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NMDA receptor dependence of mGlu-mediated depression of synaptic transmission in the CA1 region of the rat hippocampus

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1 The depression of synaptic transmission by the specific metabotropic glutamate receptor (mGlu) agonist (1S,3**R**)-1-aminocyclopentane-1,3-dicarboxylate ((1S,3**R**)-ACPD) was investigated in area CA1 of the hippocampus of 4-10 week old rats, by use of grease-gap and intracellular recording techniques.

2 In the presence of 1 mM Mg^{2+}, (1S,3**R**)-ACPD was a weak synaptic depressant. In contrast, in the absence of added Mg^{2+}, (1S,3**R**)-ACPD was much more effective in depressing both the α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) and N-methyl-D-aspartate (NMDA) receptor-mediated components of synaptic transmission. At 100 μM, (1S,3**R**)-ACPD depressed the slope of the field excitatory postsynaptic potential (e.p.s.p.) by 96±1% (mean±s.e.mean; $n=7$) compared with 23±4% in 1 mM Mg^{2+}-containing medium ($n=17$).

3 The depressant action of 100 μM (1S,3**R**)-ACPD in Mg^{2+}-free medium was reduced from 96±1 to 46±6% ($n=7$) by the specific NMDA receptor antagonist (**R**)-2-amino-5-phosphonopentanoate (AP5; 100 μM).

4 Blocking both components of GABA receptor-mediated synaptic transmission with picrotoxin (50 μM) and CGP 55845A (1 μM) in the presence of 1 mM Mg^{2+} also enhanced the depressant action of (1S,3**R**)-ACPD (100 μM) from 29±5 to 67±6% ($n=6$).

5 The actions of (1S,3**R**)-ACPD, recorded in Mg^{2+}-free medium, were antagonized by the mGlu antagonist (+)-α-methyl-4-carboxyphenylglycine ((+)-MCPG). Thus, depressions induced by 30 μM (1S,3**R**)-ACPD were reversed from 48±4 to 8±6% ($n=4$) by 1 mM (+)-MCPG.

6 In Mg^{2+}-free medium, a group I mGlu agonist, (**RS**)-3,5-dihydroxyphenylglycine (DHPG; 100 μM) depressed synaptic responses by 74±2% ($n=18$). In contrast, neither the group II agonists ((2S,1'S,2'S)-2-(2'-carboxycyclopropyl)glycine; L-CCG-1; 10 μM; $n=4$) and ((2S,1'**R**,2'**R**,3'**R**)-2-(2',3'-dicarboxycyclopropyl)glycine; DCG-IV; 100 nM; $n=3$) nor the group III agonist ((**S**)-2-amino-4-phosphonobutanoic acid; L-AP4; 10 μM; $n=4$) had any effect.

7 The depolarizing action of (1S,3**R**)-ACPD, recorded intracellularly, was similar in the presence and absence of Mg^{2+}. AP5 did not affect the (1S,3**R**)-ACPD-induced depolarization in Mg^{2+}-free medium. Thus, 50 μM (1S,3**R**)-ACPD induced depolarizations of 9±3 mV ($n=5$), 10±2 mV ($n=4$) and 8±2 mV ($n=5$) in the three respective conditions.

8 On resetting the membrane potential in the presence of 50 μM (1S,3**R**)-ACPD to its initial level, the e.p.s.p. amplitude was enhanced by 8±3% in 1 mM Mg^{2+} ($n=5$) compared with a depression of 37±11% in the absence of Mg^{2+} ($n=4$). Addition of AP5 prevented the (1S,3**R**)-ACPD-induced depression of the e.p.s.p. (depression of 4±5% ($n=5$)).

9 It is concluded that activation by group 1 mGlu agonists results in a depression of excitatory synaptic transmission in an NMDA receptor-dependent manner.

Keywords: mGlu receptors; (1S,3**R**)-1-aminocyclopentane-1,3-dicarboxylate; (**RS**)-3,5-dihydroxyphenylglycine; (2S,1'**R**,2'**R**,3'**R**)-2-(2',3'-dicarboxycycloproyl)glycine; (2S,1'S,2'S)-2-(2'-carboxycyclopropyl)glycine; (S)-2-amino-4-phosphonobutanoic acid; (+)-α-methyl-4-carboxyphenylglycine; N-methyl-D-aspartate; hippocampus; depolarization; receptor cross-talk

Introduction

Interactions between metabotropic glutamate receptors (mGlu) and their ionotropic counterparts, in particular the N-methyl-D-aspartate (NMDA) receptor, are believed to be important in the regulation of synaptic transmission and plasticity (Bliss & Collingridge, 1993). Several interactions between mGlu and NMDA receptors have been documented. First, it was shown that the specific mGlu agonist (1S,3**R**)-1-aminocyclopentane-1,3-dicarboxylate ((1S,3**R**)-ACPD) was able to potentiate reversibly responses of hippocampal neurones to NMDA (Harvey *et al.*, 1991). Second, it was found that application of NMDA potentiated the calcium mobilising response induced in cultured cerebellar granule cells by (1S,3**R**)-ACPD (Irving *et al.*, 1992). Third, it was shown that NMDA receptor activation augments (1S,3**R**)-ACPD-induced currents in CA3 pyramidal neurones (Lüthi *et al.*, 1994). These three interactions occur postsynaptically, which is consistent with the predominantly postsynaptic localization of NMDA receptors. During the course of investigations into the effects of (1S,3**R**)-ACPD on excitatory synaptic transmission in the hippocampus, we have uncovered another interaction between these two receptor systems. We have found that NMDA receptor activation facilitates the ability of (1S,3**R**)-ACPD to depress synaptic transmission in the CA1 region of the hippocampus.

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Methods

Experiments were performed on 400 μm thick transverse hippocampal slices obtained from female rats of 140–200 g in weight (approximately 4–10 weeks of age). Area CA3 was surgically removed and slices perfused with medium (28–30°C), which composed (mM): NaCl 124, KCl 3, $NaHCO_3$ 26, NaH_2PO_4 1.4 (omitted in some experiments), $MgSO_4$ 1, $CaCl_2$ 2, D-glucose 10 (bubbled with 95% O_2/5% CO_2; pH 7.4), at a rate of approximately 2 ml min^{-1}. Drugs were applied in the perfusate. The Schaffer collateral-commissural pathway was stimulated continuously with single shocks delivered once every 30 s. Electrical activity was recorded by use of two different approaches.

Grease-gap recordings were performed as described previously (Harvey & Collingridge, 1993). In brief, the alveus was partially dissected from the slice and placed under the wick of a Ag/AgCl electrode. The wick and alveus assembly was covered by an insulating grease mixture and d.c. potentials were recorded between this electrode and a similar one placed in contact with the perfusate. This method of extracellular recording was used since it provides a more suitable approach than conventional microelectrode recording for quantitative pharmacological investigation (e.g. Blake *et al.*, 1988).

Intracellular recordings were performed as described previously (Davies *et al.*, 1995). In brief, recordings were obtained from the somatic region using electrodes filled with 2 M potassium methylsulphate (78±2 MΩ). Data were obtained from 10 neurones which had resting membrane potentials of −65±1 mV and resting input resistances of 42±3 MΩ.

The amplitudes and/or initial slopes of field excitatory postsynaptic potentials (field e.p.s.ps) were displayed on-line using programmes written in-house. Intracellularly recorded excitatory postsynaptic potentials (e.p.s.ps) were analysed with commercial software.

Drugs

1S,3R-1-aminocyclopentane-1,3-dicarboxylate ((1S,3R)-ACPD), (RS)-3,5-dihydroxyphenylglycine (DHPG), (2S,1'R,-2'R,3'R)-2-(2',3'-dicarboxycyclopropyl)glycine (DCG-IV), (S)-2-amino-4-phosphonobutanoic acid (L-AP4), (+)-α-methyl-4-carboxyphenylglycine ((+)-MCPG), (2S,1'S,2'S)-2-(2'-carboxycyclopropyl)glycine (L-CCG-1), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and (R)-2-amino-5-phosphonopentanoate (AP5) were all obtained from Tocris Cookson, (Bristol, U.K.). Picrotoxin was purchased from Sigma (Poole, Dorset, U.K.). CGP 35348 (3-aminopropyl (diethoxymethyl)phosphinic acid) and CGP 55845A (3-[1-(S)-(3,4-dichlorophenyl)-ethyl]amino 2 (S)-hydroxypropyl-(P-benzyl)-phosphinic acid) were gifts from Dr M.F. Pozza (Ciba Geigy, Basel, Switzerland).

Statistical analysis

Results were analysed with paired and unpaired Student's *t* tests as appropriate; *n* signifies the number of times a result was obtained which, unless otherwise stated, is the same as the number of slices tested. Each slice was obtained from a separate rat.

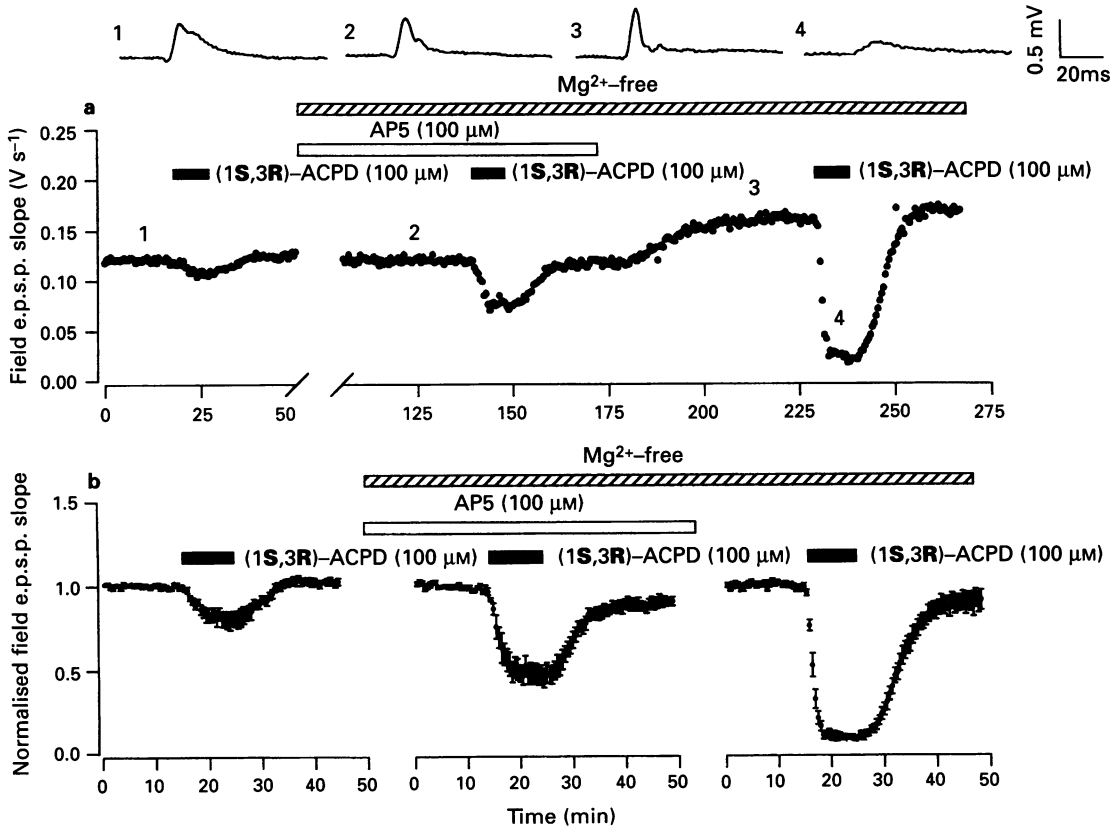

Figure 1 Depression of field e.p.s.ps by (1S,3R)-ACPD is enhanced in Mg^{2+}-free medium in an AP5-sensitive manner. (a) Data from an individual experiment to illustrate the differential effects of (1S,3R)-ACPD (100 μM) on synaptic transmission. The traces above the plot are individual synaptic responses corresponding to the time points 1–4, whereas the lower panel is a plot of the field e.p.s.p. slope for the duration of the experiment. (During the break in the graph, Mg^{2+}-free medium was perfused. This led to a large increase in the size of the synaptic response. Once the response had stabilized the stimulus intensity was reduced and a new baseline obtained. AP5 was then applied and led to a decrease in the response amplitude). (b) Illustrates the pooled data obtained from 7 similar experiments. Each point is the normalised slope±s.e.mean of the field e.p.s.p. In this and subsequent figures, solutions were applied for the times indicated by the bars above the graphs.

Results

(1S,3R)-ACPD-induced depressions are enhanced by synaptic NMDA receptor activation

In the presence of 1 mM Mg^{2+} and absence of GABA receptor antagonists, (1S,3R)-ACPD was usually weak at depressing the field e.p.s.p. For example, 100 μM (1S,3R)-ACPD depressed the response by 23±4% ($n=17$; Figure 1). However, its effects were variable between slices (range 0 to 75% depression). In contrast, under recording conditions which favoured NMDA receptor activation, (1S,3R)-ACPD was considerably more potent at depressing field e.p.s.ps (Figure 2). Four separate protocols were used. In the first, slices were perfused with Mg^{2+}-free medium for at least 45 min which results in pronounced activation of NMDA receptors following low frequency stimulation (Coan & Collingridge, 1985). Under these conditions, (1S,3R)-ACPD was considerably more potent at depressing field e.p.s.ps (Figures 1 and 2a). For example, 100 μM (1S,3R)-ACPD depressed the response by 96±1% ($n=7$). This increased sensitivity to (1S,3R)-ACPD was due, at least in part, to NMDA receptor activation since, in the same 7 slices, (1S,3R)-ACPD-induced depressions were reduced to 46±6% in the presence of AP5 (100 μM; Figure 1). In contrast, AP5 (100 μM) did not affect depressions induced by 300 μM (1S,3R)-ACPD in 1 mM Mg^{2+}-containing medium (data not shown; $n=3$).

In the second protocol, γ-aminobutyric $acid_A$ ($GABA_A$) (50 μM picrotoxin) and $GABA_B$ (1 μM CGP 55845A or 500 μM CGP 35348) antagonists were added to 1 mM Mg^{2+}-containing medium, since blockade of GABA inhibition enables activation of NMDA receptors by low frequency stimulation in the presence of Mg^{2+} (Herron *et al.*, 1985; Dingledine *et al.*, 1986). In these experiments, depressions induced by 100 μM (1S,3R)-ACPD were enhanced from 29±5 to 67±6% ($n=6$; Figure 3) following the addition of the GABA antagonists. The enhanced sensitivity to (1S,3R)-ACPD was partially dependent on NMDA receptor activation since in paired experiments depressions induced by (1S,3R))-ACPD (30–100 μM) were reduced from 53±4 to 47±4% by AP5 (100 μM; $P<0.05$; $n=5$ data not shown).

In the third protocol, the AMPA/kainate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 10 μM) was added to the GABA antagonists, in the presence of 1 mM Mg^{2+}, to isolate the NMDA receptor-mediated field e.p.s.p. (Blake *et al.*, 1988). In 18 of 25 slices tested (1S,3R)-ACPD (10–200 μM) induced a dose-dependent depression of the NMDA receptor-mediated field e.p.s.p. (Figures 2b and 4a). However, in the 7 other slices, (1S,3R)-ACPD resulted in a transient enhancement of the field e.p.s.p. (Figure 4b). In the fourth protocol, pharmacologically-isolated NMDA receptor mediated field e.p.s.ps were further enhanced by perfusion with Mg^{2+}-free medium for at least 45 min. In all 15 slices tested, (1S,3R)-ACPD (10–100 μM) induced a dose-dependent depression of the NMDA receptor-mediated field e.p.s.p. In 11 cases, the depressions reversed to control levels (Figure 4c) whilst in 4 slices recovery was followed by a potentiation of synaptic transmission, which lasted for at least 60 min (Figure 4d). The potency of (1S,3R)-ACPD at depressing NMDA receptor-mediated synaptic responses was similar in the presence and absence of Mg^{2+} and was similar to that for depressing dual-component field e.p.s.ps (Figure 2).

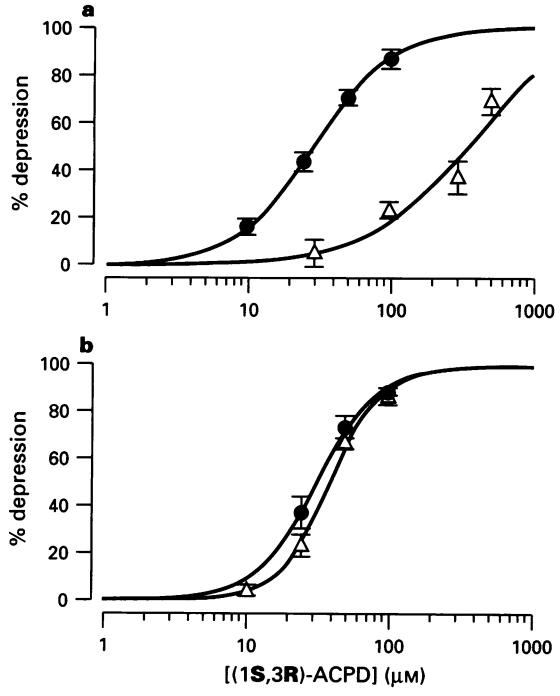

Figure 2 Dose-response curves for (1S,3R)-ACPD-induced depressions of field e.p.s.ps. In (a), the graph shows the dose-response relationships for the synaptic depressions of the field e.p.s.p. amplitude induced by (1S,3R)-ACPD in the absence (●) and presence (△) of 1 mM Mg^{2+} in the bathing medium. The plot in (b) shows the dose-response curves for (1S,3R)-ACPD-induced depressions of the field e.p.s.p. amplitude of the pharmacologically-isolated NMDA receptor-mediated component of synaptic transmission in the absence (●) and presence (△) of added Mg^{2+}. Each point is the mean±s.e.mean (vertical lines) of between 3 and 17 experiments. Approximate dose-response relationships were constructed by use of a pragmatic logistic equation, assuming that there was no effect in the absence of drug and that the maximal effect did not exceed a 100% depression. Doses at which 50% depressions were obtained were 350 μM (a, 1 mM Mg^{2+}), 29 μM (a, Mg^{2+}-free), 39 μM (b, 1 mM Mg^{2+}), 32 μM (b, Mg^{2+}-free).

Pharmacology of mGlu-induced depression of field e.p.s.ps

Further experiments were performed to establish the pharmacology of the (1S,3R)-ACPD-induced depression in Mg^{2+}-free medium. We first used the most extensively characterized mGlu antagonist (+)-MCPG, which is effective at both group I and group II mGlu receptors (for review see Watkins & Collingridge, 1994). Depressions induced by 30 μM (1S,3R)-ACPD were reversed from 48±4 to 8±6% by 1 mM (+)-MCPG ($n=4$; Figure 5).

We next compared the actions of subgroup selective agonists. The group I specific agonist (RS)-3,5-dihydroxyphenylglycine (DHPG) invariably depressed synaptic transmission. The peak depression induced by 100 μM DHPG was 74±2% ($n=18$). In 5 cases, the effects of DHPG were fully reversible (Figure 6a). In the other slices ($n=13$), however, application of DHPG lead to a long-lasting depression of synaptic transmission (data not shown). In contrast to DHPG, neither L-CCG-1 (10 μM; $n=4$; Figure 6b) and DCG-IV (100 nM; $n=3$; data not shown), compounds active at group II mGlu receptor, nor the group III selective agonist L-AP4 (10 μM; $n=4$; Figure 6b) had any appreciable effect on synaptic transmission.

Effects of (1S,3R)-ACPD measured intracellularly

Since activation of NMDA receptors can enhance the postsynaptic excitatory action of (1S,3R)-ACPD in hippocampal neurones (Lüthi *et al.*, 1994), we were concerned that part or all of the enhanced depression of the field e.p.s.ps, recorded in Mg^{2+}-free conditions, may be due to enhanced postsynaptic depolarization, mediated by group I mGlu receptors (Davies *et al.*, 1995; Gereau & Conn, 1995b). We therefore performed some experiments with intracellular recording techniques to both measure and manipulate the membrane potential.

For these experiments we applied (1S,3R)-ACPD at a concentration of 50 μM for 10 min. In the presence of 1 mM Mg^{2+}, (1S,3R)-ACPD caused a depolarization (of 9±3 mV

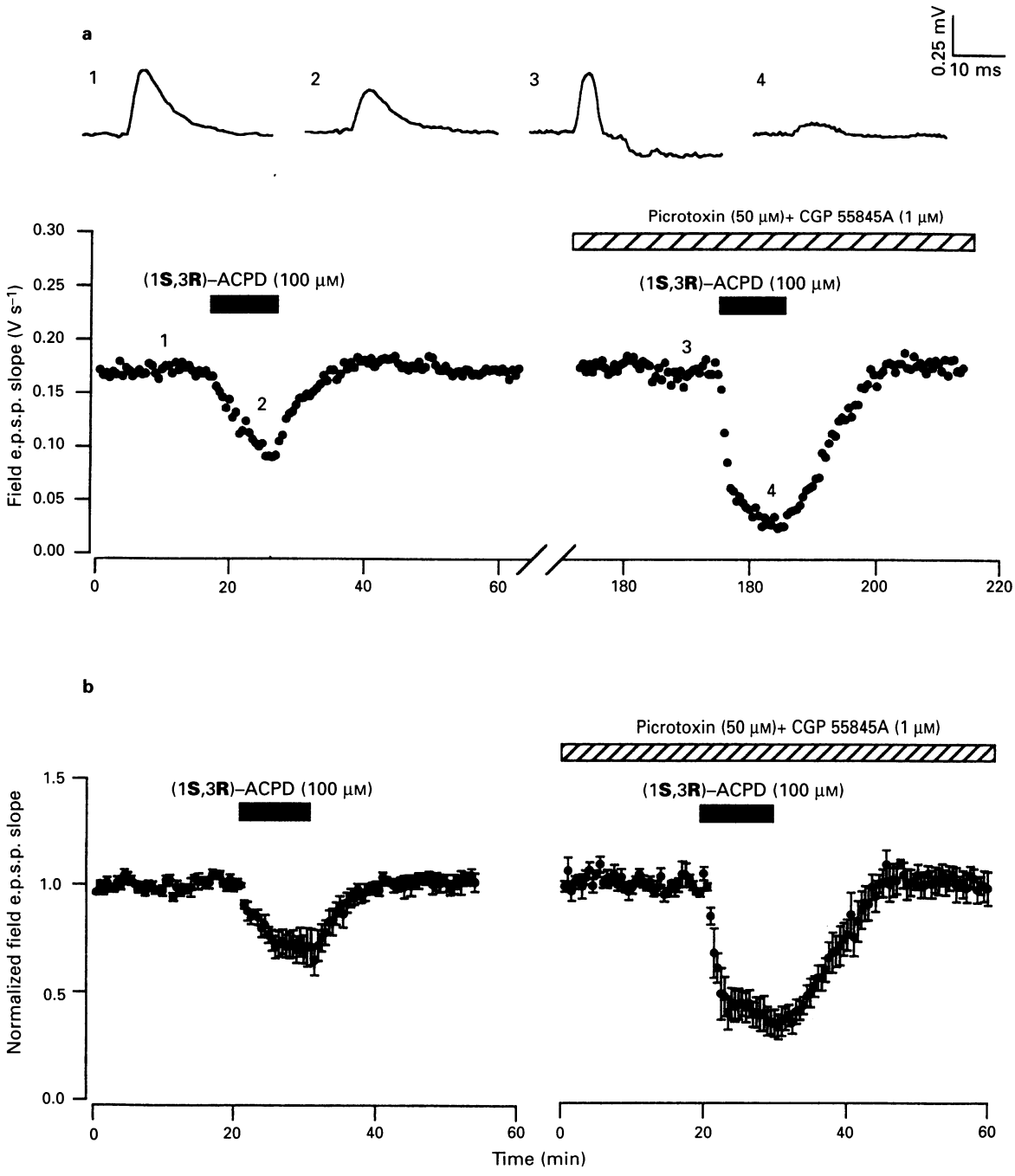

Figure 3 Depression of field e.p.s.ps by (1S,3**R**)-ACPD is enhanced by blockade of GABA-mediated synaptic transmission . (a) Data from an individual experiment and (b) pooled data for 5 slices. The GABA antagonists were applied for at least 45 min before the second challenge with (1S,3**R**)-ACPD.

from a holding membrane potential of -71 ± 2 mV; $n = 5$) and an increase in membrane input resistance. At the peak of the depolarization the e.p.s.p. was depressed, the inhibitory post-synaptic potential (i.p.s.p.) was enhanced and, in 4/5 neurones, the e.p.s.p. elicited a single action potential. However, when the membrane potential was reset to the initial level, there was no depression of the e.p.s.p. Indeed, in 3/5 neurones the e.p.s.p. was slightly enhanced in size (reflected by an increase of $8 \pm 3\%$, $n = 5$). In addition, the input resistance was $110 \pm 5\%$ of control and there was an inhibition of spike frequency adaptation. The i.p.s.p. remained larger than control in 2 neurones but was depressed in the other 3. Following a 20–30 min wash all the effects were fully reversible ($n = 4$).

In 3 of these neurones (1S,3**R**)-ACPD was re-applied 40–50 min following perfusion with Mg^{2+}-free medium. In each neurone the size of the depolarization was similar to that observed in the presence of Mg^{2+}. The mean depolarization in the presence and absence of Mg^{2+} was 12 ± 4 and 11 ± 3 mV, respectively. As seen in the presence of Mg^{2+} at the peak of the depolarization, the e.p.s.p. was depressed and the i.p.s.p. enhanced in amplitude. When the membrane potential was reset, the e.p.s.p. was still reduced relative to control in each neurone tested. In the absence of Mg^{2+} the e.p.s.p. was depressed by $36 \pm 15\%$ relative to control. This compares with an increase of $10 \pm 5\%$ for the same 3 neurones in the presence of Mg^{2+}. (1S,3**R**)-ACPD-induced changes of input resistance and spike frequency adaptation, obtained after resetting the membrane potential, were similar to the equivalent measures made before perfusing with Mg^{2+}-free medium. Again, the i.p.s.p. was affected variably between cells.

To determine whether the depression in the amplitude of the e.p.s.p. seen in Mg^{2+}-free medium was due to the enhanced activation of NMDA receptors, a comparison of the effects of 50 μM (1S,3R)-ACPD were made in Mg^{2+}-free medium in the presence and absence of 50 μM AP5 in the same 3 neurones. Under these conditions, (1S,3R)-ACPD produced similar depolarizations (9±3 and 10±4 mV, respectively). There were also depressions of the e.p.s.p. and enhancements of the i.p.s.p

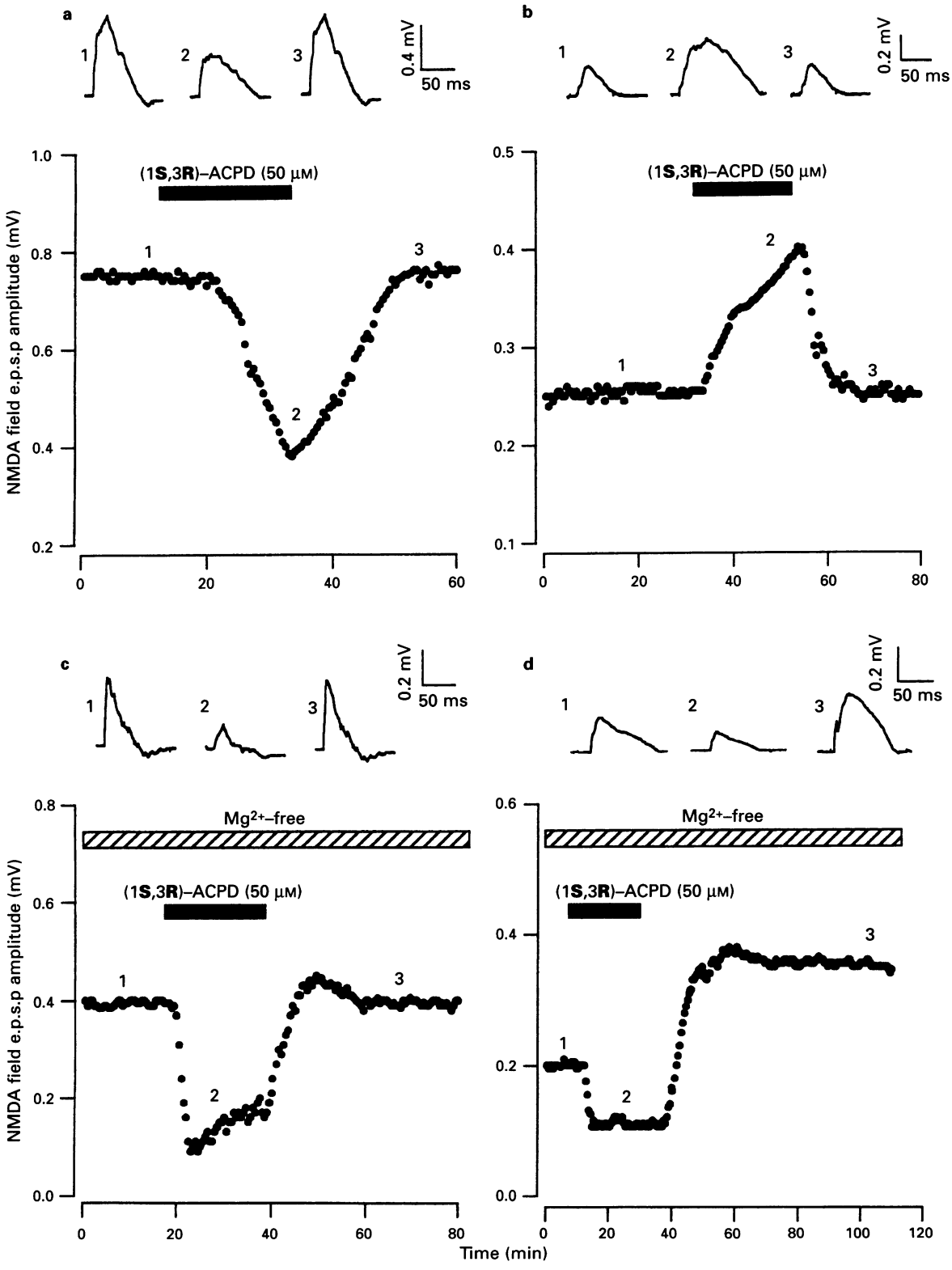

Figure 4 Effects of (1S,3R)-ACPD on NMDA receptor-mediated synaptic transmission. The plots in (a) and (b) illustrate the two distinct effects of (1S,3R)-ACPD on pharmacologically-isolated NMDA receptor-mediated field e.p.s.ps observed in slices perfused with medium containing 1 mM Mg^{2+}. (a) Application of (1S,3R)-ACPD (50 μM) for 20 min caused a slowly developing attenuation of the isolated NMDA receptor-mediated field e.p.s.p. that reversed to baseline levels following washout. (b) In contrast, application of (1S,3R)-ACPD (50 μM) to another slice resulted in a transient enhancement of the NMDA receptor-mediated field e.p.s.p. In slices where Mg^{2+} was omitted from the perfusate (c and d), (1S,3R)-ACPD (50 μM) depressed NMDA receptor-mediated field e.p.s.ps. Following washout of (1S,3R)-ACPD, synaptic transmission either returned to control (c) or a potentiated level (d). The traces above each plot are the averages of 3 successive synaptic responses, corresponding to the time points 1–3.

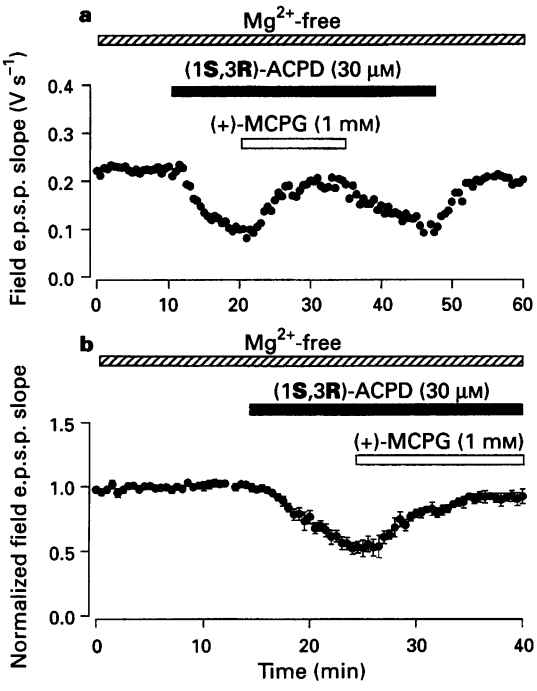

Figure 5 Actions of (+)-MCPG on (1S,3R)-ACPD-induced depressions in the absence of Mg^{2+}. (a) Shows data from an individual experiment and (b) illustrates pooled data from 4 similar experiments.

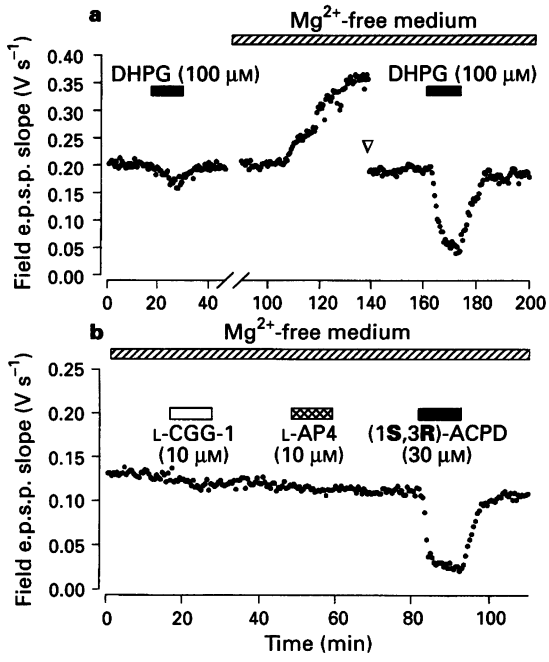

Figure 6 Comparison of the actions of DHPG, L-CCG-1 and L-AP4 in the absence of Mg^{2+}. (a) A single experiment showing the differential effects of the group I selective agonist, DHPG (100 μM), in the presence and absence of 1 mM Mg^{2+} in the bathing medium. (The stimulus intensity was reduced at the time indicated by an arrowhead). (b) A single experiment to illustrate the lack of effect of group II or group III selective mGlu agonists.

at the depolarized membrane potential. When the membrane potential was reset the e.p.s.p. was no longer appreciably depressed while the i.p.s.p. was again affected variably from neurone to neurone. Thus, the e.p.s.p. was depressed by 40±13% in Mg^{2+}-free medium and by 6±7% in the presence of AP5. (1S,3R)-ACPD-induced changes in input resistance and spike frequency adaptation were similar to those observed in the absence of AP5 ($n=3$).

Figure 7 presents a neurone where (1S,3R)-ACPD was applied sequentially in the presence of 1 mM Mg^{2+} and in the absence of Mg^{2+} before and following the addition of AP5. Note that the (1S,3R)-ACPD-induced depression of the e.p.s.p., recorded in Mg^{2+}-free medium, was associated with a decrease in the initial slope (mainly AMPA receptor-mediated) and latter components (mainly NMDA receptor-mediated) of the response. Figure 8 presents pooled data for all these neurones, and includes data from neurones where 50 μM (1S,3R)-ACPD was applied only once.

Discussion

The present study demonstrates that the effectiveness of (1S,3R)-ACPD to depress excitatory synaptic transmission in the Schaffer collateral-commissural pathway is influenced by the level of NMDA receptor activation.

Variability in the effects of (1S,3R)-ACPD

This result may explain, at least in part, some of the variability found in the effectiveness of (1S,3R)-ACPD, or the racemic mixture (1SR,3RS)-ACPD (often incorrectly referred to as trans-ACPD; see Watkins & Collingridge, 1994), in depressing excitatory synaptic transmission in area CA1. Results found have varied from no effect to almost complete abolition of the synaptic response, over similar dose ranges (Baskys & Malenka, 1991; Desai & Conn, 1991; Harvey *et al.*, 1991; Pacelli & Kelso, 1991; Desai *et al.*, 1992; Bolshakov & Siegelbaum, 1994; Gereau & Conn, 1995a; Manzoni & Bockaert, 1995; Vignes *et al.*, 1995b). Consistent with this hypothesis, previous studies where (1S,3R)-ACPD was most effective were conducted under conditions that favour NMDA receptor activation/Ca^{2+} entry, such as blockade of GABA receptor-mediated synaptic inhibition (e.g., Gereau & Conn, 1995a; Manzoni & Bockaert, 1995; Vignes *et al.*, 1995b).

An important additional variable is the age of rats from which the slices are prepared. In young animals (1SR,3RS)-ACPD causes a pronounced depression of e.p.s.cs, most probably via a presynaptic site of action (Baskys & Malenka, 1991; Bolshakov & Siegelbaum, 1994). In addition, e.p.s.cs from young animals are sensitive to agonists which activate group II and group III mGlu receptors (Baskys & Malenka, 1991; Vignes *et al.*, 1995b).

Subtypes of mGlu receptors involved in (1S,3R)-ACPD-induced depressions

The generally small depressions of e.p.s.ps observed in the presence of 1 mM Mg^{2+} can be attributed to the postsynaptic depolarizations; indeed the entire depressant effect of 50 μM (1S,3R)-ACPD recorded intracellularly was eliminated on resetting the membrane potential to the initial level. Depolarizations in CA1 pyramidal neurones are induced by the group I selective agonist DHPG but not by group II or III selective agonists (Davies *et al.*, 1995; Gereau & Conn, 1995b). Furthermore, (1S,3R)-ACPD-induced depolarizations are blocked by antagonists active at group I mGlu receptors (Davies *et al.*, 1995). Therefore, the indirect (1S,3R)-ACPD-induced depression of e.p.s.ps (seen as a result of depolarization) is likely to be mediated by one or more members of group I mGlu receptors (i.e., $mGlu_1$ and $mGlu_5$). Since CA1 pyramidal neurones do not express $mGlu_1$ (e.g., Luján *et al.*, 1996) and since the excitatory effects of (1S,3R)-ACPD are observed in $mGlu_1$ null mice (Conquet *et al.*, 1994) the most likely subtype is $mGlu_5$.

Similarly, the NMDA receptor-dependent depression of field e.p.s.ps by (1S,3R)-ACPD is also likely to be mediated by a group I mGlu since DHPG but not L-CCG-1, DCG-IV or L-

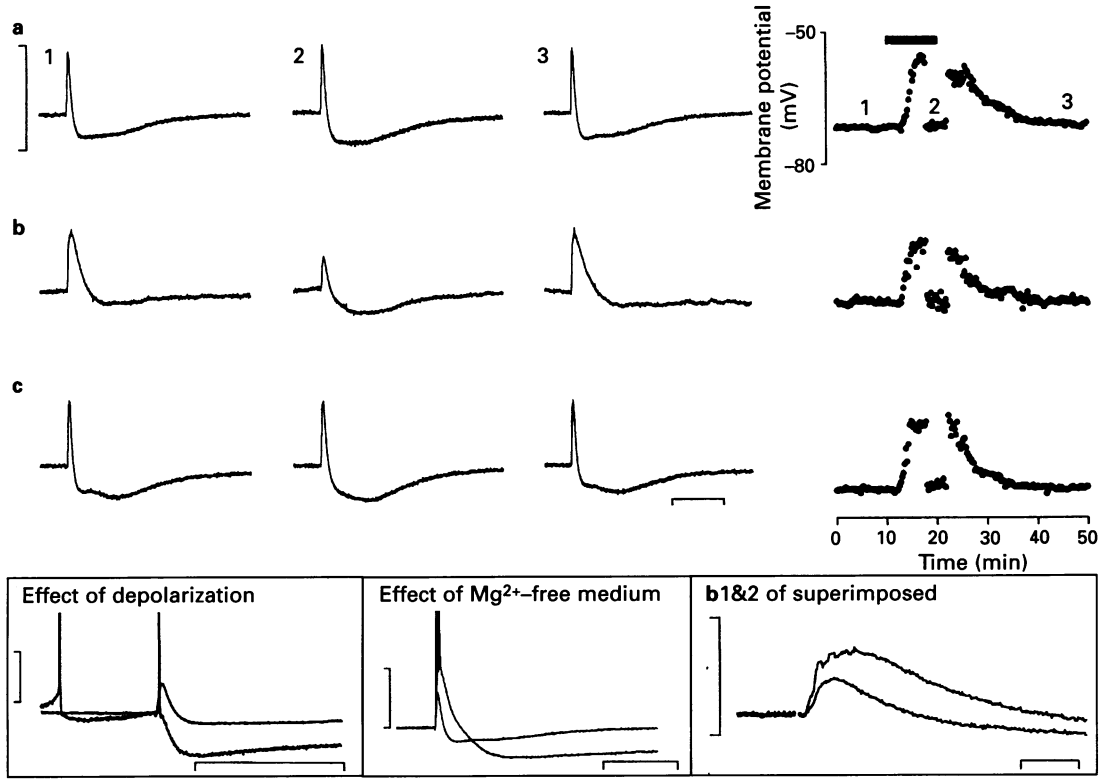

Figure 7 Effects of (1S,3R)-ACPD recorded intracellularly. The traces are averages of 4 successive sweeps showing e.p.s.p.-i.p.s.p. sequences before (1), during (2) and following washout (3) of 50 μM (1S,3R)-ACPD in the presence of 1 mM Mg^{2+} (a), in Mg^{2+}-free medium (b) and in Mg^{2+}-free medium containing 50 μM AP5 (c). The right-hand traces plot the membrane potential, measured every 15 s, and indicate when the traces were obtained. The bar above the plot in (1) indicates the application of (1S,3R)-ACPD. Note that the response in the presence of (1S,3R)-ACPD (2) was recorded after the membrane potential had been reset. The left-hand inset shows the response at the peak of the (1S,3R)-ACPD-induced depolarization in Mg^{2+}-containing medium (i.e., before the membrane potential was reset) aligned and superimposed upon (a1). The middle inset shows the response before and 30 min following perfusion of Mg^{2+}-free medium, before the stimulus intensity was reduced. The right-hand inset shows the (1S,3R)-ACPD-induced depression of the e.p.s.p., recorded in Mg^{2+}-free medium, on a faster time-base. Note the decrease of the initial slope and of the latter part of the response. The membrane potential of the neurone was −71 mV (−0.1 nA holding current, resting membrane potential −66 mV). Vertical calibration bars show 20 mV, horizontal calibration bars show 200 ms (20 ms for right-hand inset).

AP4 produced a similar depression in Mg^{2+}-free medium. The antagonism of the (1S,3R)-ACPD-induced synaptic depression by (+)-MCPG is consistent with this conclusion. It is also consistent with the results of Manzoni & Bockaert (1995) who found large depressions of field e.p.s.ps and e.p.s.cs with either (1S,3R)-ACPD or DHPG, since this study picrotoxin was used to block $GABA_A$ receptor-mediated synaptic inhibition.

Other effects of (1S,3R)-ACPD

In this study we have concentrated on the depressant action of (1S,3R)-ACPD on excitatory synaptic transmission. However, other effects of (1S,3R)-ACPD were also noted. First, there were variable effects on the i.p.s.p. (when compared at the same membrane potential). We assume this reflects two opposing actions of (1S,3R)-ACPD. One, to depress synaptic transmission via a presynaptic action on the synapses made both between Schaffer collateral-commissural fibres and inhibitory interneurones and between inhibitory interneurones and the recorded cell and two, to excite these interneurones sufficiently to facilitate their synaptic activation (Desai & Conn, 1991; Pacelli & Kelso, 1991; Desai *et al.*, 1994; McBain *et al.*, 1994; Jouvenceau *et al.*, 1995). Second, the depression of the e.p.s.p. was associated with a widening of the response, even when the synaptic inhibition was facilitated (data not shown). This might be due to a direct potentiation of the NMDA receptor-mediated conductance by (1S,3R)-ACPD (Aniksztejn *et al.*, 1992; Harvey & Collingridge, 1993). Third, washout of (1S,3R)-ACPD sometimes resulted in a long-term

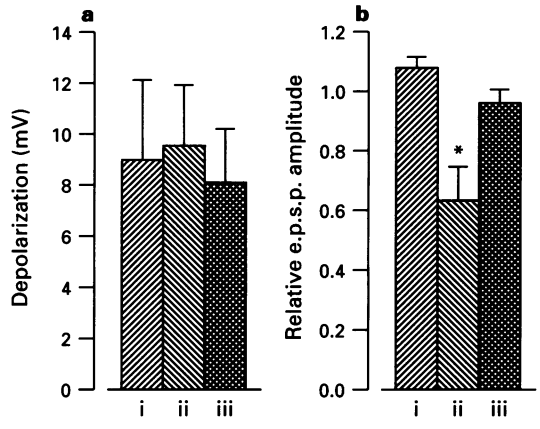

Figure 8 Summary of the effects of (1S,3R)-ACPD on membrane potential and e.p.s.p. amplitude. Steady-state depolarization (a) and change of e.p.s.p. amplitude (b) induced by 50 μM (1S,3R)-ACPD (i) 1 mM Mg^{2+}-containing medium, (ii) Mg^{2+}-free medium and (iii) Mg^{2+}-free medium containing 50 μM AP5. *$P<0.01$ versus Mg^{2+}-containing medium, $P<0.05$ versus Mg^{2+}-free medium plus AP5 (unpaired Student's t test). Each column represents the mean± s.e.mean (vertical lines) for either 5 or 6 neurones.

potentiation (LTP) of the isolated NMDA receptor-mediated synaptic response, as described previously in studies in the dentate gyrus (O'Connor *et al.*, 1994). Following washout of

(1S,3R)-ACPD, there was no LTP of AMPA receptor-mediated synaptic transmission in contrast to previous findings (Bortolotto & Collingridge, 1993; Bortolotto & Collingridge, 1995). This was not surprising since in the present experiments area CA3 was removed; a condition which prevents the generation of this effect (Bortolotto *et al.*, 1995). Also in the present study (1S,3R)-ACPD produced no long-term changes in the level of synaptic inhibition, possibly due to a species difference (Liu *et al.*, 1993). Finally, (1S,3R)-ACPD never induced long-term depression (LTD) in contrast to its described effects in the dentate gyrus (O'Mara *et al.*, 1995). However, in the majority of cases, DHPG did induce LTD. This DHPG-induced LTD is the subject of a separate investigation.

On the NMDA receptor-dependence of the (1S,3R)-ACPD-induced effect

The most intriguing aspect of the present finding is the implication of the NMDA receptor-dependence for the locus of the effect. Although we did not specifically address the locus of the synaptic depression it can be assumed, on the basis of other studies (Baskys & Malenka, 1991; Bolshakov & Sigelbaum, 1994; Gereau & Conn, 1995a), that it is presynaptic. The present finding of large depressions of both the AMPA and NMDA receptor-mediated components of synaptic transmission is more readily compatible with a presynaptic change. The most direct mechanism would require both mGlu receptors of the group I subgroup, and NMDA receptors to be located on Schaffer collateral-commissural terminals. Although feasible, there is no direct evidence that NMDA receptors are located presynaptically at this pathway. Activation of NMDA receptors in the CA1 region of the hippocampus can depress the presynaptic fibre volley (Collingridge *et al.*, 1983), but this effect may be due to ionic changes resulting from postsynaptic activation of NMDA receptors (Collingridge *et al.*, 1991). On the basis of the current knowledge of receptor distribution at this synapse, therefore, it seems necessary to propose the involvement of a retrograde messenger. One possibility is that postsynaptic NMDA receptor activation generates a retrograde messenger which acts synergistically with presynaptic mGlu receptors. An alternative possibility is that the NMDA receptor and mGlu interactions are postsynaptic and lead to the formation of the retrograde messenger, which causes the depression in transmitter release.

A number of candidates for the putative retrograde messenger exist. Activation of mGlu receptors can result in arachidonic acid formation (Dumuis *et al.*, 1990), and this fatty acid has already been implicated in retrograde signalling with respect to the generation of LTP (Williams *et al.*, 1989) and LTD (Bolshakov & Siegelbaum, 1995). Another possibility is nitric oxide (NO), since NMDA receptor activation leads to NO formation and since NO donors depress synaptic transmission at the Schaffer collateral-commissural pathway (Boulton *et al.*, 1994). A third possibility is adenosine which is also released by NMDA receptor activation and depresses transmission at this synapse (Manzoni *et al.*, 1994). Further experiments are required to test these and other possibilities.

A postsynaptic interaction between NMDA receptors and group I mGlu receptors has been described previously in cultured cerebellar granule cells (Irving *et al.*, 1992). In this system, Ca^{2+} entry following NMDA receptor activation greatly facilitates the ability of (1S,3R)-ACPD to release Ca^{2+} from intracellular stores, possibly due to a Ca^{2+}-dependence in the activation of the phospholipase C. Similar mechanisms may operate in cerebral cortex (Challiss *et al.*, 1994), striatum (Morari *et al.*, 1994) and hippocampus (Irving & Collingridge, unpublished observations). The reciprocal facilitatory interaction also occurs postsynaptically since activation of group I mGlu receptors potentiates depolarization induced by NMDA in the hippocampus (Fitzjohn *et al.*, 1996). A Ca^{2+}-dependence in mGlu-stimulated phosphoinositide breakdown in synaptosomes has been demonstrated (Vignes *et al.*, 1995a) so it is also possible that NMDA and mGlu receptor interactions can occur presynaptically.

NMDA receptor-independent facilitation of mGlu receptors

It is likely that mechanisms independent of NMDA receptors also act to facilitate the activation of mGlu receptors. First, the enhanced sensitivity to (1S,3R)-ACPD in Mg^{2+}-free medium was not fully reversed by AP5, perhaps because of actions of Mg^{2+} unrelated to antagonism of NMDA receptors (e.g., Coan & Collingridge, 1985). Second, in the presence of picrotoxin AP5 had only a small effect on the increased sensitivity to (1S,3R)-ACPD. In cerebellar granule cells, potassium-induced depolarization can provide the Ca^{2+} required to facilitate the actions of (1S,3R)-ACPD (Irving *et al.*, 1992). This suggests that the facilitation of mGlu receptors need not be specifically caused by NMDA receptor-mediated Ca^{2+} fluxes and that activation of voltage-gated Ca^{2+} channels alone could provide a sufficient source of Ca^{2+}.

In summary, the present work has revealed a further degree of complexity in the interactions between glutamate receptors at a central glutamatergic synapse. The finding that activation of NMDA receptors can facilitate the activation of group I mGlu receptors may have implications for synaptic plasticity, where both of these classes of glutamate receptors are strongly implicated (Bliss & Collingridge, 1993).

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