Differential Roles for Group 1 mGluR Subtypes in Induction and Expression of Chemically Induced Hippocampal Long-Term Depression

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Volk, Lenora J., Christine A. Daly, and Kimberly M. Huber. Differential roles for group 1 mGluR subtypes in induction and expression of chemically induced hippocampal long-term depression. J Neurophysiol 95: 2427–2438, 2006. First published January 8, 2006; doi:10.1152/jn.00383.2005. Although metabotropic glutamate receptors (mGlurRs) mGluR1 and mGluR5 are often found to have similar functions, there is considerable evidence that the two receptors also serve distinct functions in neurons. In hippocampal area CA1, mGluR5 has been most strongly implicated in long-term synaptic depression (LTD), whereas mGluR1 has been thought to have little or no role. Here we show that simultaneous pharmacological blockade of mGluR1 and mGluR5 is required to block induction of LTD by the group 1 mGluR agonist, (RS)-3,5-dihydroxyphenylglycine (DHPG). Blockade of mGluR1 or mGluR5 alone has no effect on LTD induction, suggesting that activation of either receptor can fully induce LTD. Consistent with this conclusion, mGluR1 and mGluR5 both contribute to activation of extracellular signal-regulated kinase (ERK), which has previously been shown to be required for LTD induction. In contrast, selective blockade of mGluR1, but not mGluR5, reduces the expression of LTD and the associated decreases in AMPA surface expression. LTD is also reduced in mGluR1 knockout mice confirming the involvement of mGluR1. This shows a novel role for mGluR1 in long-term synaptic plasticity in CA1 pyramidal neurons. In contrast to DHPG-induced LTD, synthetically induced LTD with paired-pulse low-frequency stimulation persists in the pharmacological blockade of group 1 mGlurRs and in mGluR1 or mGluR5 knockout mice. This suggests different receptors and/or upstream mechanisms for chemically and synthetically induced LTD.

INTRODUCTION

Group 1 metabotropic glutamate receptors (mGlurRs) are composed of two subtypes, mGluR1 and mGluR5. Both receptors are coupled to the Gq subtype of heterotrimeric G proteins and activate phospholipase C. mGluR1 and mGluR5 both contribute to increased neuronal excitability, intracellular Ca2+ increases (Ireland and Abraham 2002; Rae and Irving 2004; Thuault et al. 2002), synaptic plasticity (Gubellini et al. 2003; Sung et al. 2001), and pain (Karim et al. 2001). However, although they have similar signaling cascades, there is evidence that mGluR1 and mGluR5, expressed in the same neuron, can serve distinct functions (Mannaioni et al. 2001; Merlin 2002) (Kettunen et al. 2003; Thuault et al. 2002; for review, see Valenti et al. 2002). In hippocampal CA1 pyramidal neurons, mGluR5 is the most highly expressed subtype (Lujan et al. 1996; Romano et al. 1995; Shigemoto et al. 1997). However, a role for mGluR1 has recently been elucidated using the selective mGluR1 antagonist LY367385 (Clark et al. 1997). In particular, mGluR1 contributes to many functions in CA1 pyramidal neurons including cell depolarization, intracellular Ca2+ increases, decreases in the afterhyperpolarization potential (AHP), short-term depression of excitatory postsynaptic currents (EPSCs), and extracellular signal-regulated kinase (ERK) activation (Berkeley and Levey 2003; Ireland and Abraham 2002; Mannaioni et al. 2001; Rae and Irving 2004).

Group 1 mGluR activation of CA1 pyramidal neurons with the selective agonist, (RS)-3,5-dihydroxyphenylglycine (DHPG), or with low-frequency (1–5 Hz) synaptic stimulation causes long-term depression (LTD) of excitatory synaptic transmission (mGluR-LTD) (Bolshakov and Siegelbaum 1994; Fitzjohn et al. 1999; Huber et al. 2000, 2001; Oliet et al. 1997; Palmer et al. 1997). mGluR-LTD in mature rodents is mediated by a persistent reduction in the number of postsynaptic amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors (AMPARs) (Nosyreva and Huber 2005; Snyder et al. 2001; Xiao et al. 2001). In addition, there is evidence for presynaptic contributions to mGluR-LTD (Bolshakov and Siegelbaum 1994; Feinmark et al. 2003; Fitzjohn et al. 2001; Oliet et al. 1997; Rammes et al. 2003; Watabe et al. 2002; Zakharenko et al. 2002). One of the most interesting properties of mGluR-LTD is that it relies on rapid (within minutes) dendritic protein synthesis (Huber et al. 2000). Likewise, the long-term decrease in AMPAR surface expression requires protein synthesis (Nosyreva and Huber 2005; Snyder et al. 2001). Recent work has revealed the signaling pathways that activate translation in response to mGlurRs. The ERK and PI3K/mTOR pathways that regulate translation initiation in many cell types, including neurons, are activated by DHPG and required for mGluR-LTD (Gallagher et al. 2004; Hou and Klann 2004; for reviews, see Kelleher et al. 2004; Klann and Dever 2004). The fact that LTD induced with either DHPG or synaptic stimulation [paired-pulse low-frequency stimulation (PP-LFS)] are both blocked by the broad range mGluR antagonist, LY341495, occlude each other, and both rely on protein synthesis and ERK suggest that these two methods of LTD induction represent the same or a similar LTD mechanism (Gallagher et al. 2004; Huber et al. 2000, 2001).

mGluR-LTD is absent in mGluR5 knockout (KO) mice, suggesting a requirement for this subtype for either induction or expression of LTD (Huber et al. 2001). Consistent with this work, the mGluR5 antagonist MPEP has been shown to reduce or abolish mGluR-LTD in rats and mice (Faas et al. 2002; Gasparini et al. 1999; Hou and Klann 2004; Huang and Hsu 2006; Huang et al. 2004). In contrast, selective mGluR1...
blockade has been reported to have either a partial or no effect on DHPG-induced LTD in area CA1 (Faas et al. 2002; Fitzjohn et al. 1999; Hou and Klann 2004). Recent work has shown that mGluR1 activity is required for the acute, short-term depression of excitatory synaptic transmission induced with DHPG (Faas et al. 2002; Mannaioni et al. 2001). Therefore mGluR1 and mGluR5 both regulate excitatory synaptic transmission onto CA1 neurons. Remarkably, the expression of DHPG-induced LTD can be reversed by broad mGluR antagonists, even when applied hours after the induction stimulus (Fitzjohn et al. 1999; Palmer et al. 1997; Watabe et al. 2002). Therefore sustained mGluR activation is required for the expression of mGluR-LTD. mGluR-LTD is enhanced in the mouse model of fragile X syndrome, and it has been suggested that group 1 mGluR antagonists may serve as potential therapies for Fragile X syndrome patients (Bear et al. 2004; Huber et al. 2002). Therefore determination of the specific mGluR subtypes required for LTD may facilitate development of pharmaceutical treatments for Fragile X syndrome or other forms of mental retardation.

In light of accumulating data for mGluR1 function in CA1 pyramidal neurons and synapses, we evaluated the role of mGluR1 in LTD of their synapses. Unexpectedly, we find a role for mGluR1 in the induction and expression of DHPG-induced LTD. Our data show that activation of mGluR1 or mGluR5 alone can induce the full complement of LTD. Consequently, simultaneous blockade of mGluR1 and mGluR5 is required to abolish DHPG-induced LTD and the associated ERK activation. mGluR1, however, is selectively required for the expression of DHPG-induced LTD. In contrast, synaptically induced LTD (with PP-LFS), is unaffected by blockade or genetic knockout of group 1 mGluRs. This data provides evidence for new and additional roles for mGluR1 at hippocampal CA1 synapses and suggests that different neurotransmitter receptors induce chemically and synthetically induced LTD.

METHODS

Drugs

D,L-AP5 (Tocris, Ellisville, MO) was prepared fresh in artificial cerebrospinal fluid (ACSF). R,S-DHPG or S-DHPG, LY367385, LY341495, MPEP (Tocris), tetrodoylammonium chloride, and TTX (Sigma) were prepared as stocks in water or equimolar NaOH (LY367385 and LY341495), aliquoted, and frozen for no more than 10 days. We observed no differences in the effectiveness of fresh or frozen MPEP or LY367385.

Electrophysiology

Hippocampal slices were prepared from Long Evans hooded or Sprague-Dawley rats or mGluR1 or mGluR5 knockout (KO) or wildtype (WT) mice. Rats were obtained from Charles River Laboratories (Boston, MA). mGluR1 KO mice were originally from John Roeder (Lu et al. 1997) and were mated with WT C57BL/6 mice. mGluR1 KO mice were originally from Jackson Laboratories (Bar Harbor, ME) to obtain heterozygotes. Rats or mice were anesthetized with pentobarbital sodium (50 mg/kg) and decapitated soon after the disappearance of corneal reflexes. The brain was removed, dissected, and sliced in ice-cold dissection buffer containing (in mM) 2.6 KCl, 1.25 NaH2PO4, 26 NaHCO3, 0.5 CaCl2, 5 MgCl2, 212 sucrose, and 10 dextrose, using a vibratome (Leica VT 1000S). The slices were transferred into a static incubation chamber in ACSF (containing 100 μM D,L-AP5) at 30°C and aerated with 95% O2–5% CO2. Slices were preincubated in antagonist and treated with DHPG (5 min) or ACSF (control). For phosphospecific (P)-ERK measurements, slices were frozen immediately after DHPG treatment and stored at −80°C as previously described (Gallagher et al. 2004). Slices were homogenized in lysis buffer containing 50 mM HEPES, pH 7.3, 150 mM NaCl, 1.5 mM MgCl2, 1 mM EGTA, 10% glycerol, 0.2 mM NaVO4, 100 mM NaF, 50 mM β-glycerophosphate, 1 mM dithiothreitol, 1 mM benzamidine, 0.01 mg/ml leupeptin, 0.1 mg/ml aprotinin, 0.5 μg/ml pepstatin A, and 1% Triton. Protein concentrations were measured with a BCA.

Bioclimical measurements of surface expressed AMPA receptors and ERK phosphorylation

Hippocampal slices were prepared as for electrophysiology experiments. After a 2- to 3-h recovery period in ACSF, slices (containing area CA1 and dentate gyrus, CA3 was cut-off) were maintained in a static incubation chamber in ACSF (containing 100 μM D,L-AP5) at 30°C and aerated with 95% O2–5% CO2. Slices were preincubated in antagonist and treated with DHPG (5 min) or ACSF (control). For phosphospecific (P)-ERK measurements, slices were frozen immediately after DHPG treatment and stored at −80°C as previously described (Gallagher et al. 2004). Slices were homogenized in lysis buffer containing 50 mM HEPES, pH 7.3, 150 mM NaCl, 1.5 mM MgCl2, 1 mM EGTA, 10% glycerol, 0.2 mM NaVO4, 100 mM NaF, 50 mM β-glycerophosphate, 1 mM dithiothreitol, 1 mM benzamidine, 0.01 mg/ml leupeptin, 0.1 mg/ml aprotinin, 0.5 μg/ml pepstatin A, and 1% Triton. Protein concentrations were measured with a BCA.

For recording, slices were transferred to a submerged recording chamber, maintained at 30°C, and perfused continuously with ACSF at a rate of 2–3 ml/min. Field potentials (FPs) were recorded with extracellular recording electrodes (1 MΩ) filled with ACSF and placed in stratum radiatum of area CA1. FPs were evoked by monophasic stimulation (200-μs duration) of Schaffer collateral/commissural afferents with a concentric bipolar tungsten stimulating electrode (FHC, Bowdoinham, ME). Stable baseline responses were collected every 30 s using a stimulation intensity (10–30 μA) yielding 50–60% of the maximal response. The initial slope of the FPs was used to measure stability of synaptic responses and quantify the magnitude of LTD. Chemically induced mGluR-LTD was elicited by application of 100 μM DHPG for 5 or 20 min as indicated. Synaptically induced LTD was induced using PP-LFS (50-ms interstimulus interval) pulses at 1 Hz for 15 (for rats) or 20 min (for mice). The group data were analyzed as follows: 1) the initial slope of the FPs were expressed as percentages of the pre-DHPG baseline average, 2) the time scale in each experiment was converted to time from the onset of DHPG, and 3) the time-matched, normalized data were averaged across experiments and expressed in the text and figures as means ± SE. The effects of all pharmacological treatments on LTD were evaluated by comparing interleaved control and treated slices. Significant differences were determined by a Student’s independent t-test. Paired t-tests were used to determine significance of reversal effects on LTD expression (Fig. 2). Probability values of P < 0.05 were considered to represent significant differences.
mGluR1 AND LONG-TERM DEPRESSION

Protein Assay (Pierce). Samples containing 20–35 μg of protein were resolved on 10% SDS-PAGE in duplicate and transferred to nitrocellulose. Membranes were blocked and incubated with P-ERK (Thr202/Tyr204, Promega: 1:5,000 dilution) or total ERK (1:1,000; Cell Signaling Technologies) according to manufacturer’s protocol.

Biotinylation experiments were performed as previously described (Chung et al. 2000; Heynen et al. 2003; Nosyreva and Huber 2005). From each rat, two to three slices were pooled together for one condition. Fifteen minutes after DHPG treatment, slices were placed on ice to stop endocytosis, washed with ice-cold ACSF, and incubated in ACSF containing 1 mg/ml Sulfo-NHS-LC-Biotin (Pierce, Rockford, IL) for 10 min on ice. To quench the biotin reaction, slices were washed three times with Tris-buffered saline (TBS) and homogenized in a modified radioimmunoprecipitation (RIPA) buffer containing: 50 mM Tris-HCl, pH 7.4, 1% Triton X100, 0.1% SDS, 0.5% Na-deoxycholate, 150 mM NaCl, 2 mM EDTA, 50 mM NaH2PO4, 50 mM NaF, 10 mM Na3P2O7, 1 mM Na2VO4, and protease inhibitor cocktail III (Calbiochem, La Jolla, CA). The homogenates were centrifuged at 14,000g for 10 min at 4°C. Fifteen micrograms of protein was removed for total (T) protein measurements; 150 μg of protein was mixed with 150 μl of UltraLink immobilized NeutrAvidin beads (Pierce) by rotating for 2 h at 4°C. The beads were washed with 10 volumes of RIPA buffer, and proteins were eluted with SDS-PAGE sample buffer supplemented with 50 mM dithiothreitol for 20 min at 90°C. Both total and biotinylated proteins were resolved by SDS-PAGE transfer to nitrocellulose membranes and probed with anti-GluR1 C-terminal antibody (1:5,000; Upstate Biotechnology). Immunoreactive bands were visualized by enhanced chemiluminescence (ECL) captured on autoradiography film (Kodak). Digital images were produced by densitometric scans of autoradiographs on a ScanJet 4300C (Hewlett Packard) and quantified using Scion Image software. Multiple film exposures were performed and quantified to ensure that the values were in the linear range of the ECL reaction. A subset of GluR1 biotinylation experiments were quantified both using chemiluminescent (ECL) and chemifluorescence using ECL Plus (Amersham) and quantified using a Storm 860 scanner (Molecular Dynamics) that yielded similar results.

The P-ERK/total ERK or surface/total GluR1 ratio was calculated for each condition. For the GluR1 ratios, duplicate conditions within one animal were averaged to obtain an animal average for that condition. Therefore the n values for the biotinylation experiments (Fig. 4) represent the number of rats as opposed to slices. Significant differences between surface/total ratios of treated slices and within-animal control slices were determined using a Wilcoxon signed-rank test. Although the raw ratio values were used for statistical comparisons, the group data are presented in Fig. 4 as a percent of condition control to compare across different treatment conditions.

All experiments were conducted according to a protocol approved by the Institutional Animal Use and Care Committee at UT Southwestern Medical Center.

RESULTS

Combined blockade of mGluR1 and mGluR5 is necessary to block DHPG-induced LTD

mGluR-LTD can be induced chemically with the specific group 1 mGluR agonist, DHPG (Ito et al. 1992). Previously, we found that LTD induced with 5 min of DHPG was absent in mGluR5 KO mice (Huber et al. 2001). We first attempted to confirm that pharmacological inhibition of mGluR5 inhibited DHPG-induced LTD (Faas et al. 2002; Hou and Klann 2004; Huang et al. 2004). MPEP is the most potent and selective commercially available mGluR5 antagonist, with an IC50 = 32 nM for mGluR5 and >100 μM for mGluR1 (Gasparini et al. 1999). We first tested the ability of MPEP to block DHPG-induced LTD in hippocampal slices prepared from Long Evans rats. Extracellular field potential recordings elicited by Schaffer collateral stimulation were obtained in area CA1. We performed these and all subsequent LTD experiments in the N-methyl-D-aspartate (NMDA) receptor antagonist d,L-AP5 (100 μM) to prevent induction of NMDA receptor–dependent LTD (Dudek and Bear 1992). AP5 had no effect on DHPG-induced LTD (100 μM; 20 min; data not shown) (Huber et al. 2001). Surprisingly, in contrast to previous reports, MPEP (10 μM), applied during the baseline and DHPG application, had no effect on the acute or long-lasting depression induced with DHPG (100 μM; 5 min; MPEP = 77 ± 2% of pre-DHPG baseline, n = 6, measured 60–65 min after DHPG application; interleaved control slices = 77 ± 3%, n = 6, P = 0.99; Fig. 1A). To test the role of mGluR1 in the induction of LTD, we applied the mGluR1 antagonist LY367385 (100 μM) before and during DHPG (100 μM; 5 min). As previously described, LY367385 reduced the acute depression observed during DHPG application but did not affect LTD (acute depression; LY367385 = 64 ± 4%, n = 5; interleaved controls = 37 ± 5%, n = 7, P < 0.01; LTD; LY367385 = 82 ± 4%, n = 5; interleaved control; 82 ± 4%, n = 7, P = 0.92; Fig. 1B) (Fitzjohn et al. 1999; Hou and Klann 2004; Mannaioni et al. 2001). Therefore mGluR1 or mGluR5 blockade alone does not affect the induction of mGluR-LTD. However, preincubation in both MPEP (10 μM) and LY367385 (100 μM) completely blocked mGluR-LTD induced with DHPG (LY367385 + MPEP = 97 ± 2%, n = 7; interleaved controls = 83 ± 4%, n = 6, P < 0.01; Fig. 1C). These data suggest that activation of mGluR1 or mGluR5 is sufficient to induce LTD and therefore inhibition of both receptors is required to block LTD induction. Interestingly, LY367385 and MPEP did not completely block the acute depression observed during DHPG application (60 ± 6% of pre-DHPG baseline, n = 7; Fig. 1C). It is unlikely that the residual acute depression was caused by subsaturating concentrations of LY367385 or MPEP, because increasing LY367385 to 150 μM did not further inhibit the acute depression during DHPG compared with 100 μM LY367385 (62 ± 6%, n = 6; P = 0.8), and MPEP had no effect on the acute depression when applied alone (Fig. 1A) or in the presence of LY367385 (cf. Fig. 1, B and C).

mGluR1 and mGluR5 contribute to DHPG-induced phosphorylation of ERK

DHPG induces phosphorylation of the mitogen-activated kinase, ERK, in area CA1 (Berkeley and Levey 2003; Gallagher et al. 2004; Roberson et al. 1999), and ERK activation is required for mGluR-LTD (Gallagher et al. 2004). Therefore ERK activation may be a biochemical measure of the LTD induction signaling cascade. Because both mGluR1 and mGluR5 contribute to the induction of LTD, we predicted that both receptors contribute to ERK activation and that activation of mGluR1 or mGluR5 alone would induce phosphorylation of ERK. We previously reported that combined application of LY367385 and MPEP blocked DHPG-induced (100 μM; 5 min) phosphorylation of ERK (Gallagher et al. 2004). In contrast to their effects on DHPG-induced LTD, MPEP or LY367385 alone inhibited DHPG-induced ERK phosphorylation compared with DHPG alone (ACSF + DHPG; 470 ± 64% of basal levels, n = 6; MPEP + DHPG = 195 ± 24%, n = 5;
FIG. 1. Activation of metabotropic glutamate receptors (mGluRs) mGluR1 or mGluR5 is sufficient to induce long-term depression (LTD) and activate extracellular signal-regulated kinase (ERK). In all experiments, N-methyl-D-aspartate (NMDA) receptors were blocked with 100 μM D,L-AP5. A–C: plotted are the average (±SE) initial slope values of field potentials (FPs) normalized to the pre-DHPG baseline (DHPG baseline). LTD was induced with a 5-min application of 100 μM DHPG. A: acute application of the mGluR5-specific antagonist MPEP (10 μM; solid line) during the baseline and DHPG application had no effect on LTD induction. B: likewise, acute application of the mGluR1-specific antagonist LY367385 (100 μM; solid line) during the baseline and DHPG application had no effect on LTD induction. C: combined, acute blockade of mGluR1 and mGluR5 (10 μM MPEP + 100 μM LY367385) abolished LTD. Inset: FPs (averages of 4–10 waveforms) from a representative experiment were taken at the times indicated by the numbers on the graph. For all panels, scale bars = 0.5 mV/5 ms. D: representative Western blot of phosphorylated (P)-ERK and Total ERK under basal (B; untreated) or DHPG (D; 100 μM; 5 min) treated conditions in the absence of antagonist (artificial cerebrospinal fluid (ACSF)) or in the presence of mGluR1 (LY367385) or mGluR5 (MPEP) antagonists. Group data show that the DHPG-induced increase in the ratio of P-ERK/total ERK is reduced by LY367385 or MPEP alone and the combined application of LY367385 and MPEP (*P < 0.01; LY367385, MPEP, or LY367385 + MPEP compared with ACSF). However, in the presence of LY367385 or MPEP, DHPG induces a significant increase in P-ERK (#P < 0.05). Data showing that LY367385 + MPEP block the DHPG-induced increase in P-ERK is replotted from Gallagher et al. 2004 for comparison to each antagonist alone.

LY367385 + DHPG = 214 ± 45%, n = 7; Fig. 1D). A one-way ANOVA and subsequent multiple comparison test (Fisher’s PLSD) indicated that the ACSF (DHPG only) group was different from either MPEP- or LY367385-treated groups [F(2,15) = 11.01, P = 0.002; MPEP; P = 0.005; LY367385; P = 0.005]. Although LY367385 and MPEP reduce the levels of phosphorylated ERK, DHPG still increased ERK phosphorylation over basal levels in the presence of each drug (MPEP, P = 0.01; LY367385, P = 0.04; t-test). Taken together, the data in Fig. 1 suggest that this moderate level ERK activation is sufficient to induce the full level of LTD.

mGluR1 activity is required for LTD expression

Previous work has shown that blockade of mGluR1 with LY367385 during DHPG application has either no effect on...
LTD induction or a partial effect (Faas et al. 2002; Fitzjohn et al. 1999; Hou and Klann 2004) (Fig. 1B). These studies differ with regard to the duration of DHPG (5 or 20 min) and/or LY367385 application. We found that the presence of LY367385 throughout the experiment reduced DHPG-LTD by ~50% using either brief (5 min) or prolonged (20 min) DHPG applications (5 min; LY367385; 91 ± 1%; n = 6; control 79 ± 2%; n = 7; P = 0.002; Fig. 4A; 20 min; LY367385 = 88 ± 4% measured at 75–80 min, n = 6; controls = 66 ± 2%, n = 6, P < 0.01; Fig. 2A). Because DHPG-LTD is unaffected when LY367385 is washed out of the slice immediately after DHPG (Fig. 1B), this suggests a role for mGluR1 in LTD expression. Previous reports have shown that DHPG-induced LTD can be transiently “reversed” by applying broad range mGluR antagonists after LTD has been established (Fitzjohn et al. 1999; Palmer et al. 1997; Watabe et al. 2002). To further examine the role of mGluR1 in expression of LTD, we tested the ability of the mGluR1 antagonist LY367385 to reverse LTD in WT and mGluR1 KO mice. Consistent with our data in rats, LY367385 transiently reversed LTD in WT mice when it is applied after LTD had been established (80–100 min after DHPG application; mGluR1 WT, reversal; 79 ± 5% measured at 95–100 min after DHPG application; P < 0.05 compared with LTD at 75–80 min). However, LY367385 had no effect in mGluR1 KO mice, confirming that LY367385 is selective for mGluR1 (mGluR1 KO reversal = 83 ± 3% measured at 95–100 min after DHPG application; P = 0.44 compared with LTD at 75–80; Fig. 2C). Interestingly, LY367385 reversed LTD in the mGluR1 WT up to the level of LTD in the mGluR1 KO such that there is no difference in the magnitude of LTD between the mGluR1 KO and WT during LY367385 application (P = 0.61; Fig. 2C). Overall, these results implicate sustained mGluR1 activity in the expression of LTD.

In contrast to mGluR1, mGluR5 blockade with 10 μM MPEP throughout the experiment had no effect on LTD (MPEP = 64 ± 3%, n = 7; interleaved controls = 62 ± 4%, n = 4; P = 0.76; Fig. 2D). In addition, MPEP 60–80 min after DHPG failed to reverse LTD expression (LTD, 55–60 min post-DHPG application; LTD, 55–60 min post-DHPG application; P = 53 ± 4%; MPEP reversal, 75–80 min post-DHPG = 55 ± 4%, n = 10; P = 0.5). Prolonging the MPEP application from 20 to 60 min also did not reverse LTD expression (LTD, 55–60 min post-

**Fig. 3.** MPEP is effective at blocking the DHPG-induced suppression of I_{AHP} but not DHPG-induced LTD. A1 and A2: time-course of I_{AHP} in a representative experiment. I_{AHP} was elicited by applying a 110-nA (−50- to +50 nA) voltage step in the presence of 0.5 μM TTX and 1 mM TEA. Example waveforms (A1) indicate that I_{AHP} was measured as the outward current peak immediately (10–15 ms) after the voltage step; 100 μM DHPG significantly reduced the I_{AHP} and subsequent wash-on of MPEP reversed this effect in the continued presence of DHPG. A3: average I_{AHP} values from each cell in 100 μM DHPG and on reversal in MPEP (*P < 0.01; DHPG vs. DHPG + MPEP). B and C: LTD induced with 20 min DHPG is not affected by MPEP in multiple rat strains (B, Sprague-Dawley rats, and Long Evans hooded rats as in Figs. 1A and 2D) or in mice (C, C57BL/6 mice).
DHPG = 57 ± 4%; MPEP reversal, 115–120 min post-DHPG = 64 ± 8%, n = 5; P = 0.29; Fig. 2E). LTD was completely blocked by the presence of MPEP and LY367385 throughout the experiment (Fig. 1C). Therefore we predicted that their combined application would completely reverse LTD. This was not the case. MPEP and LY367385 perfusion after DHPG only partially reversed LTD (LTD, 55–60 min post-DHPG = 67 ± 3%; MPEP + LY367385 reversal, 75–80 min post-DHPG = 89 ± 3%, n = 8; P < 0.01; Fig. 2F) and was not different from reversal with LY367385 alone (P = 0.78).

MPEP inhibits the DHPG-induced suppression of IAHP in hippocampal CA1 neurons but not DHPG-induced LTD

Here, we showed that MPEP inhibits ERK phosphorylation (Fig. 1D) and blocks LTD induction when combined with mGluR1 blockade (Fig. 1C). However, in light of our negative results with MPEP alone on LTD, we wanted to confirm an effect of MPEP on an electrophysiological measure in our slice preparation. We tested the ability of MPEP to block DHPG-induced suppression of the AHP. The AHP can be divided into three components, a fast, medium, and slow AHP, which are mediated by activation of different potassium conductances (Storm 1990). DHPG suppresses both the medium and slow AHP which are either partially or completely blocked by MPEP (Ireland and Abraham 2002; Mannaioni et al. 2001). Whole cell patch-clamp recordings were obtained from visualized CA1 neurons from rat hippocampal slices. Cells were voltage clamped at −50 mV, and the Ca2+ activated potassium current that mediates the AHP (I_AHP) was elicited by applying a depolarizing step to +60 mV. An outward tail current was measured as the I_AHP as described (Mannaioni et al. 2001; Pedarzani and Storm 1993) (Fig. 3A). DHPG application (100 µM) reduced I_AHP (baseline = 382 ± 32 pA; DHPG = 221 ± 21 pA, n = 10; P < 0.01). MPEP (10 µM) applied in the presence of DHPG reversed the effects of DHPG on the I_AHP (DHPG + MPEP = 266 ± 19 pA, n = 10; P < 0.01). Similar results were observed with 30 µM DHPG (baseline = 366 ± 32 pA; DHPG = 161 ± 27 pA, P < 0.01 compared with baseline; DHPG + MPEP = 229 ± 29 pA, n = 9, P < 0.01 compared with DHPG alone). Although MPEP did not completely reverse the effects of DHPG in every cell tested, on average, it reduced DHPG induced suppression of the I_AHP. Our result is consistent with that of Ireland and Abraham, (2002) who found that both mGluR1 and mGluR5 mediate IAHP suppression in CA1 neurons and suggests that our inability to block LTD is not caused by inactive MPEP.

Strain variations in LTD and mGluR expression have been noted in mice and rats (Chen et al. 2005; Manahan-Vaughan 2000a,b). To determine if our inability to block LTD with MPEP was caused by the particular rat strain used in our experiments, we tested the ability of MPEP to block LTD in a different strain of rats and in mice. Previous work has shown that MPEP blocks LTD in hippocampal slices prepared from Sprague-Dawley (SD) rats induced with the mixed isomer R,S-DHPG (100 µM; 20 min) or the active form S-DHPG (50 µM; 5 min) (Faas et al. 2002; Huang et al. 2004). Consistent with our data in Long Evans rats, we found that in slices prepared from Sprague-Dawley rats, MPEP (10 µM) had no effect on LTD induced with R,S-DHPG (100 µM; 20 min; MPEP = 60 ± 4%, n = 8; interleaved controls = 62 ± 3%,

\[ n = 8, P = 0.78; \text{Fig. 3B} \] or S-DHPG (50 µM; 5 min; MPEP = 85 ± 9%, n = 2; control = 88 ± 2%, n = 2, P = 0.73). We also saw no effect of MPEP on DHPG-induced LTD (100 µM; 20 min) in slices prepared from C57BL/6 mice (MPEP = 74 ± 5%, n = 8; control = 69 ± 5%, n = 4, P = 0.4%).
0.55; Fig. 3C). Finally, slices (from Long Evans rats) that were perfused with 25 μM MPEP throughout the experiment had normal DHPG-induced LTD (100 μM; 5 min; MPEP = 87 ± 3%, n = 6; control = 84 ± 2%, n = 8, P = 0.44). Therefore we found that, under several experimental conditions in both mice and rats, MPEP was ineffective in blocking mGluR-LTD.

**mGluR1 is required for DHPG-induced decreases in AMPA receptor surface expression**

DHPG treatment of hippocampal neurons results in a rapid endocytosis and persistent decrease in the surface expression of postsynaptic AMPA receptors that are thought to mediate LTD (Nosyreva and Huber 2005; Snyder et al. 2001; Xiao et al. 2001). The mGluR subtype that mediates DHPG-induced decreases in AMPAR surface expression is unknown. To study the role of mGluR1 and mGluR5, we tested the effects of LY367385 and MPEP on DHPG-induced decreases in GluR1 surface expression in slices using receptor biotinylation (Nosyreva and Huber 2005). Slices were preincubated in antagonists (15–30 min) before DHPG or ACSF application and throughout the experiment. Slices were treated with DHPG (100 μM; 5 min), and surface proteins were biotinylated 15 min after DHPG application. Figure 4A shows the time-course for receptor biotinylation overlaid onto the electrophysiological equivalent of Fig. 4D. Like LTD, the combined application of LY367385 and MPEP blocked DHPG-induced decreases in GluR1 surface expression (LY367385 + MPEP + DHPG = 109 ± 12% of control slices, n = 7, P = 0.7; Fig. 4C). Because LTD is reduced by the continued presence of LY367385, but not MPEP, we predicted that decreases in GluR1 surface expression should also rely on mGluR1. Incubation in LY367385, but not MPEP, blocked DHPG-induced decreases in GluR1 surface expression (MPEP + DHPG = 84 ± 4% of condition control, n = 9, P = 0.02; ACSF + DHPG = 77 ± 4%, n = 8, P = 0.02; Fig. 4C; LY367385 + DHPG = 106 ± 12% of condition control, n = 12, P = 0.53; ACSF = 80 ± 5%, n = 10, P < 0.01; Fig. 4D). These results are consistent with our findings that mGluR1 antagonists alone reduce expression of DHPG-LTD (Fig. 2A), whereas blockade of mGluR5 alone has no effect (Fig. 2D).

**Synaptically induced LTD does not require group I mGluR activation**

Previously it was shown that mGluR-dependent LTD can be elicited by synaptic stimulation using PP-LFS (50-ms interstimulus interval; 1 Hz) (Huber et al. 2000; Kemp and Bashir 1999). This conclusion was based on the finding that PP-LFS–induced LTD is blocked by the broad range mGluR antagonist LY341495 (100 μM; Fig. 5E) (Huber et al. 2000). However, there is very little data addressing the specific mGluR(s) required for PP-LFS–induced LTD (but see Faas et al. 2002). We next examined the role of group I mGlRs in synthetically induced LTD using PP-LFS. All experiments were performed in 100 μM d,l-AP5. PP-LFS–induced LTD is normal in mGluR1 KO mice (mGluR1 KO = 85 ± 3%, n = 15; mGluR1 WT littermates = 84 ± 3%, n = 11, P = 0.78; Fig. 5A) and in mGluR5 KO mice (mGluR5 KO = 73 ± 6%, n = 5; mGluR5 WT littermates = 75 ± 6%, n = 6, P = 0.77; Fig. 5B). This result is in stark contrast to the effect of mGluR1 KO (Fig. 2C) and mGluR5 KO (mGluR5 KO = 96 ± 1%, n = 5; mGluR5 WT littermates = 61 ± 4%, P < 0.01; Fig. 5B) (Huber et al. 2001) on DHPG-induced LTD. We next sought to determine if inhibition of both mGluR1 and mGluR5 was necessary to block PP-LFS–induced LTD. Blockade of mGluR1 with 100 μM LY367385 in mGluR5 KO mice had no effect on PP-LFS–induced LTD (mGluR5 KO + LY367385 = 81 ± 5%, n = 10, P = 0.2 compared with mGluR5 KO without antagonists, Fig. 5C). In addition, pharmacological blockade of group I mGlRs in Long Evans rats with 10 μM MPEP and 100 μM LY367385 had no effect on PP-LFS–induced LTD (LY367385 + MPEP = 76 ± 4%, n = 11; interleaved controls = 79 ± 3%, n = 11, P = 0.47; Fig. 5D). In light of the surprising finding that group I mGluR activation is not required for induction of PP-LFS LTD, we confirmed that LY341495 (100 μM) inhibited PP-LFS–induced LTD (100 μM LY341495 = 93 ± 4%, n = 5; interleaved controls = 61 ± 4%, n = 5, P < 0.01; Fig. 5E). Taking this data together suggests that specific blockade of group II and III mGlRs or combined blockade of all three mGluR groups is required to block PP-LFS–induced LTD. While LY341495 inhibits group I, II, and III mGlRs at 100 μM, it is effective against primarily group II and III mGlRs at 20 μM (Capogna, 2004; Kingston et al. 1998). Selective blockade of group II and III mGlRs with 20 μM LY341495 has no effect on PP-LFS LTD in Long Evans rats (20 μM LY341495 = 76 ± 3%, n = 6; Fig. 5F). A cocktail of 10 μM MPEP, 100 μM LY367385, and 20 μM LY341495 was used to block all three mGluR groups and also was ineffective against PP-LFS–induced LTD (20 μM LY341495 + LY367385 + MPEP = 71 ± 5%, n = 6, P = 0.45 compared with 20 μM LY341495 alone; Fig. 5F). These data suggest that blocking all mGlRs may not be sufficient to block PP-LFS LTD and that 100 μM LY341495 may have effects at other as yet unidentified mGlRs or other neurotransmitter receptors. Future experiments are required to determine the identity of these receptors.

**DISCUSSION**

Our data indicate a novel and unexpected role for mGluR1 in DHPG-induced synaptic plasticity at CA1 excitatory synapses. We found that activation of either mGluR1 or mGluR5 can induce the full complement of LTD. Consistent with these data, both mGluR1 and mGluR5 induced activation of ERK, indicative of an induction mechanism different from the mGluR5-specific mechanism described originally by Capogna et al. (2004). Additionally, our data suggest that mGluR1 is required for the induction of LTD. The differences we observed in the induction of LTD in Long Evans rats and mGlR KO mice may be due to species differences or to differences in the endogenous mGluR expression in the different strains of mice. Previous work has shown that mGluR5 is more highly expressed in Long Evans rats than in Sprague-Dawley rats. Future experiments are required to determine if differences in mGluR expression levels are a contributing factor in the differential effects of mGluR5 KO on LTD in these strains.

**FIG. 5.** Group I mGluR activation is not required for synthetically induced LTD. In all experiments, NMDA receptors were blocked with 100 μM d,l-AP5. A–F: average (±SE) initial slope values of FPs normalized to the pre-DHPG or pre-pair-pulse low-frequency stimulation (PP-LFS) baseline. LTD was induced with PP-LFS (2 pulses with 50-ms interstimulus interval at 1 Hz) for 15 min. D–F: or 20 min. A, B, and C or with 20 min of 100 μM DHPG (B2). A: synthetically induced LTD is normal in mglR1 KO mice. B: synthetically induced LTD is normal in mglR5 KO mice (B2). In contrast, LTD induced with 20 min of DHPG is drastically reduced in the mglR5 KO mouse (B2). C: synthetically induced LTD in mglR5 KO mice is not affected by the addition of the mglR1 antagonist LY367385 (100 μM). D: pharmacological blockade of group I mglRs with LY367385 (100 μM) and MPEP (10 μM) has no effect on LTD in Long Evans rats. F: inhibition of groups II and III mglRs with 20 μM LY341495 or inhibition of groups I, II, and III mglRs with 20 μM LY341495 + 100 μM LY367385 + 10 μM MPEP has no effect on synthetically induced LTD.
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A

PP-LFS
WT
mGluR1 KO

FP Slope (% Baseline)

Time from onset of PP-LFS (min.)

B1

PP-LFS
WT
mGluR1 KO

FP Slope (% Baseline)

Time from onset of PP-LFS (min.)

B2

DHPG

FP Slope (% Baseline)

Time from onset of PP-LFS (min.)

C

PP-LFS

FP Slope (% Baseline)

Time from onset of PP-LFS (min.)

D

PP-LFS
Control
LY + MPEP

FP Slope (% Baseline)

Time from onset of PP-LFS (min.)

E

PP-LFS
Control
LY341495

FP Slope (% Baseline)

Time from onset of PP-LFS (min.)

F

PP-LFS
20uM LY-495 + MPEP + LY-385

FP Slope (% Baseline)

Time from onset of PP-LFS (min.)

mGluR1 WT, n=11
mGluR1 KO, n=15

mGluR5 WT, n=6
mGluR5 KO, n=5

100uM LY341495, n=5

20uM LY341495, n=6
20uM LY341495 + LY367385 + MPEP, n=6
which is required for mGluR-LTD (Gallagher et al. 2004). Furthermore, mGluR1 is specifically required for the expression of DHPG-induced LTD and the associated decrease in AMPAR surface expression.

Previous work in the hippocampus and other brain regions has shown that mGluR1 and mGluR5 can mediate similar functions in neurons (Gubellini et al. 2003; Ireland and Abraham 2002; Karim et al. 2001; Lee et al. 2002; Merlin 2002; Rae and Irving 2004). However, in these studies, mGluR1 or mGluR5 blockade alone had a partial or complete effect on the physiological or behavioral measure. Here, we found that blockade of either mGluR1 or mGluR5 had no effect on LTD induction, whereas the combined blockade completely prevented LTD (Fig. 1). This result suggests that mGluR1 and mGluR5 can fully substitute or compensate for each other and both can activate the signaling cascades required for LTD induction. The fact that both mGluR1 and mGluR5 induced ERK activation supports this conclusion and suggests that this level of activation is sufficient for maximal LTD.

In our attempts to block DHPG-induced LTD with MPEP, we tested mice and two different rat strains, used 5 or 20 min of DHPG application, used either R,S-DHPG or S-DHPG, and different durations of MPEP application and two different concentrations (10 and 25 μM). All of these conditions yielded no effect of MPEP on LTD. Like previous studies, we used 10 μM MPEP for most of these experiments, which is almost 300 times the IC50 value for MPEP against mGluR5. Furthermore, MPEP is a noncompetitive antagonist for mGluR5, and at 10 μM, its effects on agonist-stimulated phosphoinositide turnover are saturating. MPEP (10 μM) inhibits >95% of agonist-stimulated phosphoinositide turnover at cloned mGluR5 and >75% in rat hippocampus (Gasparini et al. 1999). We do not think the differences are caused by developmental changes in the mGluR subtype required for LTD, because the age range of our rats and mice is similar as that used in previous studies (3–6 wk) (Faas et al. 2002; Hou and Klann 2004; Huang and Hsu 2006; Huang et al. 2004). Our inability to block DHPG-induced LTD with MPEP cannot be explained by inactive MPEP in our preparation because we observed effects of MPEP on ERK activation (Fig. 1D), suppression of the IAH (Fig. 2), and LTD induction when combined with LY367385 (Fig. 1C). There are other examples or reports that differ in their findings of the contribution of mGluR1 and mGluR5 to other mGluR-dependent functions in CA1 and striatal neurons (Gubellini et al. 2001; Ireland and Abraham 2002; Mannaioni et al. 2001; Rae and Irving 2004; Sung et al. 2001). Although there may be technical reasons for these differences, we suggest that mGluR1 function is either up-regulated or maintained in our preparation so that is able to substitute for mGluR5 during its blockade. In contrast to results with MPEP, LTD induced with a either a brief (50 μM; 5 min) (Huber et al. 2001) or prolonged (100 μM; 20 min) DHPG application is completely absent in mGluR5 KO mice, (Fig. 5B). These results suggest that mGluR1 can compensate for mGluR5 when it is blocked pharmacologically, but not in the absence of mGluR5 protein. Our findings also suggest that there may be alterations in mGluR1 expression, localization, or function in CA1 neurons of mGluR5 KO mice.

Although our data show that mGluR1 and mGluR5 can both induce LTD, we found that only mGluR1 is important for expression of LTD and surface GluR1 decreases (Figs. 2 and 4). This finding is consistent with studies that have found that mGluR1 and mGluR5 mediate distinct functions in neurons (for review, see Valenti et al. 2002). The reversal of DHPG-induced LTD with nonselective mGluR antagonists has been previously shown (Fitzjohn et al. 1999; Palmer et al. 1997; Watabe et al. 2002). Data with pharmacological blockade or genetic knockout of mGluR1 indicate that activation of mGluR1 is important for the expression of LTD (Fig. 2). These data suggest that persistent mGluR1 activity contributes to LTD expression and that there is another component of LTD expression that does not require group I mGluR activity. Other studies have discovered that mGluR1 activity is required for the expression of long-term potentiation in medial vestibular neurons and epileptiform bursts in CA3, suggesting that mGluR1 may be a common mechanism to sustain mGluR-dependent plasticity in the brain (Grassi et al. 2002; Merlin 2002). Our findings support studies that have shown a role for mGluR1 in hippocampal-dependent learning and suggest that LTD in CA1 may contribute to these behaviors (Aiba et al. 1994; Maciejak et al. 2003; Petersen et al. 2002).

Previous work using single cell recordings of CA1 pyramidal neurons has shown that mGluR1 has many functions in these neurons including acute depression of EPSCs, cell depolarization (or inward current), increases in intracellular [Ca2+], and suppression of IAHP (Ireland and Abraham 2002; Mannaioni et al. 2001; Rae and Irving 2004). Furthermore, an immunohistochemical study reported that DHPG induced phosphorylation of ERK in CA1 pyramidal cell bodies and was inhibited by LY367385 or MPEP, consistent with our Western blotting results (Berkeley and Levey 2003). These studies and our present work have relied on the specificity of LY367385 to make conclusions regarding mGluR1 function (Clark et al. 1997; Valenti et al. 2002). The fact that LTD reversal is not observed with LY367385 in the mGluR1 KO mouse strongly supports that its effects are specific for mGluR1 (Fig. 2C). Many studies have shown a functional role of mGluR1 in CA1 pyramidal neurons, but showing the presence of mGluR1 protein has been more elusive (Ferraguti et al. 2004; Lujan et al. 1996; Martin et al. 1992). The many functions attributed to mGluR1 in CA1 neurons may be mediated by a low diffuse expression of mGluR1. Alternatively, the currently available antibodies may not detect the relevant mGluR1 isoforms.

We and others have previously shown that DHPG results in an endocytosis and persistent decrease in the surface expression of AMPARs that is thought to mediate LTD in mature neurons (Nosyreva and Huber 2005; Snyder et al. 2001; Xiao et al. 2001). Here we observed that mGluR1 blockade completely blocked DHPG-induced decreases in GluR1 surface expression (Fig. 4). From our LTD data, we expected that LY367385 would only partially block the decrease in GluR1 surface expression. It is likely that we are unable to detect partial effects of LY367385 on GluR1 surface expression because of large variability in the receptor biotinylation assay compared with LTD measurements. Alternatively, the mGluR1-dependent GluR1 endocytosis may only mediate one-half of the LTD. Based on the effects of mGluR1 blockade on LTD (Fig. 4A), we interpret this result as an effect of LY367385 on the expression (as opposed to induction) of GluR1 surface decreases.

Results to date indicate that DHPG- and PP-LFS–induced LTD (in AP5) represent the same LTD mechanism. Both are
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blocked by LY341495, absent in the Gq KO mouse, rely on protein synthesis and ERK activation, are enhanced in the fragile X syndrome mouse model, and are similarly developmentally regulated (Huber et al. 2000, 2001, 2002; Nosyreva and Huber 2005; Zhu et al. 2002). These data and the fact that PP-LFS–induced LTD occludes DHPG-induced LTD indicate that the two forms of plasticity converge on a common protein synthesis–dependent mechanism (Huber et al. 2001). Because PP-LFS–induced LTD is absent in Gq knockout mice, it is possible that other Gq-coupled neurotransmitter receptors are sufficient to induce LTD when group 1 mGlurRs are blocked (Kirkwood et al. 1999; Kleppisch et al. 2001; Scheiderer et al. 2004). It is unclear why 100 μM LY341495 blocked PP-LFS–induced LTD. At high concentrations, LY341495 may have nonspecific effects at these other receptor types or there are other mGlur subtypes yet to be identified (Fitzjohn et al. 1998).

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