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Pharmacology of postsynaptic metabotropic glutamate receptors in rat hippocampal CA1 pyramidal neurones

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1 Activation of metabotropic glutamate receptors (mGluRs) in hippocampal CA1 pyramidal neurones leads to a depolarization, an increase in input resistance and a reduction in spike frequency adaptation (or accommodation). At least eight subtypes of mGluR have been identified which have been divided into three groups based on their biochemical, structural and pharmacological properties. It is unclear to which group the mGluRs which mediate these excitatory effects in hippocampal CA1 pyramidal neurones belong. We have attempted to address this question by using intracellular recording to test the effects of a range of mGluR agonists and antagonists, that exhibit different profiles of subtype specificity, on the excitability of CA1 pyramidal neurones in rat hippocampal slices.

2 (2S,1'S,2'S)-2-(2'-carboxycyclopropyl)glycine (L-CCG1) caused a reduction in spike frequency adaptation and a depolarization (1-10 mV) associated with an increase in input resistance (10-30%) at concentrations ($\geq$50 μM) that have been shown to activate mGluRs in groups I, II and III. Similar effects were observed with concentrations (50-100 μM) of (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid ((1S,3R)-ACPD) and (1S,3S)-ACPD that exhibit little or no activity at group III mGluRs but which activate groups I and II mGluRs.

3 Inhibition of the release of endogenous neurotransmitters through activation of $GABA_B$ receptors, by use of 200 μM (±)-baclofen, did not alter the effects of (1S,3R)-ACPD (50-100 μM), (1S,3S)-ACPD (100 μM) or L-CCG1 (100 μM). This suggests that mGluR agonists directly activate CA1 pyramidal neurones.

4 Like these broad spectrum mGluR agonists, the racemic mixture ((SR)-) or resolved (S)-isomer of the selective group I mGluR agonist 3,5-dihydroxyphenylglycine ((SR)-DHPG (50-100 μM) or (S)-DHPG (20-50 μM)) caused a reduction in spike frequency adaptation concomitant with postsynaptic depolarization and an increase in input resistance. In contrast, 2S,1'R,2'R,3'R-2-(2',3'-dicarboxycyclopropyl)glycine (DCG-IV; 100 μM) and (S)-2-amino-4-phosphonobutanoic acid (L-AP4; 100-500 μM), which selectively activate group II mGluRs and group III mGluRs, respectively, had no effect on the passive membrane properties or spike frequency adaptation of CA1 pyramidal neurones.

5 The mGluR antagonists (+)-α-methyl-4-carboxyphenylglycine ((+)-MCPG; 1000 μM) and (S)-4-carboxyphenylglycine ((S)-4CPG; 1000 μM), which block groups I and II mGluRs and group I mGluRs, respectively, had no effect on membrane potential, input resistance or spike frequency adaptation *per se*. Both of these antagonists inhibited the postsynaptic effects of (1S,3R)-ACPD (50-100 μM), (1S,3S)-ACPD (30-100 μM) and L-CCG1 (50-100 μM). (+)-MCPG also reversed the effects of (SR)-DHPG (75 μM). (The effect of (S)-4CPG was not tested.) Their action was selective in that both antagonists did not reverse the reduction in spike frequency adaptation induced by carbachol (1 μM) or noradrenaline (10 μM) whereas atropine (10 μM) and propranolol (100 μM) did.

6 From these data it is concluded that the mGluRs in CA1 pyramidal neurones responsible for these excitatory effects are similar to the mGluRs expressed by non-neuronal cells transfected with cDNA encoding group I mGluRs.

Keywords: ACPD, DCG-IV, depolarization, hippocampus, L-CCG1, L-AP4, (+)-MCPG, mGluR, spike frequency adaptation, (S)-4CPG, (S)-DHPG.

Introduction

Stimulation of postsynaptic metabotropic glutamate receptors (mGluRs) activates non-specific cationic conductances (Crépel *et al.*, 1994; Zheng *et al.*, 1994) and inhibits a number of different potassium conductances including the slow calcium-dependent afterhyperpolarizing current (I_{AHP}), the voltage-dependent M current and a leak current (Charpak *et al.*, 1990; Guérineau *et al.*, 1994). In hippocampal pyramidal neurones the consequences of these effects is postsynaptic depolarization, an overall increase in input resistance (Stratton *et al.*, 1989; Charpak *et al.*, 1990; Desai & Conn, 1991) and a loss of spike frequency adaptation (alternatively referred to as 'accommodation'). Physiologically, these effects may be important for improving the integrity of synaptic transmission through neuronal circuits and promoting the induction of synaptic plasticity, e.g., long-term potentiation (LTP) (McGuiness *et al.*, 1991). However, it has not yet been established which of the eight mGluR subtypes, so-far identified, mediates these postsynaptic effects in CA1 pyramidal neurones.

Combined pharmacological and biochemical studies have enabled classification of mGluRs into three groups. Group I comprises mGluRs 1 and 5 which promote inositol-1,4,5-trisphosphate (IP_3) production. The remaining mGluR subtypes inhibit adenosine 3':5'cyclic-monophosphate (cyclic AMP) formation in expression systems and are subdivided on the basis of their sensitivity to (S)-2-amino-4-phosphonobutanoate (L-AP4) (Nakanishi, 1992; 1994; Pin & Duvoisin, 1995). Thus, group III mGluRs are activated by L-AP4 whereas group II mGluRs are not.

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The subtype specificity of a number of mGluR agonists and antagonists has now been determined by screening these compounds in biochemical assays on brain slices and non-neuronal cell lines transfected with cDNA for individual mGluR subtypes. In these latter experiments mGluRs 1, 2 and 4 have been used as representatives of groups I, II and III mGluRs, respectively. 2S,1'S,2'S-2-(2'-carboxycyclopropyl)-glycine (L-CCG1) is an agonist for group II mGluRs at concentrations ≤ 1 μM but at higher concentrations also activates groups I and III mGluRs (Hayashi *et al.*, 1992). The (1S,3R)- and (1S,3S)-isomers of 1-aminocyclopentane-1,3-dicarboxylic acid (ACPD) activate both groups I and II mGluRs but have little or no activity at group III mGluRs (Hayashi *et al.*, 1992; Nakanishi, 1992; Watkins & Collingridge, 1994). In contrast, (S)-3,5-dihydroxyphenylglycine ((S)-DHPG), (2S,1'R,2'R,-3'R)-2-(2',3'-dicarboxycyclopropyl)glycine (DCG-IV) and L-AP4 are selective agonists for group I, group II and group III mGluRs, respectively (Ito *et al.*, 1992; Nakanishi, 1992; Hayashi *et al.*, 1993). The mGluR antagonists (+)-α-methyl-4-carboxyphenylglycine ((+)-MCPG) and (S)-4-carboxyphenylglycine ((S)-4CPG) (Eaton *et al.*, 1993; Jane *et al.*, 1993) block group I mGluRs but (+)-MCPG is an antagonist and (S)-4CPG is an agonist at group II mGluRs (Cavanni *et al.*, 1994; Hayashi *et al.*, 1994; Thomsen *et al.*, 1994).

We have now tested systematically these agonists and antagonists in order to determine the pharmacology of the postsynaptic mGluRs that mediate depolarization, increase in input resistance and reduction in spike frequency adaptation in CA1 pyramidal neurones.

A preliminary report of these findings has been published in abstract form (Davies & Collingridge, 1994).

Methods

Experiments were performed on hippocampal slices obtained from female Wistar rats (4–6 weeks old) as described previously (Davies *et al.*, 1990). In brief, animals were anaesthetized with halothane (3.5%) and subsequently decapitated. Coronal slices (400 μm thick) containing hippocampus were cut with a Campden vibroslicer. The hippocampal region was dissected from these slices and area CA3 removed. The resultant CA3-ectomized hippocampal slices were stored at room temperature for at least 1 h before being transferred to an interface recording chamber maintained at 30–32°C. Slices rested on a nylon mesh at the interface of a warmed perfusing artificial cerebrospinal fluid containing (mM): NaCl 124, KCl 3, $NaHCO_3$ 26, $CaCl_2$ 2, $MgSO_4$ 1, D-glucose 10, NaH_2PO_4 1.25, bubbled with a 95% O_2/5% CO_2 mixture. Intracellular recordings were obtained from neurones in *stratum pyramidale* using glass microelectrodes (40–90 MΩ) filled with potassium methylsulphate (2 M) connected to an Axoclamp-2 amplifier used in switch-clamp mode (Axon Instruments, Foster City, CA, U.S.A.). To study spike frequency adaptation and input resistance pyramidal cells were activated directly by passing current pulses (amplitude ±0.1–0.5 nA, duration 500–700 ms) through the intracellular recording electrode every 30–60 s to depolarize or hyperpolarize the neurone, respectively. The effects of mGluR agonists on input resistance and spike frequency adaptation were studied at a fixed membrane potential by injecting d.c. current to compensate for any agonist-induced depolarization. Changes in spike frequency adaptation were quantified in terms of the number of action potentials fired during a depolarizing step in the presence of the mGluR agonist as a percentage of that in control medium. As such the increase in spike firing reflects a combination of the increased depolarization induced by the current step (due to the increase in the cell input resistance) and the reduction in spike frequency adaptation *per se*. Drugs were administered by addition to the superfusing medium and were applied for a sufficient period to allow their full equilibration. Experiments were performed either in control medium or in medium containing 20 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 50 μM D-2-amino-5-phosphonopentanoate (D-AP5) to block any possible activation of ionotropic excitatory amino acid receptors by the mGluR agonists or antagonists (Jane *et al.*, 1993). The results from both sets of experiments were similar and for this reason have been pooled. Since DCG-IV is a potent agonist at NMDA receptors experiments with this agonist were invariably performed in the combined presence of 100 μM ketamine and 100 μM D-AP5 to produce a multiplicative block of the NMDA receptor system.

Drugs

Atropine, (±)-baclofen, ketamine and propranolol were obtained from Sigma. DCG-IV was a gift from Drs. Shinozaki and Ohfune (Tokyo). L-AP4, CNQX, D-AP5, L-CCG1, (1S,3R)-ACPD, (1S,3S)-ACPD, (SR)-DHPG and (S)-DHPG were all gifts from Professor J.C. Watkins (Bristol). (+)-MCPG, (−)-MCPG and (S)-4CPG were synthesized and resolved according to previously published methods (Birse *et al.*, 1993). Each drug was dissolved in distilled water or equimolar NaOH at 100–1000 times its final bath applied concentration.

Statistical analysis

Results were analysed with either Student's *t* tests or analysis of variance (ANOVA) with $P<0.05$ being taken as indicating statistical significance. *n* signifies the number of times a result was obtained, which is the same as the number of slices tested (each slice was obtained from a separate rat).

Results

The present data were collected from 66 neurones with resting membrane potentials more negative than −55 mV, action potential amplitudes of >85 mV and resting input resistances in the range 30–50 MΩ.

Effects of mGluR agonists

In the first series of experiments the potency of the broad spectrum mGluR agonists (1S,3R)-ACPD, (1S,3S)-ACPD and L-CCG1 was assessed. In 11 neurones (1S,3R)-ACPD, at a concentration of 50 μM, caused a depolarization of 5±2 mV, an increase in input resistance of 19±4% and a reduction in spike frequency adaptation (quantified in terms of the increase in action potential firing during a 500 ms depolarizing step) of 243±52%. With 100 μM (1S,3R)-ACPD all these effects were more pronounced ($n=10$); i.e. (1S,3R)-ACPD caused a depolarization of 10±3 mV, an increase in input resistance of 29±7% and an increase in depolarization-induced action potential firing of 323± 63%. There was considerable intercell variability in the effects of a fixed concentration of (1S,3R)-ACPD; for example, in three of the eleven neurones tested, 50 μM (1S,3R)-ACPD caused little or no depolarization whereas in others it caused a depolarization up to 12 mV, a value similar to the mean depolarization induced by 100 μM. However, for each agonist application, a maximum effect was obtained after a 5–10 min perfusion and persisted at a constant level for the time (up to 1 h) which the agonist was present. To assess whether each neurone was sensitive to each agonist we tested individual agonists in turn on the same neurone. In these experiments all three agonists, when applied at 100 μM, increased the excitability of the neurone under investigation (Figure 1; $n=10$).

We tested next the effectiveness of group selective mGluR agonists. The racemic mixture ((SR)-; 50–100 μM) and the resolved (S)-isomer (20–50 μM) of the group I-selective agonist, DHPG, caused a depolarization of 4–15 mV, an increase in input resistance of 20–35% and an increase in depolarization-induced action potential firing of 220–433% ($n=15$; Figure 2a). The apparently higher potency of (S)-DHPG over (1S,3R)-ACPD was borne out in experiments in which both agonists were applied to the same neurone. Thus, in three

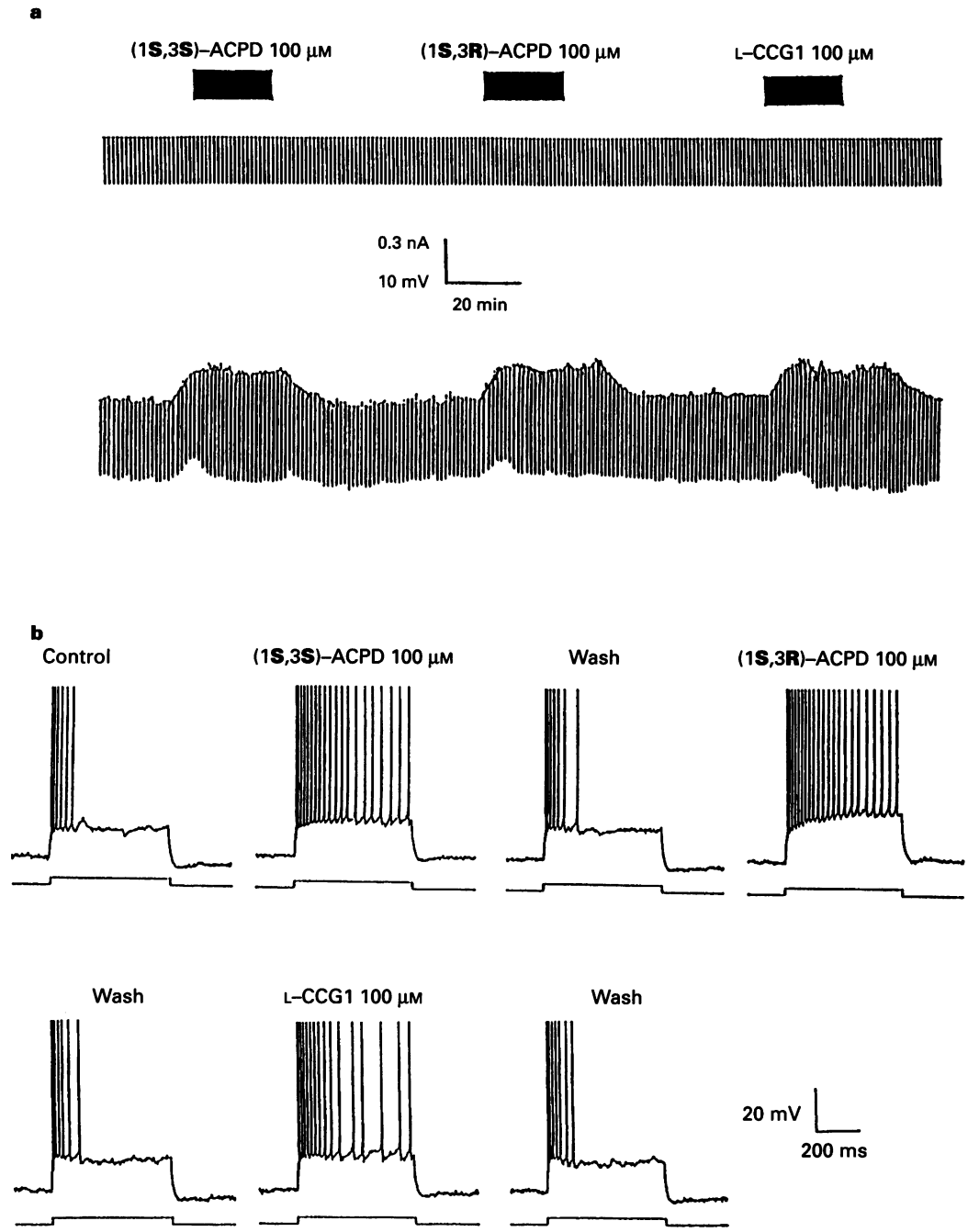

Figure 1 The effect of broad spectrum mGluR agonists on the excitability of CA1 pyramidal neurones: (a) shows a chart record illustrating the effect of sequential applications of (1S,3S)-ACPD, (1S,3R)-ACPD and L-CCG1 on the membrane potential and input resistance of a CA1 pyramidal neurone. In the bottom trace the large deflections are voltage responses to constant current pulses (−0.4 nA, 500 ms; top trace) and reflect cell input resistance. The initial membrane potential of the cell was −66 mV. The period of drug application is indicated by the horizontal solid bars. The mean depolarizations and increases in input resistance were 9±2 mV and 25±5% for (1S,3R)-ACPD, 7±1 mV and 23±2% for (1S,3S)-ACPD, and 7±2 mV and 21±4% for L-CCG1, respectively ($n=10$). (The initial depolarization of the neurone in (a) without a noticeable increase in cell input resistance was a consistent finding in this study (see also Figure 3) and may reflect the dual action of mGluR agonists in inhibiting potassium conductances and activating non-specific cationic conductances). (b) Illustrates responses recorded in a separate neurone which had an initial membrane potential of −67 mV. The upper traces show, from left to right, the response evoked by a depolarizing current pulse (+0.5 nA) in control medium, after 20 min in the presence of (1S,3S)-ACPD, following a 30 min washout and after 20 min in the presence of (1S,3R)-ACPD. Bottom traces continue from the top and illustrate the responses evoked following washout of (1S, 3R)-ACPD, after 20 min in the presence of L-CCG1 and following a 30 min washout. Each of the three agonists reduced the level of spike frequency adaptation in a reversible manner. The mean increases in depolarization-induced action potential firing were 309±42% for (1S,3R)-ACPD, 289±47% for (1S,3S)-ACPD, and 204±52% for L-CCG1, respectively ($n=10$). Unless otherwise stated, in this and all subsequent figures illustrating spike frequency adaptation, action potentials are truncated and traces are single responses which accurately reflect the average response recorded when steady state drug-mediated effects had been obtained. d.c. current injection was used to compensate for membrane depolarization induced by the agonists such that all responses were evoked at the same membrane potential.

neurones the depolarizations induced by 30 μM (S)-DHPG and 100 μM (1S,3R)-ACPD were 9±2 mV and 8±2 mV, respectively (not illustrated). In contrast, the respective group II and group III selective agonists DCG-IV (100 μM; $n=3$) and L-AP4 (500–1000 μM; $n=5$, $P>0.05$) invariably had no postsynaptic effects (Figures 2b and c).

One problem with experiments in brain slices when applying agonists which increase neuronal excitability is that the effects monitored in the neurone under study may reflect indirect actions through activation of complex neuronal circuits and the release of other neurotransmitters. To address this possiblity we repeated the applications of the effective mGluR agonists in the presence of 200 μM (±)-baclofen to block both excitatory and inhibitory synaptic transmission (Thompson *et al.*, 1992) and the release of other neurotransmitters which could have similar excitatory actions (e.g., catecholamines; Bowery, 1993). Under these conditions, (1S,3R)-ACPD (100 μM), (1S,3S)-ACPD (100 μM), L-CCG1 (100 μM) and (S)-DHPG (50 μM) caused a

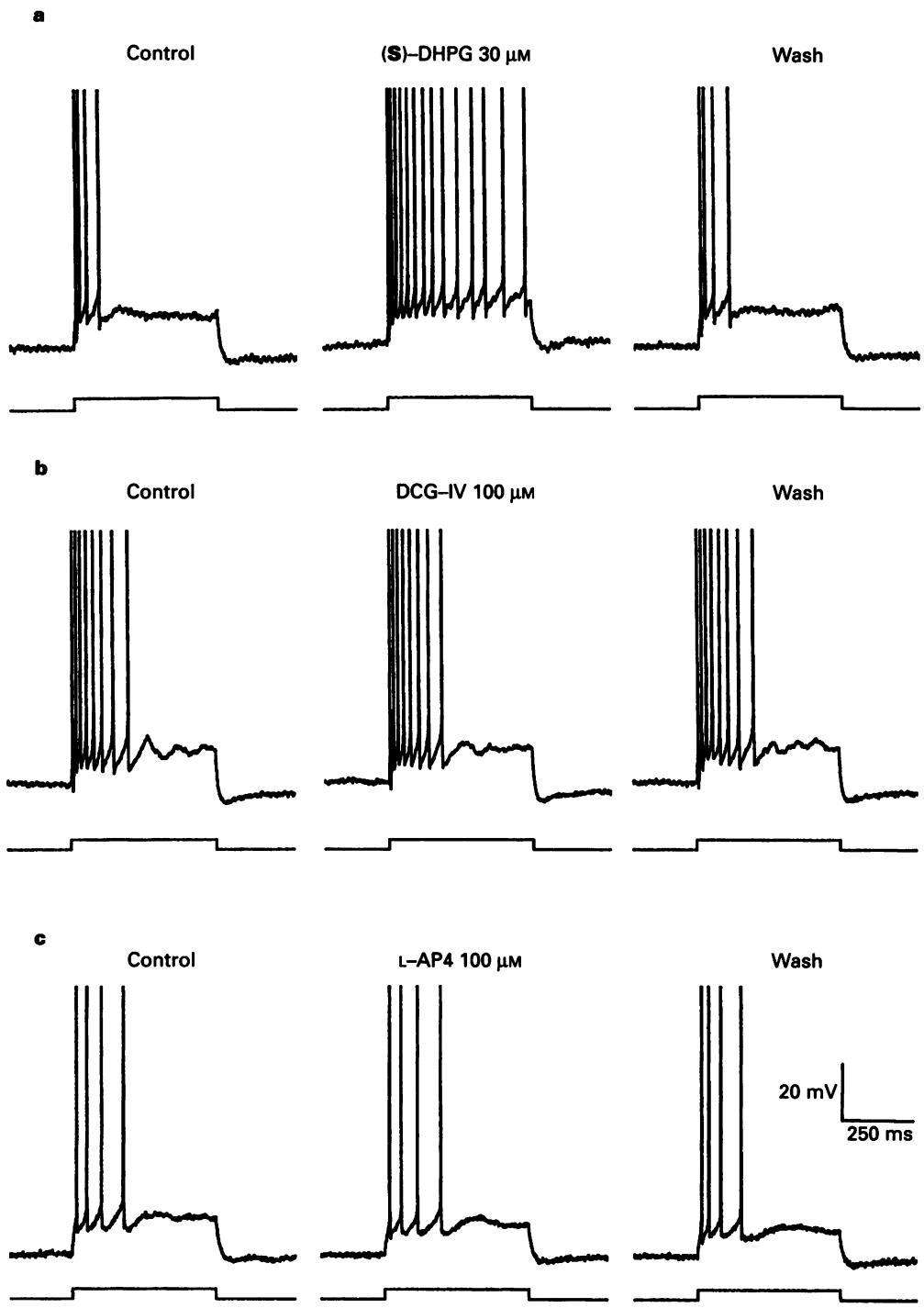

Figure 2 The effect of group-selective mGluR agonists on spike frequency adaptation: (a), (b) and (c) show the effects of (S)-DHPG, DCG-IV and L-AP4, respectively, on spike frequency adaptation. In each case, traces represent the voltage responses to depolarizing current pulses recorded immediately before drug application, in the presence of the mGluR agonist (i.e. after a 20 min exposure) and following a 30 min washout period after termination of the agonist application. In the three cells the depolarizing current pulses and initial membrane potentials were +0.3 nA and −64 mV (a), +0.2 nA and −63 mV (b), and +0.3 nA and −66 mV (c), respectively. Note that only the group I selective agonist, (S)-DHPG reduced spike frequency adaptation. In 8 neurones, 30 μM (S)-DHPG induced a mean depolarization, increase in input resistance and depolarization-induced action potential firing of 8±1 mV, 30±1% and 286±65%, respectively.

postsynaptic depolarization, an increase in input resistance and an increase in depolarization-induced action potential firing that was similar to that observed under control conditions ($n = 8$; Figure 3).

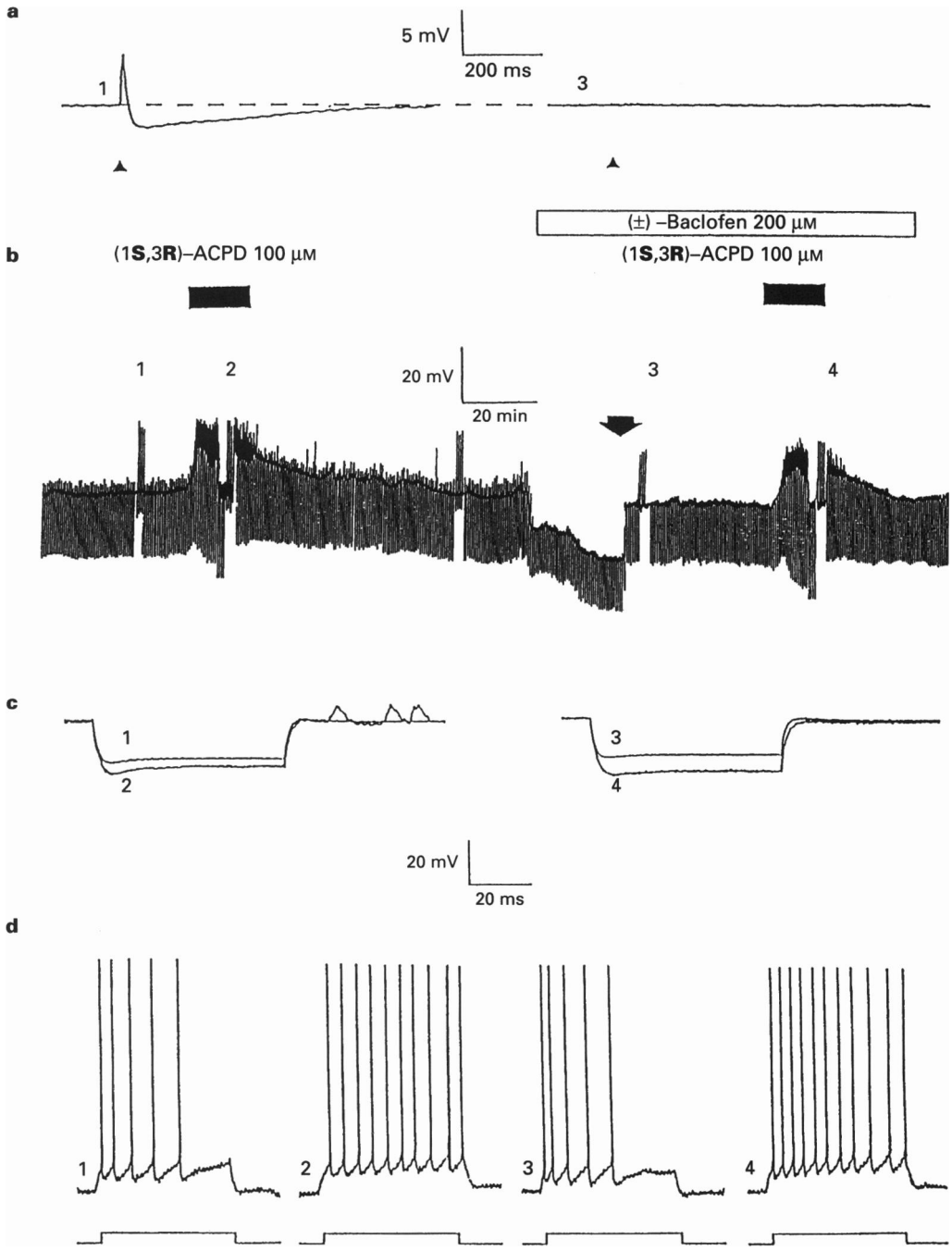

Figure 3 mGluR agonists activate postsynaptic mGluRs: (a) shows synaptic potentials in response to a single stimulus delivered with a bipolar stimulating electrode placed in *stratum radiatum* of area CA1 in (1) control medium and (3) in the presence of 200 μM (±)-baclofen. Traces are averages of four consecutive responses and the point of stimulation is marked by a (▲). The chart record illustrated in (b) portrays the membrane potential of the neurone with the downward deflections reflecting the voltage responses to current pulses (−0.4 nA, duration 500 ms) which were used to measure the input resistance of the cell. The small upward deflections during the baseline and washout from the first application of (1S,3R)-ACPD are a representation of the evoked excitatory postsynaptic potentias (e.p.s.p.s) (see (a)). In the presence of (1S,3R)-ACPD the larger upward deflections represent the firing of action potentials. The absolute amplitude of these events is truncated due to the slow response time of the chart recorder. The arrow marks the point where d.c. current injection was applied to restore the membrane potential of the cell to that prior to (±)-baclofen application. (c) Shows, on an expanded time scale, examples of the input resistance of the cell at the times indicated by the numbers 1-4. Note that (1S,3R)-ACPD induces spontaneous e.p.s.p.s in control medium (c2) but not in the presence of (±)-baclofen (c4). Each trace is an average of four consecutive responses. (d) Shows examples of responses to depolarizing current steps (+0.15 nA) recorded at the times indicated by 1–4. The initial membrane potential of the cell was −62 mV. Note that in (a-d) although (±)-baclofen abolished all synaptic transmission it did not inhibit the effects of (1S,3R)-ACPD. Thus, in three neurones the mean depolarizations, increases in input resistance and depolarizatio-induced action potential firing induced by (1S,3R)-ACPD in the absence and presence of (±)-baclofen (200 μM) were 11 ± 3 mV and 10 ± 2 mV, 33 ± 4% and 32 ± 6%, and 367 ± 63% and 345 ± 73%, respectively.

Effects of mGluR antagonists

We next determined the effectiveness of (+)-MCPG and (S)-4CPG in reversing the postsynaptic effects induced by mGluR agonists. In untreated slices neither antagonist had any effect on membrane potential, input resistance or spike frequency adaptation, ($n=3$ for each antagonist; see Figure 6). (+)-MCPG (1000 μM) reversed substantially the depolarization,

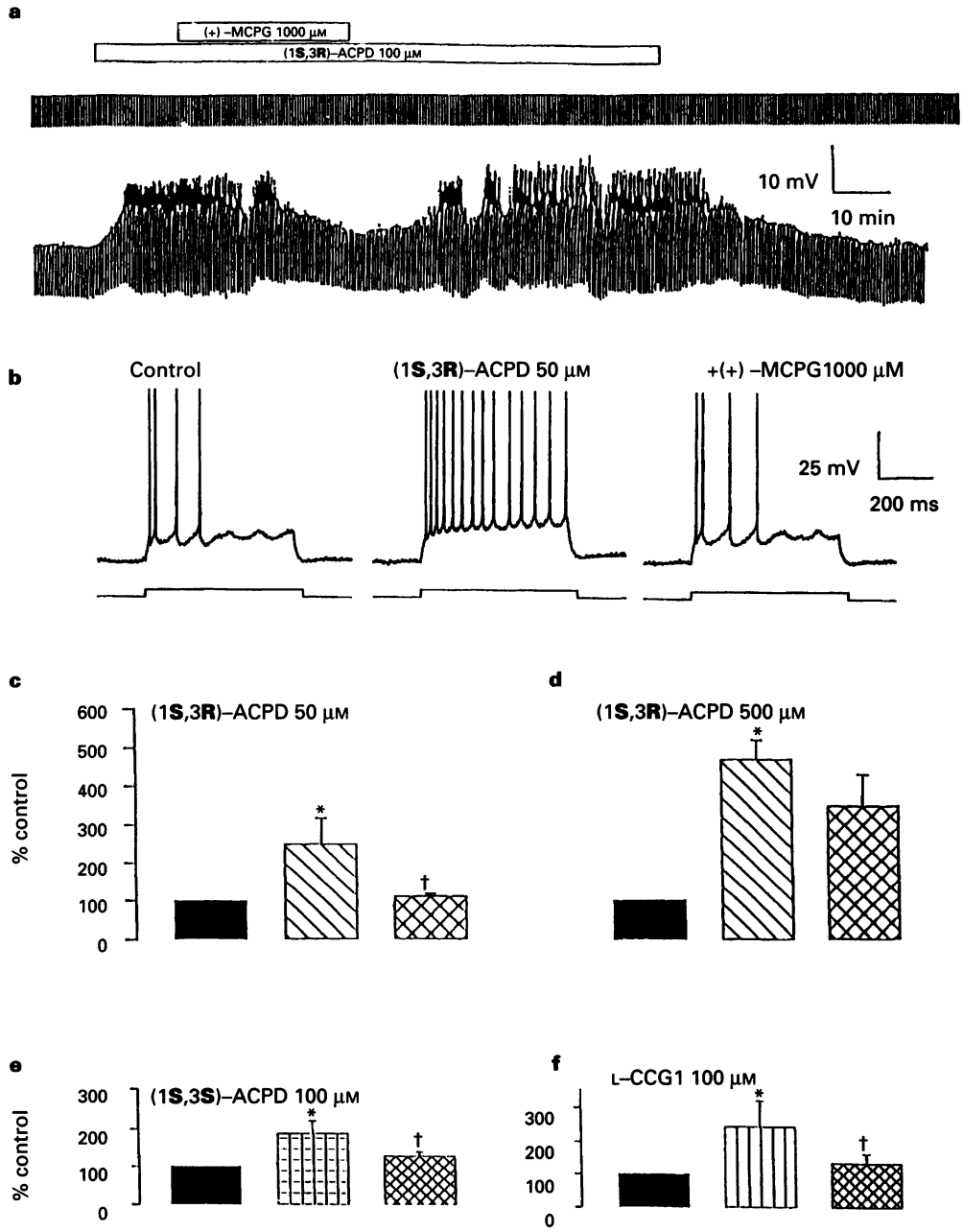

Figure 4 (+)-MCPG reverses the depolarization, increase in input resistance and reduction in spike frequency adaptation induced by mGluR agonists: (a) is a chart recorder trace showing the membrane potential and hyperpolarizing voltage responses of the cell (lower trace) to 500 ms long current pulses (downward deflections in upper trace). The dark upward voltage deflections in (a) represent the firing of action potentials. The absolute magnitude of these events has been truncated due to the slow response time of the chart recorder. (1S,3R)-ACPD induced a depolarization and an increase in input resistance that was reversed by (+)-MCPG. The initial membrane potential of the neurone and current step amplitude for this neurone were -63 mV and -0.25 nA. In four neurones the depolarizations and increases in input resistance induced by 50 μM (1S,3R)-ACPD in the absence and presence of (+)-MCPG (1000 μM) were 6 ± 2 mV and 1 ± 1 mV, and $20\pm6\%$ and $4\pm3\%$, respectively. (b) In a separate neurone (+)-MCPG abolished the reduction in spike frequency adaptation induced by 50 μM (1S,3R)-ACPD. The initial membrane potential and depolarizing current step for this neurone were -62 mV and $+0.3$ nA. (c) Shows histograms in which pooled data for the number of action potentials fired during the depolarizing step in the presence of (1S,3R)-ACPD (hatched column) and in the presence of (+)-MCPG plus (1S,3R)-ACPD (cross-hatched column) is plotted as a percentage of control (solid column). Percentages are expressed as a mean $\pm$ s.e.mean ($n=5$). (d-f), as for (c), except that in (d) 500 μM (1S,3R)-ACPD ($n=5$), in (e) 100 μM (1S,3S)-ACPD ($n=4$; checked column) and in (f) 100 μM L-CCG1 ($n=4$; vertical lined column) were used. The effect of (+)-MCPG (1000 μM) in each case is indicated by the cross-hatched columns in the respective histograms. (For L-CCG1 the depolarizing step was 700 ms long.) For (e) and (f) the magnitude of the depolarizations induced by (1S,3S)-ACPD and L-CCG1 in the absence and presence of (+)-MCPG were 9 ± 3 mV and 2 ± 1 mV, and 7 ± 2 mV and 2 ± 1 mV, respectively. * and † represent statistical significance at the $P<0.05$ level for mGluR agonist treated *versus* control, and for mGluR agonist plus (+)-MCPG-treated *versus* mGluR agonist-treated neurones, respectively.

increase in input resistance and increase in depolarization-induced action potential firing induced by 50–100 μM (1S,3R)-ACPD ($n=7$; Figure 4a-c). These effects were stereoselective as (-)-α-methyl-4-carboxyphenylglycine had no effect ($n=2$; not illustrated). (+)-MCPG (1000 μM) was less effective at reversing the effects of 500 μM (1S,3R)-ACPD ($n=5$; Figure 4d); an observation consistent with the competitive nature of its block (Eaton *et al.*, 1993). (+)-MCPG (1000 μM) also reversed the effects of (1S,3S)-ACPD (30–100 μM, $n=6$; Figure 4e), L-CCG1 (50–100 μM, $n=6$; Figure 4f) and (SR)-DHPG (75 μM). The depolarizations and increases in input resistance and depolarization-induced action potential firing induced by (SR)-DHPG in the absence and presence of (+)-MCPG were 11±3 mV and 3±2 mV, 29±8% and 13±6%, and 306±71% and 144±27%, respectively ($n=3$). (S)-4CPG (1000 μM) was also effective in reversing the excitatory effects induced by (1S,3R)-ACPD (50–100 μM; $n=3$), (1S,3S)-ACPD (50–100 μM; $n=3$) and L-CCG1 (100 μM; $n=2$) (Figure 5).

Selectivity of mGluR antagonist action

To ensure that (+)-MCPG and (S)-4CPG were acting at the level of the receptor itself, rather than down-stream of receptor activation, we tested these antagonists against reduction of spike frequency adaptation induced by activation of muscarinic acetylcholine receptors and noradrenoceptors (Cole &

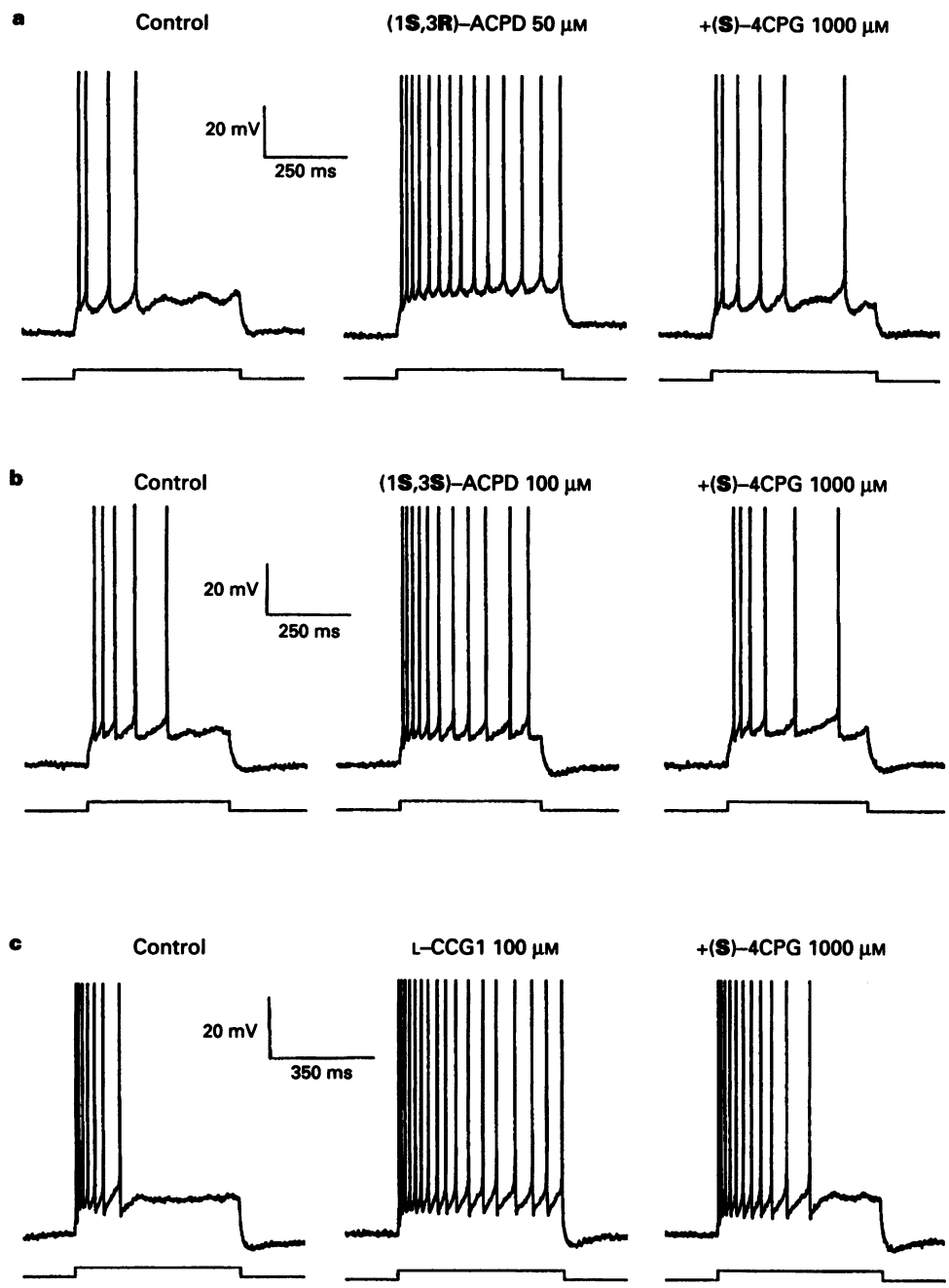

Figure 5 (S)-4CPG antagonizes the reduction in spike frequency adaptation induced by broad spectrum mGluR agonists. In (a) traces show from left to right the response to a +0.25 nA depolarizing step in control medium, in the presence of (1S,3R)-ACPD and in the combined presence of (1S,3R)-ACPD and (S)-4CPG. In this neurone the initial membrane potential was −63 mV. (b) and (c) are similar to (a) but (1S,3S)-ACPD and L-CCG1 were used, respectively. The magnitude of the current pulses and initial membrane potentials for these two neurones were +0.3 nA and −65 mV (b), and +0.3 nA and −58 mV (c). Note that (S)-4CPG reversed the reduction in spike frequency adaptation independently of the mGluR agonist used to induce these effects.

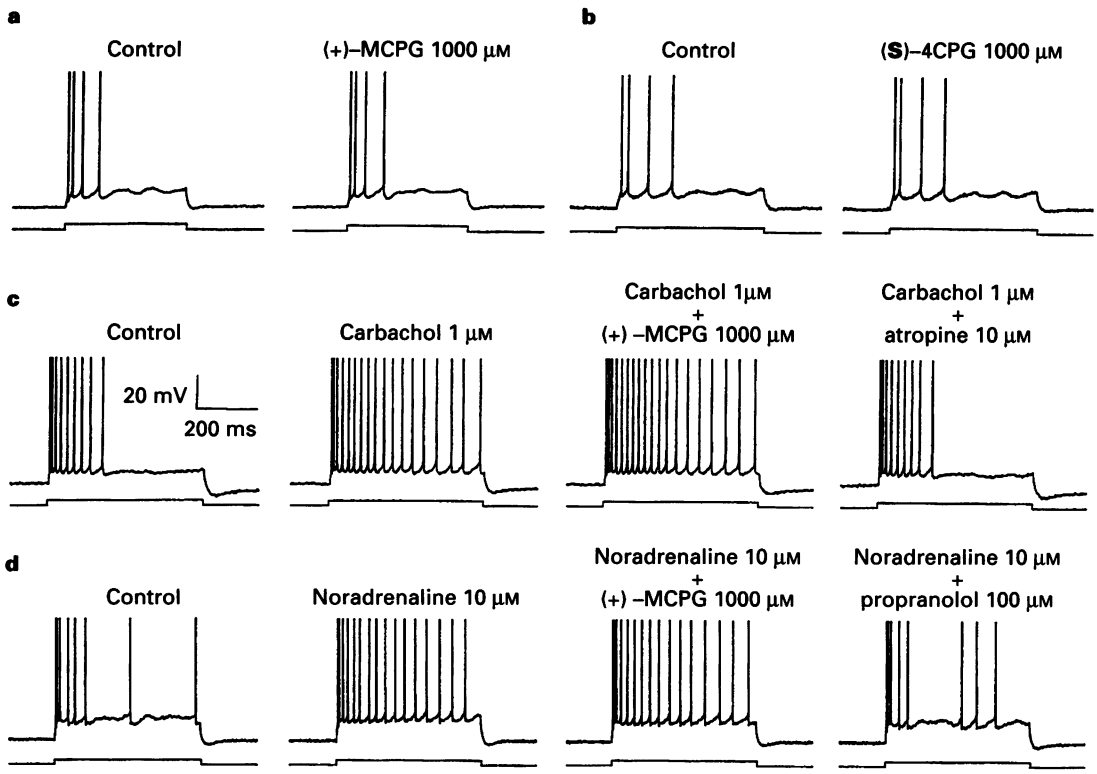

Figure 6 Selectivity of the antagonist actions of (+)-MCPG: (a) and (b) illustrate that in separate neurones neither (+)-MCPG nor (S)-4CPG affect spike frequency adaptation. In (c) traces show from left to right the response to a +0.3 nA depolarizing step in control medium immediately before carbachol application, in the presence of carbachol, in the combined presence of carbachol and (+)-MCPG and in the combined presence of carbachol and atropine. Responses recorded in the presence of drugs were obtained 20 min after the start of perfusion of these compounds. In three neurones the increases in depolarization-induced action potential firing were 261 ± 43% in carbachol alone, 273 ± 67% in carbachol plus (+)-MCPG, and 104 ± 4% in carbachol plus atropine. (d) is similar to (c) but noradrenaline and propranolol were used in place of carbachol and atropine, respectively. In these experiments the increases in depolarization-induced action potential firing were 261 ± 38% in noradrenaline alone, 266 ± 36% in noradrenaline plus (+)-MCPG, and 113 ± 5% in noradrenaline plus propranolol ($n=4$). The initial membrane potentials of the neurones were −62 mV (a), −63 mV (b and c) and −64 mV (d).

Nicoll, 1983; Madison & Nicoll, 1982). Neither (+)-MCPG (1000 μM) nor (S)-4CPG (1000 μM) affected the increase in depolarization-induced action potential firing produced by either carbachol (1 μM; $n=3$ and $n=2$, respectively) or noradrenaline (10 μM; $n=4$ and $n=2$, respectively; Figure 6). In contrast, the effects of these agonists were reversed by atropine (10 μM; $n=3$) and propranolol (100 μM; $n=4$), respectively.

Discussion

Agonist pharmacology

The present study confirms that (1S,3R)-ACPD and (1S,3S)-ACPD reduce spike frequency adaptation and cause postsynaptic depolarization with an associated increase in input resistance in CA1 pyramidal neurones (Desai *et al.*, 1992). These effects appear to be due to a direct activation of mGluRs, in CA1 pyramidal neurones, because they persist in the presence of (±)-baclofen which inhibits the release of most neurotransmitters and hyperpolarizes neurones to membrane potentials far beyond the threshold for firing action potentials. We have demonstrated also that L-CCG1 and (S)-DHPG mediate similar effects to ACPD. In contrast, L-AP4 and DCG-IV *per se* are ineffective at postsynaptic mGluRs in these neurones. That L-AP4 has no effect on naive preparations is consistent with a report by Harris *et al.* (1987) who demonstrated that L-AP4 depolarized CA1 pyramidal neurones only after the slice had previously been exposed to quisqualate, i.e. following so-called 'priming'. Although quisqualate was not used in the present study it has been reported that ACPD is also able to 'prime' certain effects of L-AP4 (Whittemore & Cotman, 1991); however, this phenomenon was not observed in the present study.

The agonist pharmacology of the mGluR(s) responsible for the excitatory effects in CA1 pyramidal neurones that were analysed in the present study is similar to that of the mGluRs that mediate depolarization of spinal cord motoneurones (Eaton *et al.*, 1993; Ishida *et al.*, 1993). In both sets of neurones the effectiveness of the agonists tested correlates well with their capacity to generate the production of IP_3 in brain slices (Ito *et al.*, 1992; Cartmell *et al.*, 1993; Schoepp, 1993; Schoepp *et al.*, 1994) and to activate mGluR1, rather than mGluR2 or 4, transfected cells (Nakagawa *et al.*, 1990; Ito *et al.*, 1992; Nakanishi, 1992; Kristensen *et al.*, 1993; Tanabe *et al.*, 1993). In keeping with this, the initial studies characterizing the effects of the postsynaptic mGluR on CA1 pyramidal neurones were performed with quisqualate (Stratton *et al.*, 1989; Charpak *et al.*, 1990) which strongly promotes the production of IP_3 in brain slices (Palmer *et al.*, 1989; Abe *et al.*, 1992; Schoepp, 1993) and mGluR1 transfected cells (Aramori & Nakanishi, 1992) but only weakly inhibits cyclic AMP formation in brain slices (Genazzani *et al.*, 1993) and mGluR2 or mGluR4 transfected cells (Nakanishi, 1992; Tanabe *et al.*, 1992).

Antagonist pharmacology

As regards antagonist pharmacology, we have demonstrated that 1000 μM (+)-MCPG reverses the effects of 50–100 μM (1S,3R)-ACPD. These data are consistent with our initial observations that 500–1000 μM of the racemic mixture of MCPG ((±)-MCPG) inhibits the reduction in spike frequency adaptation induced by 30–50 μM (1S,3R)-ACPD (Bashir *et al.*, 1993). A recent report by Manzoni *et al.* (1994) confirms

these findings; however, Chinestra *et al.*, (1993) suggest that (±)-MCPG is not an antagonist at mGluRs in the hippocampal CA1 region. Possible factors that might explain this discrepancy are (i) the weak spike frequency adaptation observed by the latter authors under control conditions and (ii) their inability to demonstrate that (±)-MCPG was active in any of their test systems. We have now extended our study to show that (+)-MCPG reverses the depolarization, increase in input resistance and reduction in spike frequency adaptation induced by mGluR agonists, independently of the mGluR agonist used to induce these effects, and that, as in the spinal cord and the thalamus (Jane *et al.*, 1993), it is the (+)-isomer of MCPG that is the active enantiomer. However, (+)-MCPG is a weak competitive antagonist (Birse *et al.*, 1993; Watkins & Collingridge, 1994). It is therefore important to consider carefully the agonist and antagonist concentrations when examining its effects.

A further advance towards characterizing the mGluR subtype in CA1 pyramidal neurones is the observation that (S)-4CPG is also an antagonist of the mGluR that mediates changes in the passive membrane properties and spike frequency adaptation in these neurones. Like (+)-MCPG this compound is also a relatively weak antagonist of mGluRs (Watkins & Collingridge, 1994) and therefore was used at 1000 μM.

This profile of antagonism of mGluRs in CA1 pyramidal neurones is similar to that of mGluRs which depolarize spinal cord motoneurones (Birse *et al.*, 1993; Eaton *et al.*, 1993) and CA3 pyramidal neurones (Gerber *et al.*, 1993; Guérineau *et al.*, 1994). In the nucleus tractus solitarius (NTS), however, neither 250 μM (±)-MCPG nor 500 μM (**SR**)-4CPG reversed significantly an inward current induced by 25 μM (**1S,3R**)-ACPD (Glaum *et al.*, 1993). This does not, however, necessarily indicate that different classes of mGluR mediate these similar excitatory effects in different regions of the CNS and could be explained by the low potency of (±)-MCPG and (**SR**)-4CPG at inhibiting these excitatory effects of ACPD. The pharmacology of postsynaptic mGluRs on hippocampal CA1 pyramidal neurones does differ, however, from presynaptic mGluRs on glutamate terminals in the CA1 region and spinal cord (Baskys & Malenka, 1991; Pook *et al.*, 1993; Vignes *et al.*, 1995).

The antagonist pharmacology strongly implicates group I mGluRs in the mediation of the postsynaptic excitation of hippocampal CA1 pyramidal neurones since both (+)-MCPG and (S)-4CPG are antagonists at mGluR1 while (+)-MCPG is an antagonist at mGluR2 and (S)-4CPG is an agonist at these receptors. Both antagonists are inactive at mGluR4 (Hayashi *et al.*, 1994; Thomsen *et al.*, 1994). Consistent with this categorization, (+)-MCPG and (S)-4CPG are antagonists of (**1S,3R**)-ACPD-stimulated IP_3 production in brain slices (Watkins & Collingridge, 1994).

However, a comparison of these results with those generated previously with L-2-amino-3-phosphonopropionate (L-AP3) is complicated by the disparities that exist as to the capacity of this compound to antagonise either IP_3 production, or electrophysiological effects, in brain slices and cells transfected with group I mGluRs (Aramori & Nakanishi, 1991; Abe *et al.*, 1992; Desai *et al.*, 1992; Hu & Storm, 1992; Schoepp, 1993; Saugstad *et al.*, 1995).

Which subtype of mGluR excites CA1 pyramidal neurones?

Although the profiles of agonist and antagonist pharmacology indicate group I mGluRs as the mediators of postsynatpic depolarization, increase in input resistance and inhibition of spike frequency adaptation in CA1 pyramidal neurones, the identity of the actual mGluR(s) is not defined. *In situ* hybridisation and immunohistochemical studies have demonstrated expression of both mGluR5 and mGluR1 (Abe *et al.*, 1992; Shigemoto *et al.*, 1993; Fotuhi *et al.*, 1994) in CA1 neurones. In mice in which the mGluR1 gene has been functionally deleted (1S,3R)-ACPD produces postsynaptic excitatory effects indistinguishable from controls (Aiba *et al.*, 1994; Conquet *et al.*, 1994). This shows that receptors other than mGluR1 can mediate these effects (but does not necessarily preclude a role of mGluR1 in normal animals). The simplest interpretation of these data, however, is that mGluR5 mediates these excitatory effects in CA1 pyramidal neurones.

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