, D-glucose 12, gassed with 5% CO_2/95% O_2. In addition, for most experiments, 2 mM $MgSO_4$ and 50 μM D-2-amino-5-phosphonopentanoate (D-AP5) were included in the standard medium to eliminate slow synaptic responses mediated by N-methyl-D-aspartate (NMDA) receptors and to isolate the main component of the monosynaptic response which is mediated by

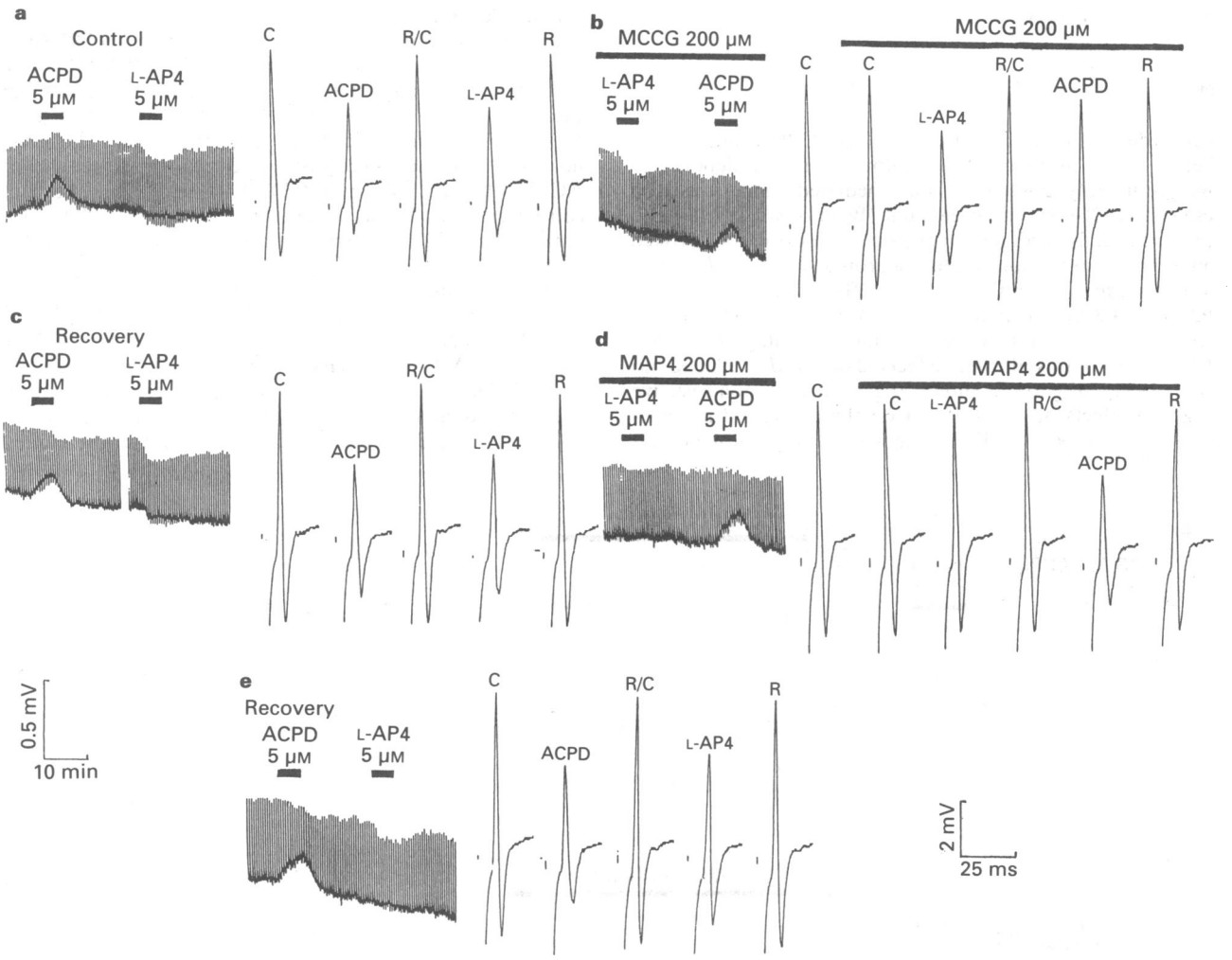

Figure 3 Selective antagonist effects of MCCG and MAP4 on (1S,3R)-ACPD and L-AP4-induced responses in neonatal rat motoneurones. All responses recorded in the same spinal cord preparation; Mg^{2+}/D-AP5-containing medium. (a–e) Each two-part section shows slow (left hand sequences, direct chart recordings) and fast (right hand sequences, oscilloscope traces) DR-VRPs. (a) Control responses showing the depolarizing base line shift produced by (1S,3R)-ACPD (5 μM), and small depressant effects of L-AP4 (5 μM) on the slow DR-VRP and the depressant effects of both (1S,3R)-ACPD and L-AP4 on the fast component of the DR-VRP. (b) Recorded 15 min after addition of 200 μM MCCG to the medium. This antagonist, which itself had no overt action on the slow or fast components of the synaptic response, selectively reduced the depression of the fast component of the DR-VRP produced by (1S,3R)-ACPD without altering the effects of L-AP4 on slow and fast components of the DR-VRP or the depolarizing effect of (1S,3R)-ACPD. (c) Responses recorded 35 min after return to normal (antagonist-free) medium. (d) Recorded 15 min after changing to medium containing 200 μM MAP4, which had no effect on the amplitude of the evoked synaptic responses. The depressant effects of L-AP4 on both the slow and fast components of the DR-VRP were antagonized by MAP4, without antagonism of either the deloparizing effect of (1S,3R)-ACPD or the depression of the fast component of the DR-VRP produced by (1S,3R)-ACPD. (e) Recorded 25 min after returning to normal medium, showing near-complete recovery of the L-AP4-induced depression. C, control; R/C, recovery responses for fast component of DR-VRP following removal of depressant agonists and representing control responses preceding the next addition of depressant; R, recovery at end of sequence of medium changes. Left hand calibration (0.5 mV, 10 min) applies to slow, chart recorded components of DR-VRP; right hand calibration (2 mV, 25 ms) refers to oscilloscope traces of fast components of DR-VRP. For abbreviations, see text.

receptors of the α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and/or kainate types (Long *et al.*, 1990). The rate of superfusion of the medium over the cord was 1 ml min^{-1}. After control responses to dorsal root stimulation were obtained, flow of standard medium was changed to agonist-containing medium (5 ml) at various concentrations, in order to cause depression of the monosynaptic response within the range of approximately 5–95% (usually 25–75%). Following the determination of control levels of depression by L-AP4, (1S,3S)-ACPD, (1S,3R)-ACPD or L-CCG-I the depression of the response was then measured in each case in a medium containing MAP4 or MCCG (both 200–500 μM). Some experiments were also conducted with standard medium (excluding $MgSO_4$ and D-AP5) that contained tetrodotoxin (TTX; 10^{-5} M for 2 min, then 10^{-7} M continuously) in order to investigate the effects of the antagonists on depolarization directly generated in motoneurones by (1S,3R)-ACPD, or by the inotropic receptor agonists NMDA and AMPA.

Results

As recorded from ventral roots (1S,3R)-ACPD produces two different effects in neonatal rat motoneurones – a depolarization, presumably postsynaptically mediated, since it is also present in TTX-containing medium (Birse *et al.*, 1993) – and a presynaptically-mediated depression of dorsal root-evoked monosynaptic excitation of motoneurones (Pook *et al.*, 1992). The latter effect is simulated by (1S,3S)-ACPD (Pook *et al.*, 1992), by L-CCG-I (Ishida *et al.*, 1993) and by L-AP4 (Evans *et al.*, 1982). A previously reported mGluR antagonist, (+)-MCPG, antagonizes all these effects (Jane *et al.*, 1993; Kemp *et al.*, 1994). Figure 2 shows the relatively non-selective antagonist effects of (+)-MCPG on the depolarization produced by (1S,3R)-ACPD in neonatal rat motoneurones (Figure 2a) and on the (1S,3R)-ACPD- and L-AP4-induced depression of monosynaptic excitation of these motoneurones (Figure 2b).

In contrast, MCCG and MAP4 both showed differential antagonism of these three responses, each antagonist producing a characteristic pattern of activity. Figure 3a and b shows that neither the postsynaptic motoneurone depolarization produced by (1S,3R)-ACPD, nor the presynaptic depression of monosynaptic excitation produced by L-AP4, was affected by 200 μM MCCG; however, this antagonist attenuated the presynaptic depression produced by (1S,3R)-ACPD. On the other hand, MAP4 (Figure 3c and d) greatly attenuated the presynaptic depression produced by L-AP4 without affecting the depolarization of presynaptic depression produced by (1S,3R)-ACPD. Neither MCCG nor MAP4 affected either the slow or fast components of the DR-VRP when added alone to the superfusion medium (Figure 3b and d). Rapid recovery was obtained after each antagonist was washed out (Figure 3c and e).

In addition to its effect as an antagonist of (1S,3R)-ACPD-induced depression of the monosynaptic DR-VRP, MCCG (200 μM) also antagonized the depression of monosynaptic excitation produced by (1S,3S)-ACPD (Figure 4a) and L-CCG-I (Figure 4b). In contrast, MAP4 (200 μM) had little or no effect on the depression produced by either (1S,3S)-ACPD (Figure 5a) or L-CCG-I (Figure 5b). At higher concentrations (1 mM), however, MAP4 did show some antagonism of the synaptic depression affected by L-CCG-I (Figure 5c).

Dose-response curves were constructed for the depression of monosynaptic excitation produced by L-AP4, L-CCG-I and (1S,3S)-ACPD in the presence and absence of MAP4 (300 μM) or MCCG (300 μM). MAP4 produced a consistently parallel shift to the right in the curve for L-AP4 (Figure 6a), a lesser and non-parallel shift to the right in the curve for L-CCG-I (Figure 6b) and no significant shift in the curve for

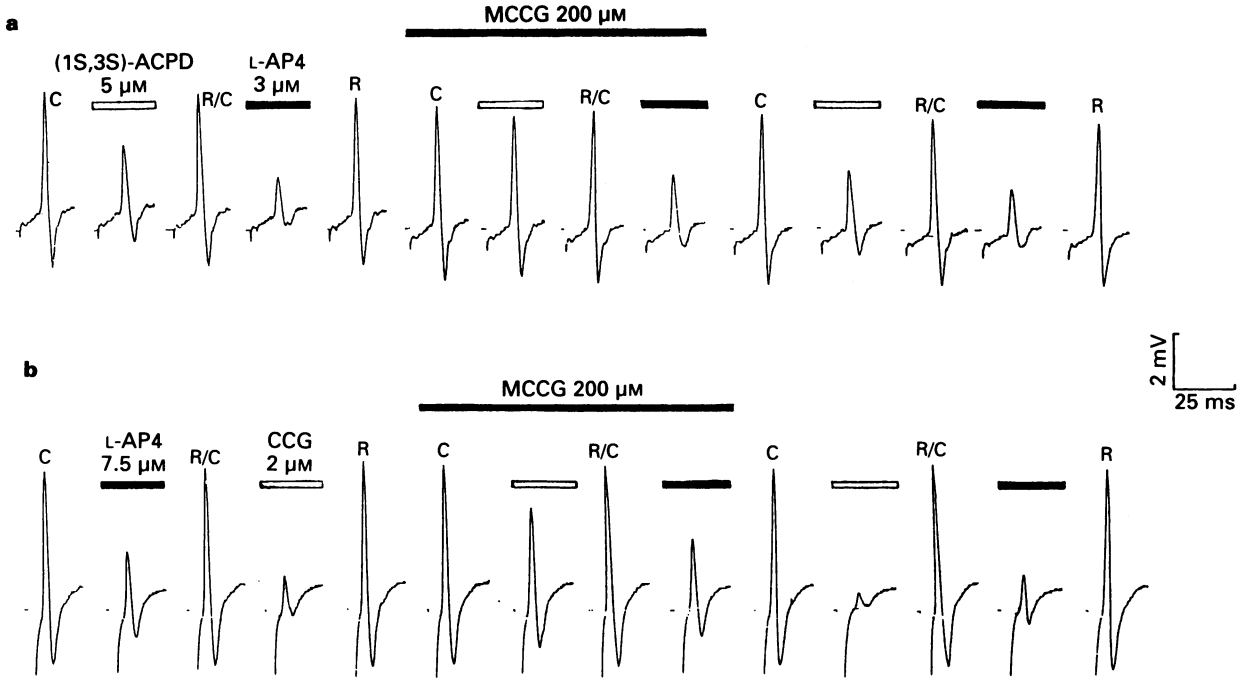

Figure 4 Selective antagonism by MCCG of (1S,3S)-ACPD and L-CCG-I-induced depression of monosynaptic DR-VRP in neonatal rat motoneurones. (a) Shows depression of synaptic response by (1S,3S)-ACPD (5 μM) and L-AP4 (3 μM). MCCG (200 μM) almost completely abolished the depression produced by (1S,3S)-ACPD and was less effective versus L-AP4. (b) Shows selective antagonism by MCCG (200 μM) of L-CCG-I (2 μM)-induced depression of the monosynaptic DR-VRP relative to L-AP4 (7.5 μM)-induced depression. (a) and (b) are from different preparations; Mg^{2+}/D-AP5-containing medium. The centre sequences of responses were recorded 20 min after beginning superfusion with antagonist-containing medium. The two sequences of responses showing recovery from the antagonists were recorded 30–35 min after return to antagonist-free medium. C, control responses recorded before each addition of depressant agonist. R/C, recovered response acting as control for subsequent agonist-induced depression. R, recovery, recorded at end of each sequence of medium changes. For abbreviations, see text.

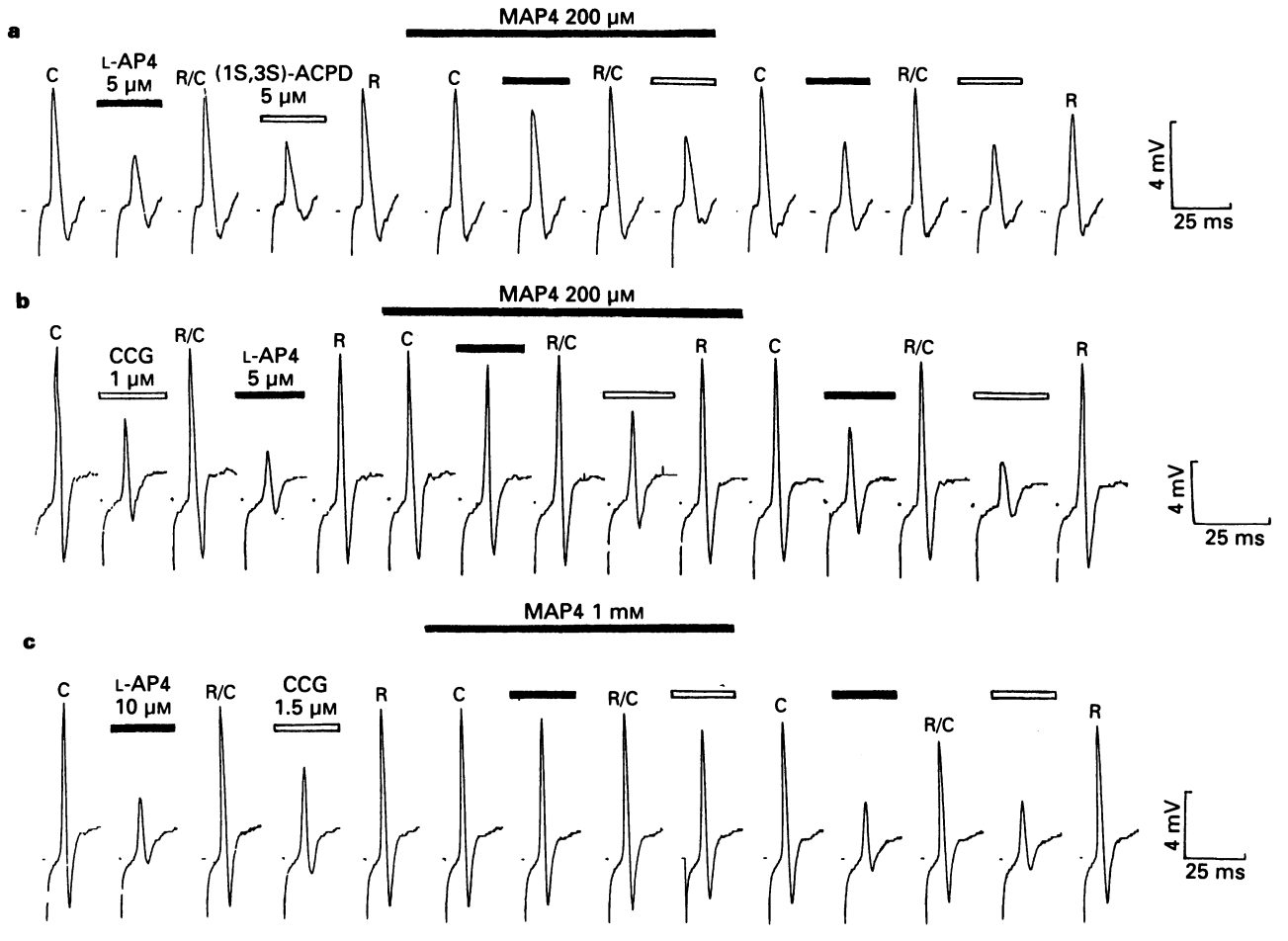

Figure 5 Three separate preparations showing selective antagonism by MAP4 of L-AP4-induced synaptic depression relative to (1S,3S)-ACPD- and L-CCG-I-induced depression. (a) MAP4 (200 μM) antagonized the depression produced by 5 μM L-AP4 with little or no effect on the depression produced by 5 μM (1S,3S)-ACPD. (b) MAP4 (200 μM) selectively antagonized the depression produced by 5 μM L-AP4 with little or no effect on the depression produced by 1 μM L-CCG-I. (c) MAP4 (1 mM), almost abolished the depression produced by 10 μM L-AP4 and also antagonized the lesser depression produced by 1.5 μM L-CCG-I. Other details as Figure 4. For abbreviations, see text.

(1S,3S)-ACPD (Figure 6c). MCCG produced a parallel rightward shift in the curve for L-CCG-I (Figure 6e), a similar parallel shift in the case of (1S,3S)-ACPD (Figure 6f) and either no effect or a slight potentiation (Figure 6d) in the case of L-AP4. A feature of such experiments was the tendency of the preparation to become more sensitive to the agonists after prolonged treatment with an antagonist. This is reflected in the recovery dose-response curves shown in Figure 6b,e and f.

Table 1 gives apparent K_D values calculated for the interactions between MAP4 or MCCG and the receptors activated by L-AP4, L-CCG-I and (1S,3S)-ACPD. The most potent action was the antagonism by MAP4 of L-AP4-induced synaptic depression; the apparently non-competitive action of MAP4 versus L-CCG-I was more than ten fold weaker. The apparent K_D values calculated for the antagonism by MCCG of the depressant responses produced by (1S,3S)-ACPD and L-CCG-I were relatively close to one another, but the difference between them did reach statistical significance.

It was important to establish the selectivity of MAP4 and MCCG as antagonists of presynaptic excitatory amino acid (EAA) receptors mediating synaptic depression, relative to postsynaptic metabotropic and ionotropic excitatory receptors causing depolarization. Table 2 indicates that, in a TTX-containing medium, and at a concentration (250 μM) which antagonized presynaptic depressant responses, neither of the two antagonists significantly affected similar magnitude depolarizations produced by (1S,3R)-ACPD (25 μM) or N-methyl-D-aspartate (NMDA, 7 μM). Each of the antagonists produced a small depression of the depolarizations induced by (S)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA, 1.2–1.5 μM). However, this was significant only in the case of MAP4 versus AMPA-induced depolarizations, and the failure to achieve complete recovery of the AMPA depolarizations after washout in these cases may have reflected a slight change in the responsiveness of the preparations during the course of these experiments. At higher concentrations, slight potentiation of depolarizations produced by AMPA (in the case of MCCG) or NMDA (in the case of MAP4) were sometimes observed (Figure 7).

Discussion

These results indicate that MAP4 and MCCG, which had relatively little effect on responses mediated by ionotropic EAA receptors (Table 2; Figure 7), discriminate effectively between three types of responses produced in neonatal rat motoneurones by specific metabotropic glutamate receptor agonists as recorded electrophysiologically: (a) the depolarization produced by (1S,3R)-ACPD; (b) the depression of monosynaptic excitation effected by L-CCG-I, (1S,3S)-ACPD and (1S,3R)-ACPD, and (c) the depression of monosynaptic excitation mediated by L-AP4. MAP4 was selective for L-AP4-induced depression, and MCCG preferentially antagonized (1S,3R)-ACPD-induced depression. At the same concentrations of MAP4 and MCCG used to show their selective presynaptic antagonist effects, neither of these two substances

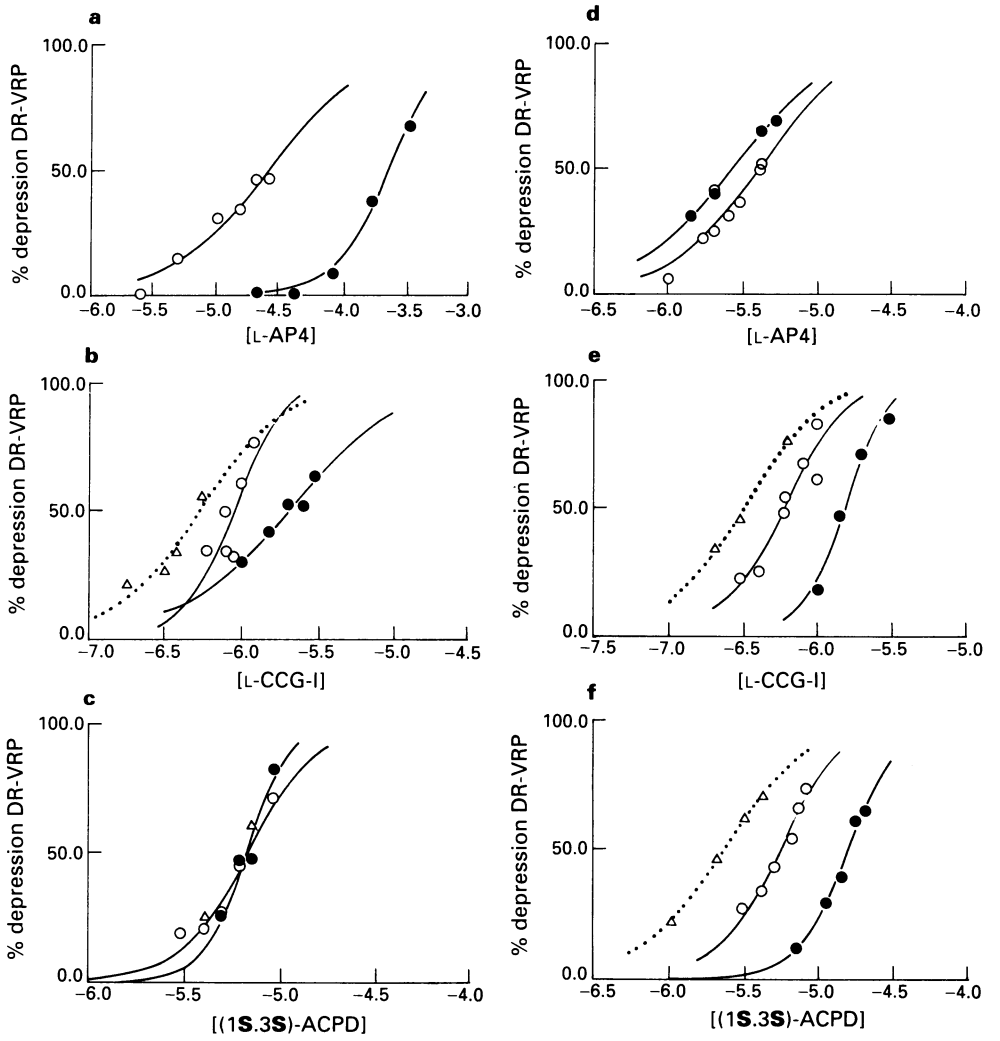

Figure 6 Dose-response curves for depression of monosynaptic DR-VRP by L-AP4, (1S,3S)-ACPD and L-CCG-I in presence and absence of MAP4 or MCCG. (a,d) Depression by L-AP4; (b,e) depression by L-CCG-I; (c,f) depression by (1S,3S)-ACPD. (a–c) Antagonist MAP4 (300 μM); (d–f) antagonist MCCG (300 μM). (○) Control responses; (●) responses recorded in presence of antagonist; (△) responses recorded after removal of antagonist. Parallel shifts to the right, showing competitive antagonism, observed for MAP4/L-AP4 (a), MCCG/L-CCG-I (e) and MCCG/(1S,3S)-ACPD (f); non-parallel shift to the right, showing non-competitive antagonism for MAP4/LCCG-I (b). A slight potentiation (shift to the left) seen for MCCG/L-AP4 (d). Note that after washout of the antagonists the preparation became more sensitive to the depressant agonists than before exposure to the antagonists. Ordinates: % depression of the monosynaptic response; abcissae, log concentration of depressant agonist. For abbreviations, see text.

antagonized the depolarizations produced in neonatal rat motoneurones by (1S,3R)-ACPD, which were previously shown to be blocked by (S)-4C3H-PG, (S)-4CPG and (+)-MCPG (Birse *et al.*, 1993; Eaton *et al.*, 1993b; Jane *et al.*, 1993; Kemp *et al.*, 1994). Such depolarizations are probably mediated postsynaptically by mGluR1 receptors since the three phenylglycine derivatives that antagonize these responses are also antagonists at mGluR1 receptors expressed in CHO cells (Hayashi *et al.*, 1994). However, the same three mGluR1 receptor antagonists also have actions at presynaptic EAA receptors, activation of which effects depression of monosynaptic excitation of neonatal rat motoneurones. (S)-4C3HPG is a moderately potent agonist and (S)-4CPG a relatively weak agonist at presynaptic EAA receptors (Pook *et al.*, 1993), while (+)-MCPG is an antagonist at these receptors, reducing depression of excitation mediated by (1S,3R)-ACPD, (1S,3S)-ACPD, L-AP4, (S)4C3HPG (and the positional isomer (S)-3C4HPG) and L-CCG-I (Kemp *et al.*, 1994 and Figure 1). Except in the case of L-AP4, such depression is likely to be mediated by mGluR2 receptors since the depressant effects of L-CCG-I, (1S,3R)-ACPD, (S)-4C3HPG, (S)-3C4HPG and (S)-4CPG on monosynaptic excitation and the antagonism of these effects by (+)-MCPG parallel their effects on mGluR2 expressed in CHO cells (Hayashi *et al.*, 1994). However, L-AP4 is not an agonist at mGluR2 receptors (Tanabe *et al.*, 1992; Nakanishi *et al.*,

Table 1 Apparent K_D values calculated for the interaction between MAP4 or MCCG and metabotropic glutamate receptors mediating the presynaptic depressant effects of L-AP4, (1S,3S)-ACPD and L-CCG-I

	Antagonist (App K_D, μM) versus		
Antagonist	L-AP4	(1S,3S)-ACPD	L-CCG-I
MAP4	22 ± 5(5)	n.e.(4)[1]	>200(6)[2]
MCCG	n.e.(3)[1]	103 ± 28(5)*	259 ± 34(5)*

Values are μM ± s.e.mean (no. of experiments).
[1]n.e. = no effect (no consistent antagonism at 300 μM).
[2]Non-parallel dose-response curves in presence and absence of antagonist.
*Difference statistically significant ($P<0.02$, Student's t test).
For abbreviations, see text.

Table 2 Effects of MCCG and MAP4 on neonatal rat motoneuronal depolarizations induced by (1S,3R)-ACPD, NMDA or AMPA

		% control depolarization	
Agonist	*Conc.* (μM)	*MCCG* (250 μM)	*MAP4* (250 μM)
(1S,3R)-ACPD	25	103 ± 6(6)[1,2]	95 ± 8(5)*[3]
NMDA	7	87 ± 6(4)[1]	100 ± 6(3)[3]
AMPA	1.2–1.5	79 ± 7(4)[2]	73 ± 2(3)*

All agonists tested in the same preparations; TTX-containing medium.
*Difference statistically significant ($P<0.05$); pairs 1,2,3 not significantly different (Mann-Whitney U-Test).

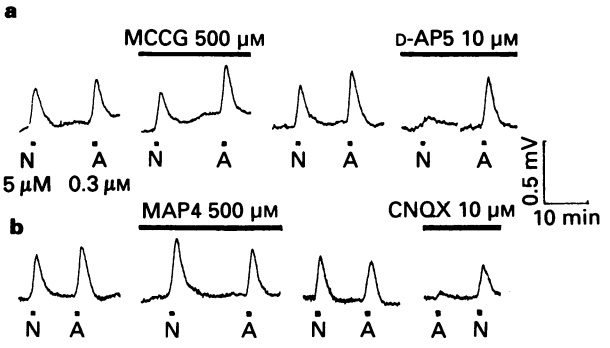

Figure 7 Effects of MCCG and MAP4 on depolarizing responses mediated by NMDA or AMPA receptors. (a) Shows lack of depressant effect of MCCG (500 μM) on either NMDA (5 μM, N)- or AMPA (0.3 μM, A)-induced depolarizations, and subsequent selective depression of NMDA-induced responses by 10 μM D-AP5. (b) Recorded in same spinal cord preparation following recovery from D-AP5-containing medium (approx 1 h), shows lack of effect of MAP4 (500 μM) on AMPA-induced responses and slight potentiation of NMDA-induced responses, with subsequent selective depression of AMPA-induced responses by CNQX (10 μM). Left to right: control responses, responses recorded 20 min after beginning MCCG- or MAP4-containing medium, responses recorded 20 min after washout or MCCG or MAP4, responses recorded 20 min after perfusion with D-AP5 or CNQX-containing medium. For abbreviations, see text.

1992). Therefore the presynaptic depressant effect of L-AP4 and its antagonism by (+)-MCPG must be mediated at receptors other than mGluR2. The present work shows that depression produced by L-AP4 is selectively blocked by MAP4 while the depressions produced by L-CCG-I, (1S,3R)-ACPD and (1S,3S)-ACPD are selectively blocked by MCCG. These results thus confirm the participation of at least two types of presynaptic receptor in the mediation of such depressant effects, and implicate mGluR2 as one of the mGluR sub-types involved.

The nature of the receptor type mediating the depression produced by L-AP4 is currently unknown, but is probably neither mGluR4 nor mGluR6, which, with mGluR7, comprise the range of L-AP4-sensitive mGluR receptors currently identified (Tanabe *et al.*, 1992; Nakajima *et al.*, 1993; Okamoto *et al.*, 1994). Sub-type mGluR4 is not sensitive to (+)-MCPG (Hayashi *et al.*, 1994), which is a less-specific L-AP4 antagonist than MAP4 (Kemp *et al.*, 1994 and Figure 1), and mGluR6 appears to be highly localized to retina (Nakajima *et al.*, 1993). This question of the identity of the L-AP4/MAP4-sensitive receptors remains to be answered.

Of the two sub-type-selective mGluR antagonists described in this work, MAP4 is the more potent. The K_D value found for the interaction of this antagonist with L-AP4-sensitive presynaptic receptors was 22 μM whereas that for MCCG versus (1S,3S)-ACPD- or L-CCG-I-sensitive receptors was in excess of 100 μM and dependent on the agonist. This agonist-dependency raises the question of the selectivity not only of the two antagonists but also of the agonists used. While MAP4 had little or no action at receptors sensitive to (1S,3S)-ACPD, this antagonist did show significant antagonist activity against L-CCG-I-induced depression of synaptic excitation. Such antagonism, however, was at least ten fold weaker than the antagonism shown at L-AP4-sensitive receptors and was probably non-competitive. MCCG had only minimal effect on L-AP4-induced synaptic depression and was more potent as an antagonist of (1S,3S)-ACPD-induced synaptic depression (apparent K_D 103 μM) than as an antagonist of L-CCG-I-induced synaptic depression (apparent K_D 259 μM). These results can be explained on the hypothesis that (1S,3S)-ACPD is a more specific agonist at non-L-AP4 presynaptic receptors on primary afferent terminals in neonatal rat spinal cord than is L-CCG-I, and that the depressant responses produced by L-CCG-I in this preparation were predominantly mediated by (1S,3S)-ACPD-sensitive receptors, but partly also by L-AP4-sensitive receptors. In this case since MAP4 had no action on (1S,3S)-ACPD-sensitive receptors, and MCCG had no action at L-AP4-sensitive receptors, the two antagonists are possibly both highly selective for the two different types of metabotropic receptor on presynaptic terminals at which they act. On this basis L-AP4 and (1S,3S)-ACPD would be regarded as the agonists of choice for the selective activation of these two receptors.

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