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Reexamination of the Effects of MCPG on Hippocampal LTP, LTD, and Depotentiation

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SUMMARY AND CONCLUSIONS

1. We examined the effects of the metabotropic glutamate receptor (mGluR) antagonist α-methyl-4-carboxyphenylglycine (MCPG) on the induction of long-term potentiation (LTP), long-term depression (LTD), and depotentiation in CA1 hippocampal neurons using extracellular recording techniques.

2. MCPG (500 μM) strongly antagonized the presynaptic inhibitory action of the mGluR agonist 1-aminocyclopentane-(1S,3R)-dicarboxylic acid yet failed to block LTP induced with either tetanic stimulation (100 Hz, 1 s) or theta-burst stimulation.

3. To test the possibility that our failure to block LTP was due to prior activation of a "molecular switch" that in its "on" state obviates the need for mGluR activation to generate LTP, we gave repeated periods of prolonged low-frequency stimulation (LFS; 1 Hz, 10 min), a manipulation reported to turn the switch "off." Although this stimulation saturated LTD, subsequent application of MCPG still failed to block LTP.

4. MCPG did not block LFS-induced depotentiation in older slices (4–6 wk) or LFS-induced LTD in older, young (11–18 days), or neonatal (3–7 days) slices.

5. These results demonstrate that MCPG-sensitive mGluRs are not necessary for the induction of LTP, LTD, or depotentiation in hippocampal CA1 pyramidal cells. The possibility remains, however, that their activation may modify the threshold for the induction of these long-term plastic changes.

INTRODUCTION

The mechanisms responsible for generating long-term potentiation (LTP) and long-term depression (LTD) in the CA1 region of the hippocampus have received considerable attention because of their potential involvement in several important nervous system functions. It is generally accepted that the induction of LTP requires synaptic activation of postsynaptic *N*-methyl-D-aspartate receptors (NMDARs) during postsynaptic depolarization resulting in a rise in postsynaptic calcium concentration (intracellular calcium concentration, $[Ca^{2+}]_i$). Similarly, homosynaptic LTD appears to require a smaller NMDAR-mediated rise in $[Ca^{2+}]_i$ (Bear and Malenka 1994). Although there is compelling evidence indicating that a rise in $[Ca^{2+}]_i$ is a mandatory trigger for LTP, there is also evidence suggesting that activation of NMDARs and a large rise in $[Ca^{2+}]_i$ alone may not be sufficient for generating stable LTP (Kauer et al. 1988; Kullmann et al. 1992), implying that synaptic activity provides some additional essential component.

With the discovery of metabotropic glutamate receptors (mGluRs) and the subsequent development of specific agonists and antagonists, it became possible to examine whether mGluR activation was an additional factor required for generating NMDAR-dependent LTP and LTD. Application of the mGluR agonist 1-aminocyclopentane-(1S,3R)-dicarboxylic acid (ACPD) was reported to generate a slowly developing but long-lasting synaptic potentiation that did not require NMDAR activation but did exhibit mutual occlusion with tetanus-induced LTP (Bortolotto and Collingridge 1993). Although this result was suggestive, more direct and compelling evidence for a critical role of synaptic mGluRs in LTP was obtained when the specific mGluR antagonist (RS)-α-methyl-4-carboxyphenylglycine (MCPG) became available and was found to block tetanus-induced LTP as well as the ACPD-induced potentiation (Bashir et al. 1993a). Interestingly, MCPG blocked LTP in a qualitatively different manner than NMDAR antagonists, which block both LTP and short-term potentiation (STP) (Malenka 1991): instead, an LTP-inducing tetanus given in the presence of MCPG still elicited STP (lasting <1 h). These results suggested that mGluR activation was primarily necessary for the stabilization of LTP.

Because of the simplicity of the experiment demonstrating a block of LTP by MCPG, it appeared that a role for mGluRs in LTP had been firmly established in a manner reminiscent of the first demonstration of the block of LTP by an NMDAR antagonist (Collingridge et al. 1983). However, subsequent experiments by different groups either failed to find any effect of MCPG on tetanus-induced LTP (Chinestra et al. 1993; Manzoni et al. 1994), or showed an effect different from originally reported in that only the very late phases of LTP (2–4 h after induction) were affected (Brown et al. 1994; Richter-Levin et al. 1994; Riedel and Reymann 1993), or reported that MCPG application immediately after the tetanus blocked LTP in young (15 or 30 days) but not adult (60 days) animals (Izumi and Zorumski 1994). Examination of LTP in mice lacking mGluR1 did little to clarify the role of mGluRs in synaptic plasticity (Aiba et al. 1994; Conquet et al. 1994; Malenka 1994), and even the relationship between the slow-onset, ACPD-induced potentiation and LTP has been seriously questioned (Chinestra et al. 1994).

Taken together, these studies suggest that activation of mGluRs is not an absolute requirement for eliciting LTP but instead may play some sort of conditional or modulatory role. Consistent with this idea, data have been presented suggesting that mGluRs activate a "molecular switch" that then negates the need for further mGluR activation to induce LTP (Borto-

lotto et al. 1994). This hypothesis could explain all of the negative results using MCPG if it is assumed that, for unknown reasons, before LTP induction this switch was turned on. Because understanding the role of mGluRs in synaptic plasticity is of fundamental importance for elucidating the mechanisms of LTP, we have independently in our two laboratories reexamined the effects of MCPG on NMDAR-dependent LTP in area CA1 of the hippocampus. MGluR antagonists have also been reported to block homosynaptic LTD (Bashir et al. 1993b; Bolshakov and Siegelbaum 1994; Stanton et al. 1991), and we have also, therefore, independently examined the effects of MCPG on LTD and depotentiation. Following a recent example (Paulsen et al. 1993), and because of the similarity of our results, we have decided to publish our findings together. However, because of some differences in procedures, the methods and results from each laboratory are presented separately.

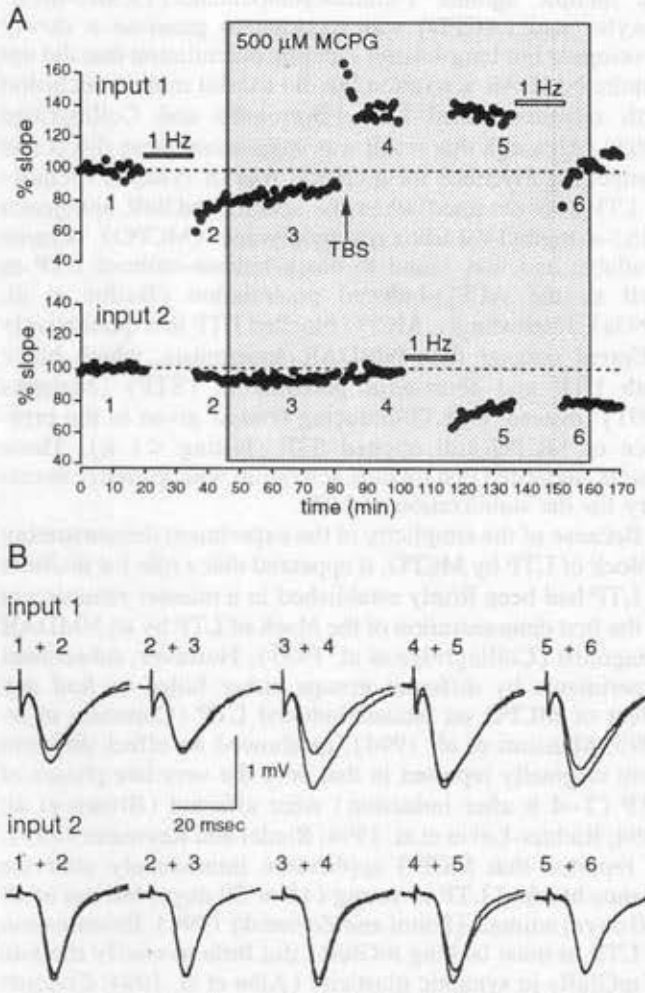

FIG. 1. Examples of synaptic plasticity in (RS)-α-methyl-4-carboxyphenylglycine (MCPG). *A*: record of a 2-pathway experiment. Before MCPG application, low-frequency stimulation (LFS) produced long-term depression (LTD) in input 1. Bath application of 500 μM MCPG did not significantly affect the baseline responses, as can be seen in input 2. After a 35-min application of MCPG, theta-burst stimulation (TBS) was delivered to input 1, which resulted in normal long-term potentiation (LTP). LFS delivered in the presence of MCPG produced LTD and depotentiation, shown in inputs 2 and 1, respectively. *B*: traces of extracellular field potentials obtained from the experiment shown in *A*. Each trace is the average of 4 consecutive sweeps at the times indicated in *A*.

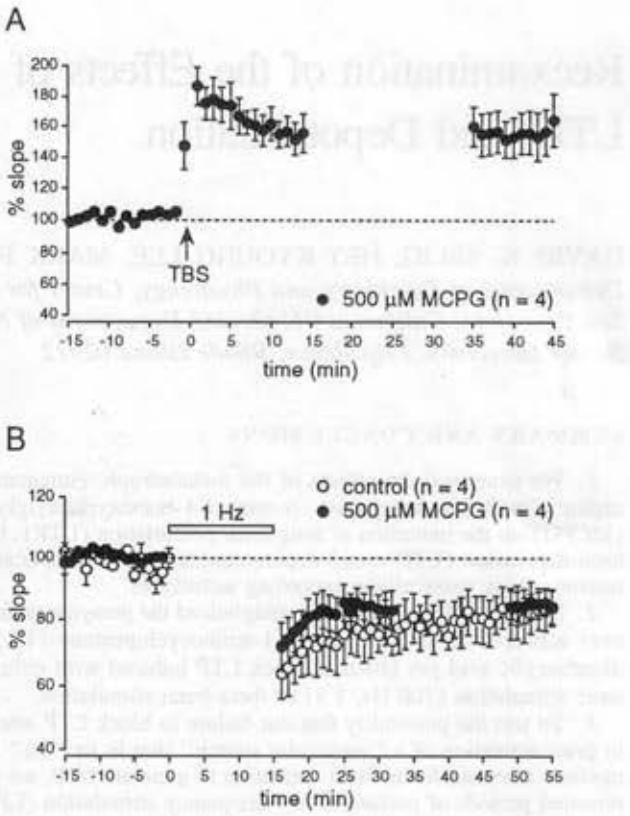

FIG. 2. LTP and LTD in MCPG. *A*: average of LTP obtained in the presence of 500 μM MCPG. There is a 19-min gap in the recording due to LFS given to the other pathway. The magnitude of LTP was 165 ± 17% (mean ± SE) of the initial baseline slope, measured 45 min post-TBS (n = 4). *B*: comparison of LTD obtained in the presence and absence of 500 μM MCPG. In the case of LTD obtained in the presence of MCPG, the gap in the data represents LFS stimulation given to the other pathway during the course of experiment. The magnitude of LTD obtained in the absence of MCPG was 84 ± 6% (measured 55 min after the delivery of 1 Hz, n = 4), whereas LTD obtained in the same slice in the presence of MCPG was 84 ± 5% (n = 4).

STUDY 1: BROWN EXPERIMENTS

Methods

Hippocampal slices were prepared from young adult Long-Evans rats (100–150 g) using standard techniques. Each animal was anesthetized using metofane vapor and was decapitated soon after the disappearance of any corneal reflexes. Slices were collected in ice-cold artificial cerebrospinal fluid (ACSF) bubbled with 95% O_2-5% CO_2 and gently transferred to an interface chamber (Medical Systems, Greenvale, NY) continually superfused with 35°C ACSF at a rate of 1 ml/min and supplied with an atmosphere of humidified 95% O_2-5% CO_2. The composition of ACSF was (in mM) 124 NaCl, 5 KCl, 1.25 NaH_3PO_4, 1.5 $MgSO_4$, 2.5 $CaCl_2$, 26 $NaHCO_3$, and 10 dextrose. The slices were left in the chamber for ≥1 h to equilibrate before the experiments were performed.

Synaptic responses were measured extracellularly in the stratum radiatum of CA1 using recording microelectrodes filled with ACSF (1–2 MΩ). Synaptic responses were evoked by stimulating Schaffer collaterals with 0.2-ms pulses 2–100 μA in amplitude delivered using bipolar stimulating electrodes (200 μm OD, FHC #16–60–3). In most cases, two stimulating electrodes were placed on either side of the recording electrode to stimulate two independent pathways. The absence of cross-pathway paired-pulse facilitation was the criterion used to determine that the pathways were independent

of each other. Baseline responses were obtained by stimulating the two inputs alternately at 0.033–0.067 Hz using a stimulation intensity that yielded a half-maximal population excitatory postsynaptic potential (EPSP) slope. To induce LTP, four episodes of theta-burst stimulation (TBS) were delivered at 0.1 Hz, using the same stimulation intensity as for baseline. TBS consists of 10 stimulus trains delivered at 5 Hz; each train consists of four pulses at 100 Hz (Larson et al. 1986). Homosynaptic LTD was induced by delivering low-frequency stimulation (LFS; 900 pulses at 1 Hz) at the same stimulation intensity as baseline (Dudek and Bear 1992). Evoked extracellular field potentials were digitized at 20 kHz and stored on an AST 386 IBM-compatible computer using Experimenter's Workbench (BrainWave Systems, Boulder, CO) until further analysis. Initial slopes of the recorded field potentials were measured as an indicator of synaptic strength.

In all two-pathway experiments ($n = 4$), the experimental design was kept approximately the same. After ~20 min of stable baseline, LFS was delivered to input 1 to test the magnitude of control LTD; then 500 μM of MCPG were bath applied to the slice. After 30 min of MCPG application, TBS was delivered to input 1 to test the magnitude of LTP in the presence of 500 μM MCPG. Then LFS was delivered after ~10 min to input 2, to assess the degree of LTD induced in a naive synaptic input in the presence of the drug. Finally, LFS was given to input 1 to assess depotentiation in MCPG.

A stock solution of MCPG [(RS)-α-methyl-4-carboxyphenylglycine; Tocris Cookson] was prepared at a concentration of 50 mM by dissolving MCPG in an equivalent concentration of NaOH solution. The stock solution was frozen in small aliquots and one of the aliquots was thawed on the day of experiment and diluted to 5-mM solution by adding normal ACSF. MCPG (5 mM) was then bath applied using a syringe pump (Harvard Apparatus, Southnatick, MA) at a rate (0.1 ml/min) that would result in an approximate concentration of 500 μM in the ACSF perfusing the slice. MCPG was applied ≥30 min before attempting induction of synaptic plasticity. ACPD (Tocris Cookson) was dissolved in normal ACSF at a concentration of 10 μM.

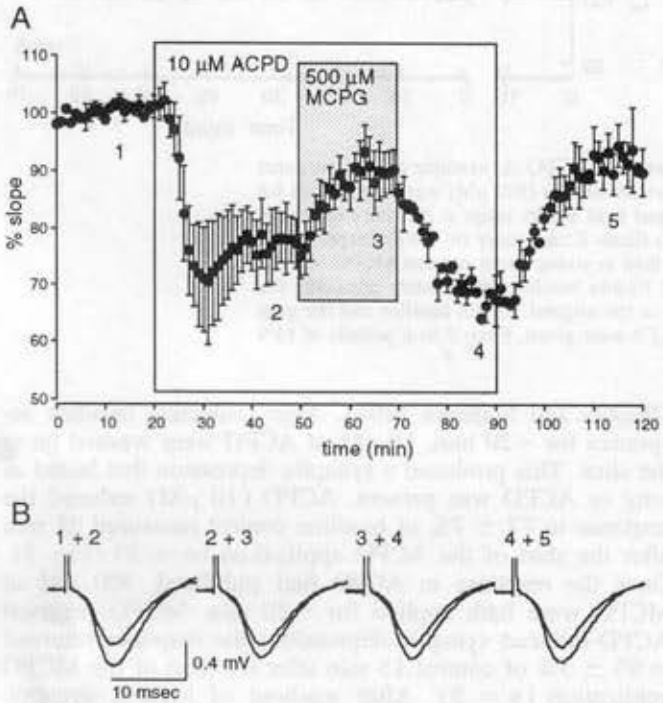

FIG. 3. MCPG (500 μM) antagonizes synaptic depression induced by 10 μM 1-aminocyclopentane-(1S,3R)-dicarboxylic acid (ACPD). *A*: average effect of MCPG on ACPD-induced synaptic depression ($n = 3$). After collecting baseline data for ~20 min, normal artificial cerebrospinal fluid (ACSF) was switched to ACSF containing 10 μM ACPD. ACPD (10 μM) produced a depression in synaptic responses, which lasted as long as ACPD was present. After ACPD-induced depression had stabilized, 500 μM of MCPG were applied for 20 min with ACSF containing 10 μM ACPD. MCPG reversed the ACPD-induced depression. The synaptic response was depressed again when MCPG was washed out. When ACSF containing 10 μM ACPD was switched back to normal ACSF, the synaptic response returned to near baseline level. *B*: extracellular field potential traces from a representative experiment. Each trace is the average of 4 consecutive sweeps at the times indicated in *A*.

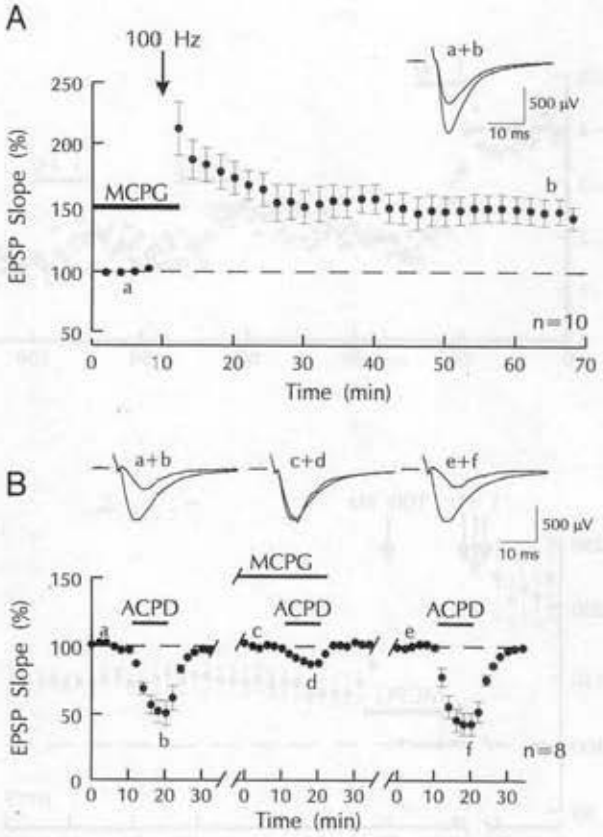

FIG. 4. MCPG does not block LTP but does antagonize the presynaptic inhibitory actions of ACPD. *A*: summary of 10 experiments in which a tetanus (100 Hz, 1 s) was given in the presence of MCPG (500 μM), which had been applied for ≥20 min. Stable LTP was elicited. *Inset*: sample field excitatory postsynaptic potentials (EPSPs) (average of 6 consecutive sweeps) taken at the times indicated by the letters. *B*: summary of 8 experiments in which ACPD (25 μM) was applied 3 times; first in the absence of MCPG (*left panel*), then in the presence of MCPG (*middle panel*), and then again after MCPG had washed out (*right panel*).

Results

TWO-PATHWAY EXPERIMENTS. Figure 1 shows the records of a representative experiment in which responses to two pathways were monitored. Before application of MCPG, 1-Hz stimulation was delivered to one pathway to confirm that the slice could exhibit LTD. Then 500 μM of MCPG were bath applied for ~35 min. MCPG did not significantly alter the baseline responses, as can be seen in the traces of extracellular field potential recordings in Fig. 1*B*. To determine whether MCPG has any effect on LTP, TBS was given to input 1. TBS produced normal LTP. To test the effect of MCPG on LTD, LFS was given to input 2. LFS produced normal LTD. In addition, when the same 1-Hz stimulation

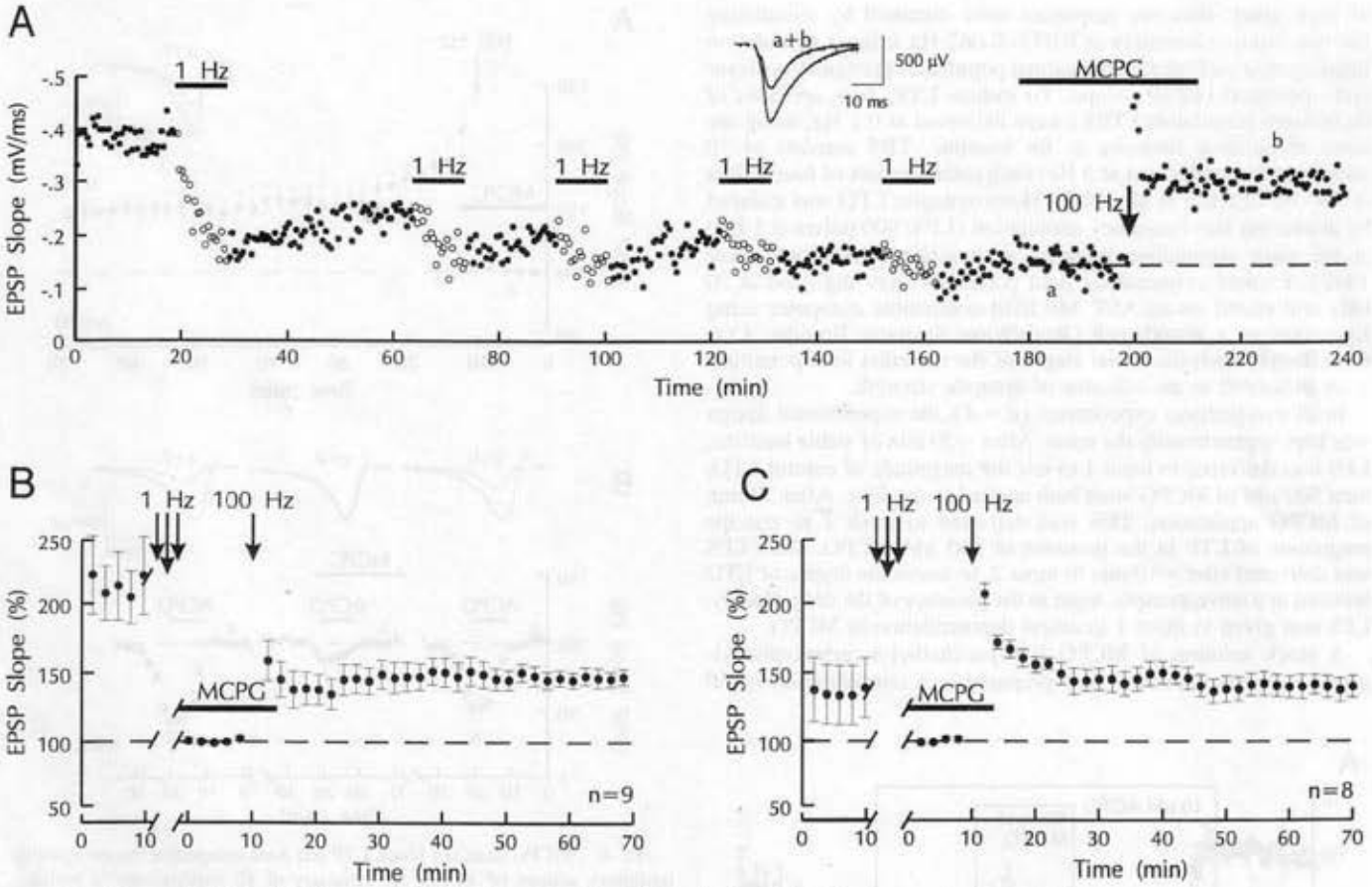

FIG. 5. Repeated periods of LFS saturate LTD but do not sensitize LTP induction to MCPG. *A*: example of an experiment in which LFS (1 Hz, 10 min) was given repeatedly until LTD was clearly saturated. MCPG (500 μM) was then applied for 20 min, at which point a tetanus (100 Hz, 1 s) still elicited LTP. *Inset*: averaged field EPSPs taken at the times indicated. *B*: summary ($n = 9$) of similar experiments performed on young (11–18 days) slices. *C*: summary ($n = 8$) of experiments performed in older (4–6 wk) slices. Note that the magnitude of LTD was less than in young slices and that MCPG still did not block LTP. Each experiment included in *B* and *C* was normalized to the 10-min baseline immediately preceding the LTP-inducing tetanus. The initial 5 points in the graphs (*B* and *C*) correspond to the original 10-min baseline and the gaps in the *X*-axis (*B* and *C*) represent the time during which repeated periods of LFS were given. From 3 to 6 periods of LFS were necessary to saturate LTD.

was given to the previously potentiated pathway (input 1), depotentiation resulted. These results suggest that MCPG at 500 μM does not significantly affect LTP or LTD formation.

The averaged data for LTP and LTD in the presence of 500 μM MCPG are shown in Fig. 2, *A* and *B*. The average magnitude of LTP obtained in the presence of MCPG was 165 ± 17% (mean ± SE) of baseline slope 45 min post-TBS ($n = 4$). Induction of LTD in a naive pathway in the same slices reduced the response to 84 ± 5% of baseline (55 min post-LFS, $n = 4$) in the presence of MCPG, which is not significantly different from naive LTD produced in the same slices in the absence of the drug (84 ± 6%, $n = 4$). These results show that, using our experimental conditions, MCPG at 500 μM does not affect LTP or LTD in CA1 cells.

EFFECT OF MCPG ON ACPD-INDUCED SYNAPTIC DEPRESSION. To determine whether MCPG at 500 μM is acting as an antagonist at mGluRs under the conditions of our experiments, its effect on ACPD-induced synaptic depression was studied. ACPD is a selective agonist at some subtypes of mGluRs (Schoepp et al. 1990). Previous reports have shown that ACPD can produce a transient depression of the synaptic responses, probably mediated by a presynaptic mechanism (Baskys and Malenka 1991). After collecting baseline responses for ~20 min, 10 μM of ACPD were washed on to the slice. This produced a synaptic depression that lasted as long as ACPD was present. ACPD (10 μM) reduced the response to 77 ± 7% of baseline control measured 25 min after the start of the ACPD application ($n = 3$) (Fig. 3). Once the response in ACPD had stabilized, 500 μM of MCPG were bath applied for ~20 min. MCPG reversed ACPD-induced synaptic depression; the response returned to 93 ± 5% of control 15 min after the start of the MCPG application ($n = 3$). After washout of MCPG, synaptic transmission was again depressed by the 10 μM ACPD, back to 73 ± 1% of control, 10 min after the MCPG was turned off ($n = 3$). The synaptic response returned near to the original baseline after washout of 10 μM ACPD (95 ± 4% of initial baseline, 25 min after ACPD washout, $n = 3$). These results demonstrate that 500 μM MCPG can reversibly antagonize an effect mediated by ACPD.

STUDY 2: UCSF EXPERIMENTS

Methods

Hippocampal slices were prepared from 4- to 6-wk-old Sprague-Dawley rats (except where noted), allowed to recover for 1–4 h,

and then transferred to a recording chamber where they were submerged beneath a continuously superfusing ACSF (28–29°C) saturated with 95% O_2-5% CO_2. The composition of our ACSF was (in mM) 119 NaCl, 2.5 KCl, 2.5 $CaCl_2$, 1.3 $MgSO_4$, 1 NaH_2PO_4, 26.2 $NaHCO_3$, and 11 glucose. Stimulating techniques, recording techniques, data collection, and data analysis were the same as those described previously (Huang et al. 1992; Mulkey and Malenka 1992). Each pathway was stimulated in alternation at either 0.1 or 0.033 Hz. For two-pathway experiments, stimulating electrodes were placed on either side of the recording electrode. Data in the text and in the summary graphs are presented as mean ± SE. The mean percent change in EPSP slope was calculated by averaging over a 10-min period taken 50–60 min after the induction of LTP and 20–30 min after the induction of LTD or depotentiation, and this value was compared with the average of the 10-min period preceding the induction protocol. LTP was elicited using a 100-Hz, 1-s tetanus. LTD and depotentiation were elicited by stimulating at 1 Hz for 10 min (LFS).

The active enantiomer of MCPG [(+)-α-methyl-4-carboxyphenylglycine; Tocris Cookson] was prepared daily by first dissolving it in an equimolar amount of NaOH and then diluting to a final concentration of 500 μM in ACSF. It was bath applied to the slice for ≥20 min before the induction of LTP, LTD, or depotentiation. Solutions containing D-2-amino-5-phosphonovaleric acid (D-APV) (Tocris Cookson) or ACPD (Tocris Cookson) were prepared daily from stocks (25 mM in water). Nifedipine (Sigma) was prepared daily as a stock (20 mM) in dimethylsulfoxide which was protected from light and diluted to its final concentration (20 μM in ACSF) immediately before application.

Results

For all of our experiments examining LTP, we attempted to match as closely as possible the conditions of previous experiments that found a dramatic effect of MCPG on LTP (Bashir et al. 1993a; Bortolotto et al. 1994). Thus we used animals of the same age, the same LTP induction protocol, and the same high concentration of MCPG (500 μM). Figure 4*A* shows that MCPG had no apparent effect on LTP induced by a 100-Hz, 1-s tetanus. The average magnitude of LTP was 147 ± 10% (n = 10) and the synaptic enhancement was stable 1 h after the tetanus. To ensure that the MCPG was effectively antagonizing mGluRs in these slices, we examined its ability to inhibit the reversible depression of synaptic transmission caused by the mGluR agonist ACPD (Baskys and Malenka 1991). We first established that ACPD (25 μM) depressed field EPSPs significantly (Fig. 4*B, left panel*) in slices that had already exhibited LTP in MCPG (Fig. 4*A*). ACPD was then reapplied to these same slices in the presence of MCPG. This resulted in a large but reversible reduction of the effects of ACPD on synaptic transmission (Fig. 4*B, middle and right panels*) (n = 8 pathways in 4 slices). These results confirm that MCPG was effective under our experimental conditions.

A possible explanation for the lack of effect of MCPG on LTP is that during the preparation of our slices, the mGluR-dependent molecular switch had been turned on and thus mGluR activation was no longer necessary to generate LTP (Bortolotto et al. 1994). Because LFS was reported to "decondition" or turn off the switch and thereby make LTP induction sensitive to blockade by MCPG (Bortolotto et al. 1994), we reasoned that prior application of LFS to our slices should reset the mGluR switch so that MCPG would subsequently block LTP. To have an objective measure that

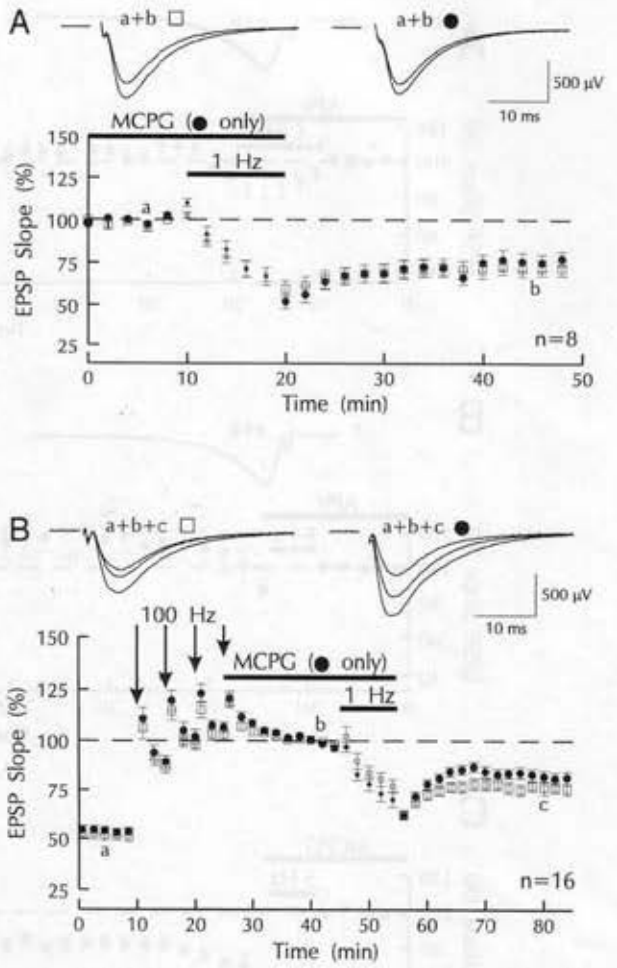

FIG. 6. MCPG does not affect LTD in young (11–18 days) slices or depotentiation in older (4–6 wk) slices. *A*: summary (n = 8) of 2-pathway experiments in which LFS was applied once to each pathway either in the presence or absence of MCPG (500 μM). Although the LFS was given to each pathway 40–60 min apart, the 2 pathways are shown superimposed to facilitate comparison. Sample field EPSPs are shown above the graph. *B*: summary (n = 16) of 2-pathway experiments in which LTP was elicited in both pathways using repetitive tetani (100 Hz, 1 s, given 4 times 5 min apart). LFS was then applied 20 min later either in the presence or absence of MCPG (500 μM). LTP was induced in each path 60–90 min apart, but as in *A*, to facilitate comparison, the 2 pathways are shown superimposed. For both sets of experiments (*A* and *B*), the time of MCPG application was randomized so that in half of the experiments the control pathway received the LTD- or LTP-inducing stimuli 1st, and in half of the experiments these stimuli were 1st given in the presence of MCPG that was then washed out so that the control pathway could be examined.

LFS was effective, we initially performed experiments in young slices (11–18 days), in which LFS elicits robust LTD (Dudek and Bear 1992; Mulkey and Malenka 1992). Furthermore, we always gave repetitive periods of LFS until LTD was saturated to ensure that the switch was completely "turned off." Figure 5 shows an example (*A*) and a summary (*B*) of nine such experiments. Repetitive periods of LFS caused a large depression (EPSP slope reduced to 48 ± 6% of baseline). After obtaining a new baseline for these maximally depressed responses, MCPG was applied and was still ineffective in blocking LTP, the magnitude of which (147 ± 6%) was identical to that recorded in naive pathways in the presence of MCPG (Fig. 4*A*). Because the role of mGluRs in LTP may change during development (Izumi

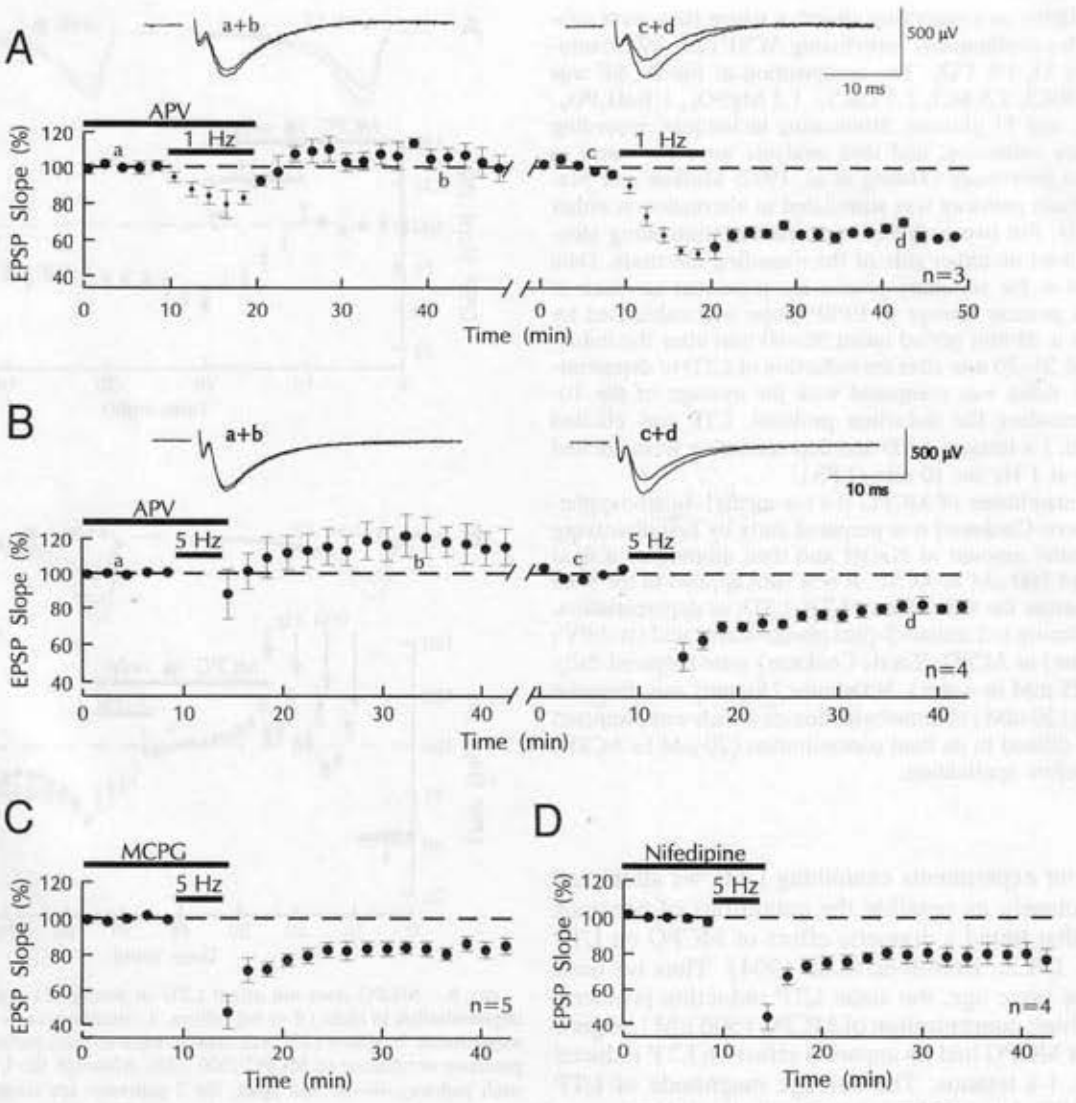

FIG. 7. LTD in neonatal (3–7 days) slices is blocked by D-2-amino-5-phosphonovaleric acid (D-APV) but not by MCPG or nifedipine. *A*: summary ($n = 3$) of experiments in which LFS was given 1st in the presence of D-APV (50 μM), then after it had been washed out. Sample field EPSPs are shown above the graph. *B*: summary ($n = 4$) of experiments in which 5-Hz stimulation for 3 min, rather than LFS, was applied 1st in the presence of D-APV, then after washout. Sample field EPSPs are shown above the graph. *C* and *D*: summary of experiments in which 5-Hz, 3-min stimulation was applied in the presence of MCPG (*C*, 500 μM, $n = 5$) or in the presence of nifedipine (*D*, 20 μM, $n = 4$).

and Zorumski 1994), we repeated these experiments using slices the same age (4–6 wk) as those in which the effectiveness of LFS in turning off the mGluR-dependent molecular switch had been demonstrated (Bortolotto et al. 1994). Figure 5*C* shows that after saturation of LTD with LFS (EPSP slope reduced to 82 ± 9% of baseline, $n = 8$), MCPG was again ineffective in blocking LTP (140 ± 9%). Thus we were unable to observe an effect of MCPG on LTP despite repeated attempts to turn off the postulated mGluR-dependent molecular switch.

We also examined the effects of MCPG on homosynaptic LTD in young (11–18 days) slices (Dudek and Bear 1992; Mulkey and Malenka 1992). In these two-pathway experiments, LFS was applied to one pathway in the absence of MCPG and to the other pathway in the presence of MCPG. Figure 6*A* shows that MCPG had no effect on LTD. LFS decreased EPSPs to 72 ± 5% of baseline in the absence of MCPG and to 76 ± 5% of baseline in its presence ($n = 8$). We further examined in older slices (4–6 wk) the effects of MCPG on depotentiation (Fujii et al. 1991) (Fig. 6*B*), which is routinely defined as the LTD that occurs on a pathway in which LTP was generated previously. Similar to our results on LTD, MCPG did not affect depotentiation. In the absence of MCPG, LFS caused a decrease in EPSP slope to 76 ± 3% of its potentiated value ($n = 16$); in the presence of MCPG the LFS-induced decrease reached 82 ± 3%.

Although we were unable to observe an effect of MCPG on LTD or depotentiation, it has been reported that in slices prepared from neonatal animals (3–7 days), a form of LTD exists that is blocked by MCPG or nitrendipine (Bolshakov and Siegelbaum 1994) and, unlike the homosynaptic LTD elicited in slightly older slices (Dudek and Bear 1992; Mulkey and Malenka 1992), is not dependent on NMDAR activation. Figure 7 summarizes our pharmacological examina-

tion of LTD in neonatal slices prepared from 3- to 7-day-old rats. Using our standard LFS, LTD was completely and reversibly blocked by D-APV (Fig. 7*A*). We then examined LTD elicited by 5-Hz stimulation applied for 3 min, the protocol used to elicit an MCPG and nitrendipine-sensitive LTD (Bolshakov and Siegelbaum 1994). Surprisingly, we found that again LTD was reversibly blocked by D-APV (Fig. 7*B*, $n = 4$) but was unaffected by either MCPG (Fig. 7*C*, $n = 5$) or nifedipine (20 μM) (Fig. 7*D*, $n = 4$). The 5-Hz stimulation decreased EPSPs to 84 $\pm$ 4% of baseline in the presence of MCPG, to 79 $\pm$ 5% of baseline in the presence of nifedipine, and to 81 $\pm$ 3% of baseline in control ACSF after washout of D-APV.

DISCUSSION

Because of the potential importance of mGluRs in generating LTP, LTD, and depotentiation in the hippocampus and the confusion in the literature concerning this issue, we have reexamined the effects of the mGluR antagonist MCPG on these phenomena. Experiments conducted independently in our two laboratories have failed to find any effect of MCPG on LTP, LTD, or depotentiation. We made significant efforts to replicate the experimental conditions in which MCPG was found to be effective. Most importantly, we have directly tested and failed to confirm what appeared to be the most likely explanation for the conflicting results concerning LTP: that mGluR activation is not absolutely required for LTP generation but instead functions as a molecular switch that when turned on negates the need for further mGluR stimulation during the induction of LTP (Bortolotto et al. 1994). It is unlikely that MCPG was ineffective in blocking mGluRs in our experiments because it was found to inhibit effectively a well-documented action of ACPD and was applied at high concentrations (500 μM) for long periods of time ($\geq$20 min) before the generation of LTP, LTD, or depotentiation. This concentration was chosen because it is the same as or higher than that used in previous studies that reported effects of MCPG on synaptic plasticity. However, it is difficult to rule out the possibility that during repetitive stimulation glutamate concentrations reach levels that are sufficient to compete with a high concentration of MCPG and thereby activate mGluRs.

We have also failed to confirm that the induction of LTD in neonatal hippocampus is blocked by MCPG or L-type calcium channel antagonists (Bolshakov and Siegelbaum 1994). Instead we found that, like LFS-induced homosynaptic LTD and depotentiation (Dudek and Bear 1992; Fujii et al. 1991; Mulkey and Malenka 1992), this form of LTD is blocked by D-APV and therefore requires activation of NMDARs. One difference between the two studies is that we used extracellular field recording to monitor synaptic strength, whereas the initial study used whole cell recording techniques. Because the cell was taken out of voltage clamp during the induction protocol (5 Hz for 3 min), this technical difference in recording techniques should have had no effect. However, changes in the intracellular milieu due to the whole cell recording may have modified the cells' condition sufficiently to account for the discrepancy in results.

It is more difficult to explain the lack of effect of MCPG on LTP in the present study. Slices of different ages were examined, different recording chambers (interface and submerged) were used, and different LTP induction protocols were tested. Taken together with previous negative results (Brown et al. 1994; Chinestra et al. 1993; Manzoni et al. 1994), we think that the experimental evidence favors the conclusion that MCPG-sensitive mGluRs are not necessary for the induction of LTP, even when synaptic strength has been maximally depressed and the hypothesized molecular switch is presumably turned off. It remains possible, however, that MCPG-sensitive mGluRs are capable of modulating the threshold for the induction of LTP and LTD, perhaps by influencing NMDAR function (Ben-Ari et al. 1992) or by affecting transmitter release (Baskys and Malenka 1991). Furthermore, it is also conceivable that MCPG-insensitive mGluRs may prove to be necessary for the generation of LTP and/or LTD.

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