Postsynaptic Induction and Presynaptic Expression of Group 1 mGluR-Dependent LTD in the Hippocampal CA1 Region

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Watabe, Ayako M., Holly J. Carlisle, and Thomas J. O’Dell. Postsynaptic induction and presynaptic expression of group 1 mGluR-dependent LTD in the hippocampal CA1 region. J Neurophysiol 87: 1395–1403, 2002; 10.1152/jn.00723.2001. Activation of metabotropic glutamate receptors (mGluRs) with the group I mGluR selective agonist (R,S)-3,5-dihydroxyphenylglycine (DHPG) induces a long-term depression (LTD) of excitatory synaptic transmission in the CA1 region of the hippocampus. Here we investigated the potential roles of pre- and postsynaptic processes in the DHPG-induced LTD at excitatory synapses onto hippocampal pyramidal cells in the mouse hippocampus. Activation of mGluRs with DHPG, but not ACPD, induced LTD at both Schaffer collateral/commissural fiber synapses onto CA1 pyramidal cells and at associational/commissural fiber synapses onto CA3 pyramidal cells. DHPG-induced LTD was blocked when the G-protein inhibitor guanosine-5′-[2-thiodiphosphate] was selectively delivered into postsynaptic CA1 pyramidal cells via an intracellular recording electrode, suggesting that DHPG depresses synaptic transmission through a postsynaptic, GTP-dependent signaling pathway. The effects of DHPG were also strongly modulated, however, by experimental manipulations that altered postsynaptic calcium influx. In these experiments, we found that elevating extracellular Ca2+ concentrations ([Ca2+]o) to 6 mM almost completely blocked the effects of DHPG, whereas lowering [Ca2+]o to 1 mM significantly enhanced the ability of DHPG to depress synaptic transmission. Enhancing Ca2+ influx by prolonging action potential duration with bath applications of the K+ channel blocker 4-aminopyridine (4-AP) also strongly reduced the effects of DHPG in the presence of normal [Ca2+]o (2 mM). Although these findings indicate that alterations in Ca2+-dependent signaling processes strongly regulate the effects of DHPG on synaptic transmission, they do not distinguish between potential pre- versus postsynaptic sites of action. We found, however, that while inhibiting both pre- and postsynaptic K+ channels with bath-applied 4-AP blocked the effects of DHPG; inhibition of postsynaptic K+ channels alone with intracellular Cs+ and TEA had no effect on the ability of DHPG to inhibit synaptic transmission. This suggests that presynaptic changes in transmitter release contribute to the depression of synaptic transmission by DHPG. Consistent with this, DHPG induced a persistent depression of both AMPA and N-methyl-D-aspartate receptor-mediated components of excitatory postsynaptic currents in voltage-clamped pyramidal cells. Together our results suggest that activation of postsynaptic mGluRs suppresses transmission at excitatory synapses onto CA1 pyramidal cells through presynaptic effects on transmitter release.

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INTRODUCTION

Activation of G-protein-coupled, group I metabotropic glutamate receptors (mGluRs) by either synaptically released glutamate (Bolshakov and Siegelbaum 1994; Kemp and Bashir 1999; Oliet et al. 1997; Otani and Connor 1998) or by pharmacological agonists such as (R,S)-3,5-dihydroxyphenylglycine (DHPG) (Fitzjohn et al. 1999; Huber et al. 2000, 2001; Kleppisch et al. 2001; Palmer et al. 1997) elicits a long-term depression (LTD) of synaptic transmission at excitatory synapses onto hippocampal CA1 pyramidal cells. DHPG also induces LTD at medial perforant path synapses onto granule cells in the dentate gyrus (Comodeca et al. 1999). While recent reports have shown that DHPG-induced LTD in the hippocampal CA1 region is dependent on activation of Gα-type G proteins (Kleppisch et al. 2001) and protein synthesis (Huber et al. 2000), relatively little is known about the mechanisms responsible for DHPG-induced LTD in the hippocampal CA1 region (however, see Schnabel et al. 1999a,b, 2001). Indeed, it is not yet clear whether the synaptic depression induced by DHPG in hippocampal CA1 pyramidal cells arises from pre- and/or postsynaptic mechanisms. Several findings, including the postsynaptic localization of group I mGluRs at these synapses (Luján et al. 1996; Romano et al. 1995; Shigemoto et al. 1997) and the fact that postsynaptic injection of protein synthesis inhibitors into CA1 pyramidal cells blocks DHPG-induced LTD (Huber et al. 2000), indicate that postsynaptic mechanisms are primarily involved. Other findings suggest, however, that activation of group I mGluRs with DHPG depresses transmission through a presynaptic inhibition of transmitter release (Gereau and Conn 1995; Herrero et al. 1998; Mannaioni et al. 2001; Manzoni and Bockaert 1995; Rodriguez-Moreno et al. 1998). Finally, DHPG-induced LTD might depend on both pre- and postsynaptic processes because studies using hippocampal slices from young rats suggest that a combination of postsynaptic induction and presynaptic expression is involved in mGluR-dependent forms of LTD induced by synaptic stimulation (Bolshakov and Siegelbaum 1994; Oliet et al. 1997). Here we investigated the synaptic locus of DHPG-induced LTD in the hippocampal CA1 region by using a combination of postsynaptic injections of pharmacological reagents to examine the role of postsynaptic processes along with extracellular manipulations designed to probe the potential involvement of...
presynaptic mechanisms. Our results suggest that activation of postsynaptic mGluRs with DHPG depresses excitatory synaptic transmission through changes in presynaptic Ca\(^{2+}\) signaling and thus indicate that retrograde signaling via an as yet unidentified messenger(s) has an important role in this form of LTD.

**Methods**

Standard techniques were used to prepare 400-μm-thick hippocampal slices from tissue obtained from 5- to 7-wk-old male C57BL/6 mice that had been anesthetized with halothane prior to being killed by cervical dislocation. Slices were maintained at 30°C in an interface-type chamber (Fine Science Tools, Foster City, CA) that was perfused with an oxygenated (95% \(O_2\)-5% \(CO_2\)) mouse artificial cerebrospinal fluid (ACSF) consisting of (in mM) 124 NaCl, 4.4 KCl, 25 NaHCO\(_3\), 1.0 NaH\(_2\)PO\(_4\), 1.2 MgSO\(_4\), 2.0 CaCl\(_2\), and 10 glucose (flow rate = 2–3 mL/min). Slices were allowed to recover for \(\geq 2\) h prior to an experiment. For an experiment, a slice was placed in a submerged-slice recording chamber and a bipolar stimulating electrode was used to activate Schaffer collateral/commissural (SC) fiber synapses onto CA1 pyramidal cells and associational/commissural fiber synapses (AC) onto CA3 pyramidal cells. The resulting field excitatory postsynaptic potentials (fEPSPs) were recorded using low-resistance glass microelectrodes (5–10 MΩ, filled with ACSF) placed in stratum radiatum of either the CA1 or CA3 region. Presynaptic fiber stimulation pulses were delivered at 0.02 Hz using a stimulation intensity that evoked fEPSPs that were 50% of the maximal fEPSP amplitude.

Whole cell current-clamp recordings were used to record EPSPs from individual CA1 pyramidal cells and to introduce reagents selectively into postsynaptic cells. Low-resistance electrodes (2–5 MΩ, access resistance ranged from 9 to 28 MΩ) were filled with a solution containing (in mM) 127.5 K-glucuronate, 17.5 KCl, 1.0 MgCl\(_2\), 0.2 EGTA, 10 HEPES, 2 Mg-ATP, and 0.3 GTP (pH = 7.2). In some experiments, we used an electrode-filling solution designed to block postsynaptic K\(^+\) channels that contained (in mM) 122.5 Cs-glucuronate, 17.5 CsCl, 1.0 TEA, 0.2 EGTA, 10 HEPES, 2 Mg-ATP, and 0.3 GTP (pH = 7.2). If needed, cells were hyperpolarized to between −70 and −75 mV using constant current injection through the recording electrode. Throughout the experiment a 50-ms-long pulse of hyperpolarizing current (0.1 nA) was injected 150 ms after each EPSP to monitor input and access resistance. Presynaptic fiber stimulation pulses were delivered once every 20 s using a stimulation intensity sufficient to evoke EPSPs between 10 and 15 mV in amplitude. Whole cell recordings were maintained for \(\geq 18–20\) min before DHPG application to allow diffusion of substances in the electrode solution into the cell.

Whole cell voltage-clamp recordings were used to record excitatory postsynaptic currents (EPSCs) in CA1 pyramidal cells. In these experiments, the slices were bathed in a modified ACSF containing 100 μM picrotoxin to block GABA\(_A\) receptor-mediated inhibitory postsynaptic currents, and the concentration of KCl was lowered to 2.4 mM. In addition, the CA3 region of the slice was removed to prevent bursting. Patch-clamp electrodes were filled with the Cs-glucuronate electrode-filling solution described in the preceding text, and cells were voltage-clamped at −60 mV. Presynaptic stimulation pulses were delivered once every 20 s and a 30-ms hyperpolarizing voltage step (−2 mV) was delivered 50 ms before each pulse of synaptic stimulation to monitor input and access resistance throughout the experiment. 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 μM) was added to the ACSF to block the AMPA receptor-mediated component of EPSCs in experiments where the effects of DHPG on NMDA receptor-mediated EPSCs were investigated.

Data acquisition and analysis were performed using Experimenters’ Workbench and Common Processing software package (Data Wave Technologies, Longmont, CO). All values are reported as means ± SE. Unless indicated otherwise, a 10-min bath application of 100 μM DHPG was used in all experiments to reliably induce a robust and persistent depression of synaptic transmission. The effects of DHPG on synaptic transmission were assessed using the average size of synaptic responses recorded over the last 5 min of a 10-min application to determine the initial effects of DHPG on synaptic transmission. The average size of synaptic responses recorded between 25 and 30 min after DHPG washout was used to determine the persistent effects of DHPG on synaptic transmission. Paired t-tests and one-way repeated-measure ANOVAs (followed by Dunnett’s test comparisons) were used to determine statistical significance for within-group comparisons. Unpaired t-tests or, where appropriate, one-way ANOVAs (followed by Dunnett’s tests) were used for between-group comparisons.

DHPG, (1S,3R)-1-aminoxycyclopentane-1,3-dicarboxylic acid (ACPD), and 2S-2-amino-2-(1S,2S-2-carboxycyclopropyl-1-yl)-(xanth-9-yl) propanic acid (LY341495) were obtained from Tocris Cookson (Ballwin, MO). All other compounds were from Sigma (St. Louis, MO).

**Results**

As reported previously (Fitzjohn et al. 1999; Palmer et al. 1997), activation of mGluRs with DHPG induced a strong depression of synaptic transmission at Schaffer collateral/commissural fiber synapses in area CA1 that persisted for \(\geq 30\) min following DHPG washout (Fig. 1A). In contrast, the mGluR agonist ACPD induced only a transient suppression of synaptic transmission that fully recovered when ACPD was washed from the recording chamber with agonist-free ACSF (Fig. 1B). DHPG, but not ACPD, also induced LTD at associational/commissural fibers synapses onto pyramidal cells in the CA3 region (Fig. 1, C and D). While the effects of DHPG on synaptic transmission in both the CA1 and CA3 regions were strongly inhibited by a high concentration of the mGluR antagonist LY341495 (Figs. 1, A and C, and 2A), the N-methyl-D-aspartate (NMDA) receptor antagonist d,L-2-amino-5-phosphonovaleric acid (APV, 100 μM) had no effect on DHPG-induced LTD in the CA1 region (n = 5, data not shown). Thus similar to previous observations in rat hippocampus (Huber et al. 2001; but see Palmer et al. 1997), activation of mGluRs with DHPG induces an NMDA receptor-independent form of LTD in mouse hippocampal slices.

The ability of LY341495 to inhibit both the transient and persistent depression of synaptic transmission induced by DHPG indicates that activation of mGluRs is required for the induction of this form of LTD. To examine whether mGluR activation is also required for the expression and/or maintenance of DHPG-induced LTD, we investigated whether blocking mGluRs with the LY341495 had any effect on the depression of synaptic transmission induced by prior DHPG application. In both the CA3 and CA1 regions, the depression of synaptic transmission induced by DHPG was strongly reversed when LY341495 was applied for 10 min beginning 20 min after DHPG was washed out of the recording chamber (Fig. 2, B–D). This result, which is similar to previous findings in rat hippocampal slices (Fitzjohn et al. 1998; Palmer et al. 1997), indicates that activation of mGluRs is required for the expression of DHPG-induced LTD. Following washout of the LY341495, however, the synaptic depression was re-established nearly intact (Fig. 2). Thus persistent activation of mGluRs does not seem to be required for the maintenance of DHPG-induced LTD because LTD reappears after mGluR
antagonists are washed from the recording chamber (see also Fitzjohn et al. 1998; Palmer et al. 1997).

To determine whether activation of postsynaptic mGluRs is specifically required for both the initial and persistent inhibition of synaptic transmission induced by DHPG, we performed whole cell recordings from CA1 pyramidal cells using a potassium gluconate-based electrode-filling solution where GDP was replaced with 1 mM guanosine-5′-O-(2-thiodiphosphate) (GDPβS) to inhibit activation of postsynaptic G proteins. While DHPG induced a robust and persistent depression of synaptic transmission in interleaved control recordings with GDP containing solutions, only a small initial depression and no persistent depression was observed in recordings where GDPβS was present in the electrode-filling solution (Fig. 3A). To control for the possibility that loading postsynaptic cells with GDPβS by itself might have effects on synaptic transmission, we monitored evoked EPSPs for ≥60 min starting within 1 min after obtaining whole cell recordings with GDPβS-containing electrodes. In all cells studied (n = 5), EPSPs grew dramatically (~4-fold on average) during the first 5–10 min of whole cell recording with GDPβS-containing electrodes perhaps due to effects of GDPβS on AMPA receptor endocytosis.
FIG. 3. Blocking postsynaptic G proteins with guanosine-5′-O-(2-thiodiphosphate) (GDPβS) suppresses both the initial and persistent synaptic depression induced by DHPG. A: whole cell current-clamp recordings were used to record EPSPs from individual CA1 pyramidal cells using electrode-filling solutions that contained either GTP (○, n = 12) or 1.0 mM GDPβS (●, n = 6). In cells where the electrode-filling solution contained GTP, EPSPs were depressed to 47.1 ± 3.2% of baseline in the presence of DHPG (indicated by ●) and were still depressed to 57.4 ± 5.2% of baseline 30 min after DHPG washout. DHPG had little effect on synaptic transmission in interleaved experiments where the electrode-filling solution contained GDPβS (EPSPs were 91.4 ± 4.3% of baseline in the presence of DHPG and 112.9 ± 2.6% of baseline following DHPG washout). Inset: example EPSPs (average of 3 responses) recorded during baseline and 30 min after DHPG washout in a control experiment (left) and in an experiment where GDPβS was present in the electrode (right). Calibration bars are 2.5 mV and 15 ms. B: postsynaptic GDPβS does not block the inhibition of excitatory synaptic transmission induced by bath application of adenosine. EPSPs were depressed 27 ± 6.5% of baseline during the last 5 min of a 10-min application of 100 μM adenosine in control cells (○, n = 7) and were depressed to 20.4 ± 5% of baseline in cells where the patch-clamp electrode was filled with a solution containing 1.0 mM GDPβS (●, n = 8). Inset: EPSPs (average of 3 responses) recorded before and at the end of the adenosine application in control experiments (left) and in experiments where GDPβS was present in the electrode solution (right). Calibration bars are 2.5 mV and 10 ms.

(Lüscher et al. 1999) and/or clearance from the slice of substances that leaked out of the electrode before seal formation. Following this initial “run-up,” however, synaptic transmission was stable for the remainder of the experiment. When normalized to a baseline determined by the average size of EPSPs recorded between 10 and 20 min of whole cell recording (which corresponds to the baseline used in the experiments shown in Fig. 3A), EPSPs recorded after 25–30 min of whole cell recording were 98.5 ± 3.3% of baseline, whereas those recorded after 55–60 min of whole cell recording were 107.4 ± 4.3% of baseline. This indicates that loading postsynaptic cells with GDPβS does not induce a slow onset enhancement of synaptic transmission that might mask a DHPG-induced depression. As an additional control, we also examined the effects of postsynaptic GDPβS on the inhibition of synaptic transmission by adenosine, which is known to regulate synaptic transmission at excitatory synapses in the CA1 region through presynaptic effects on transmitter release (Lupica et al. 1992; Prince and Stevens 1992; Scholz and Miller 1991; Wu and Saggu 1994). While postsynaptic GDPβS completely prevented the postsynaptic hyperpolarization and decrease in input resistance induced by adenosine (data not shown), it had no effect on the depression of synaptic transmission induced by a 10-min bath application of 100 μM adenosine (Fig. 3B). Together, these results suggest that both the initial and the persistent depression induced by DHPG are dependent on postsynaptic, GTP-dependent signaling pathways.

Previous studies suggest that activation of group I mGluRs with DHPG depresses synaptic transmission through presynaptic effects on transmitter release, perhaps due to an inhibition of voltage-sensitive Ca2⁺ channels (Gereau and Conn 1995; Herrera et al. 1998; Rodriguez- Moreno et al. 1998). Thus although our experiments with postsynaptic injection of GDPβS indicated that postsynaptic processes are responsible for the synaptic depression induced by DHPG, we also investigated whether changes in presynaptic Ca2⁺ signaling might be involved. In these experiments, we used the approach outlined by Wheeler et al. (1996) in their study of the role of specific subtypes of voltage-dependent Ca2⁺ channels in synaptic transmission at Schaffer collateral/commissural fiber synapses onto CA1 pyramidal cells. As described by Wheeler et al. (1996), the instantaneous Ca2⁺ flux into the presynaptic terminals (F) can be described by the equation

\[ F = N_{tot} \cdot P_o \cdot i_{Ca^{2+}}. \]

where \( N_{tot} \) is the total number of Ca2⁺ channels, \( P_o \) is the probability of channel opening, and \( i_{Ca^{2+}} \) is the Ca2⁺ flux through a single channel. While we have not used this relationship in a quantitative manner to test for potential presynaptic effects of DHPG on synaptic transmission, the fact that the relationship in Eq. 1 is multiplicative indicates that a constant value of \( F \) can be maintained if changes in one parameter are compensated for by changes in the other parameters. Thus Eq. 1 makes several qualitative predictions regarding how the DHPG-induced depression of synaptic transmission might be affected by experimental manipulations of \( N_{tot} \), \( P_o \), and/or \( i_{Ca^{2+}} \) if DHPG depresses synaptic transmission through effects on presynaptic calcium signaling. For instance, if DHPG acts by decreasing \( P_o \) and/or reducing the total number of channels available for activation (\( N_{tot} \)), Eq. 1 predicts that increasing \( i_{Ca^{2+}} \) by increasing extracellular Ca2⁺ concentrations ([Ca2⁺]o) should be able to attenuate the DHPG-induced depression of synaptic transmission. Conversely, under conditions of low-[Ca2⁺]o (where \( i_{Ca^{2+}} \) is reduced), decreases in \( P_o \) or \( N_{tot} \) induced by DHPG should have an even more dramatic effect on synaptic transmission as now even small changes in either parameter could be sufficient to
reduce Ca\(^{2+}\) influx to levels below that needed to support transmitter release.

Because adenosine depresses synaptic transmission by inhibiting presynaptic calcium channels (Wu and Saggu 1994, 1997), we first examined the effects of changes in \(i_{\text{Ca}^{2+}}\) on the depression of synaptic transmission induced by adenosine to determine whether this approach might be useful for examining potential presynaptic changes in DHPG-induced LTD. In these experiments, we examined the effects of adenosine (25 \(\mu M\)) on synaptic transmission in slices bathed in a modified ACSF where the concentration of CaCl\(_2\) was either decreased to 1.0 mM (low-Ca\(^{2+}\) ACSF) or increased to 6.0 mM (high-Ca\(^{2+}\) ACSF). As expected from previous work showing that adenosine inhibits transmitter release at these synapses through effects on presynaptic calcium channels and as predicted by Eq. 1, the depression induced by adenosine was significantly suppressed in slices bathed in high-Ca\(^{2+}\) ACSF and significantly enhanced in slices bathed in a low-Ca\(^{2+}\) ACSF (Fig. 4A). Changes in extracellular Ca\(^{2+}\) levels had a similar and even more striking affect on the ability of DHPG to inhibit synaptic transmission. As shown in Fig. 4, B and C, both the initial and persistent depression induced by DHPG were dramatically enhanced in slices bathed in low-Ca\(^{2+}\) ACSF. Conversely, when slices were bathed in high-Ca\(^{2+}\) ACSF, both phases of the DHPG-induced depression were almost completely blocked (Fig. 4, B and C). Importantly, Eq. 1 predicts that if high extracellular Ca\(^{2+}\) inhibits the effects of DHPG by producing an increase in \(i_{\text{Ca}^{2+}}\) that compensates for a DHPG-induced inhibition of presynaptic Ca\(^{2+}\) signaling, then manipulations that decrease Ca\(^{2+}\) channel activity in the presence of high [Ca\(^{2+}\)]\(_{\text{yr}}\) should restore the ability of DHPG to depress synaptic transmission. Consistent with this we found that DHPG was able to induce a significant initial and persistent depression of synaptic transmission in slices bathed in high-Ca\(^{2+}\) ACSF that also contained the Ca\(^{2+}\) channel blocker Cd\(^{2+}\) (50 \(\mu M\); Fig. 4D).

Increasing the duration of presynaptic action potentials with the potassium channel blocker 4-aminopyridine (4-AP) attenuates the effects of voltage-sensitive Ca\(^{2+}\) channel blockers on transmitter release at excitatory synapses onto CA1 pyramidal cells (Wheeler et al. 1996). This occurs because the increase in \(P_{\text{Ra}}\) of presynaptic Ca\(^{2+}\) channels produced by prolonging action potentials with 4-AP presumably compensates for the decrease in \(N_{\text{out}}\) produced by blocking specific Ca\(^{2+}\) channel subtypes (Wheeler et al. 1996). Similarly we found that the presynaptic inhibition of excitatory synaptic transmission induced by adenosine (25 \(\mu M\)) was significantly reduced in slices bathed in ACSF containing 100 \(\mu M\) 4-AP (in control experiments, a 10-min bath application of ACSF containing 25 \(\mu M\) adenosine reduced fEPSPs to 42 \pm 8.5\% of baseline, \(n = 7\), while fEPSPs were reduced to only 83.1 \pm 5\% of baseline in 4-AP-treated slices, \(n = 5\), \(P < 0.01\) compared with control). Thus to further explore whether DHPG depresses synaptic transmission through effects on presynaptic Ca\(^{2+}\) signaling, we examined the effects of 4-AP on the ability of DHPG to inhibit synaptic transmission. Equation 1 predicts that increasing \(P_{\text{Ra}}\) by prolonging presynaptic action potentials with 4-AP should inhibit the depression induced by DHPG if DHPG depresses synaptic transmission by inhibiting presynaptic Ca\(^{2+}\) signaling. Consistent with this prediction, both the initial and persistent inhibition of synaptic transmission induced by DHPG were blocked in slices bathed in normal ACSF containing 100 \(\mu M\) 4-AP (Fig. 5A1). Moreover, Eq. 1 predicts that if 4-AP acts by producing an increase in \(P_{\text{Ra}}\) that compensates for a DHPG-induced inhibition of presynaptic Ca\(^{2+}\) signaling,
then manipulations that decrease $I_{\text{Ca}^{2+}}$ should offset the effects of 4-AP on $P_o$ and restore the ability of DHPG to depress synaptic transmission. As predicted, DHPG was able to induce a significant initial and persistent depression of synaptic transmission in the presence of 4-AP when $I_{\text{Ca}^{2+}}$ was reduced by lowering $[\text{Ca}^{2+}]_o$ to 0.5 mM (Fig. 5A).

Although the results from our experiments with 4-AP and changes in extracellular $\text{Ca}^{2+}$ are consistent with the hypothesis that activation of group I mGluRs with DHPG depresses synaptic transmission by modulating $\text{Ca}^{2+}$ channel activity, they do not rule out the possibility that DHPG might instead regulate presynaptic $\text{Ca}^{2+}$ signaling indirectly via an enhancement of presynaptic $K^+$ channels and/or a decrease in the $\text{Ca}^{2+}$ sensitivity of presynaptic proteins involved in transmitter release. Perhaps more importantly, these experiments do not directly demonstrate that 4-AP and changes in $[\text{Ca}^{2+}]_o$ modify the effects of DHPG by altering $\text{Ca}^{2+}$ influx through pre- as opposed to postsynaptic $\text{Ca}^{2+}$ channels. To address this second possibility, we examined whether blocking postsynaptic, but not presynaptic, $K^+$ channels by introducing $K^+$ channel blockers into postsynaptic CA1 pyramidal cells through a patch-clamp electrode could block the inhibition of synaptic transmission by DHPG. We did not attempt to use an electrode-filling solution containing 4-AP in these experiments because 4-AP is membrane permeant and thus could potentially diffuse out of the postsynaptic cell to affect presynaptic $K^+$ channels. Instead, we examined whether the $K^+$ channel blockers $\text{Cs}^+$ or TEA might be useful for comparing how DHPG-induced LTD is affected when the same $K^+$ channel blocker is bath applied to inhibit pre- and postsynaptic $K^+$ channels or delivered intracellularly through the recording electrode to inhibit only postsynaptic $K^+$ channels. To find concentrations of bath-applied $\text{Cs}^+$ and TEA that had presynaptic effects similar to those produced by 100 $\mu$M 4-AP, we first determined the effects of 4-AP on the presynaptic fiber volley in slices where synaptic transmission was blocked by bathing slices in a modified ACSF containing 0 mM CaCl$_2$ and 10 mM MgSO$_4$. Under these conditions, a continuous application of 100 $\mu$M 4-AP induced a rapid increase in the duration of the presynaptic fiber volley that stabilized within 15–20 min after exposure to 4-AP. Forty minutes after slices were switched into ACSF containing 100 $\mu$M 4-AP the amplitude of the fiber volley was unchanged but its duration was increased to 160 ± 9% of baseline ($n = 5$). While 20–25 mM TEA produced a similar change in fiber volley duration ($n = 4$), we could not test the effects of bath-applied TEA on DHPG-induced LTD because we were unable to obtain stable baseline tEPSP recordings in slices bathed in normal ACSF containing TEA. We were also unable to determine whether inhibiting pre- and postsynaptic $K^+$ channels with bath-applied $\text{Cs}^+$ altered the effects of DHPG on synaptic transmission because we were unable to find a concentration of $\text{Cs}^+$ that produced a stable change in the fiber volley similar to that induced by 4-AP. Intracellular $\text{Cs}^+$ and TEA will, however, block several different types of postsynaptic $K^+$ channels (Chen and Wong 1992; Velumian et al. 1993), including the A-type channels most likely inhibited by bath application of 4-AP (see Brown et al. 1990; Storm 1990 for reviews). Thus we examined the effects of DHPG on synaptic transmission in cells where the electrode-filling solution contained $\text{Cs}^+$ and TEA. As can be seen from the results shown in Fig. 5B and the comparison
shown in Fig. 5C, the DHPG-induced depression of synaptic transmission observed in cells where whole cell recordings were performed with electrodes containing Cs⁺ and TEA was the same as that seen in cells where the recording electrode was filled with a K⁺-based solution. In contrast, bath application of 4-AP strongly suppressed the DHPG-induced depression of synaptic transmission in cells where the recording electrode solution contained Cs⁺ and TEA (Fig. 5B). Thus while bath application of a low concentration of a K⁺ channel blocker, which will inhibit both pre- and postsynaptic K⁺ channels, strongly inhibits the depression of synaptic transmission by DHPG, selectively blocking postsynaptic K⁺ channels alone has no effect. This suggests that inhibition of presynaptic, rather than postsynaptic, K⁺ channels is responsible for the ability of 4-AP to oppose the DHPG-induced depression of synaptic transmission and supports the notion that pharmacological activation of group I mGluRs depresses synaptic transmission through presynaptic effects.

To further explore the potential role for presynaptic changes in LTD induced by DHPG, we performed two additional experiments. First, we examined paired-pulse facilitation (using an inter-pulse interval of 50 ms) before, during, and after application of 100 μM DHPG for 10 min. While previous studies have found effects of DHPG on paired-pulse facilitation in hippocampal neurons (Greene and Conn 1995; Mannion et al. 2001), we observed no consistent effect of DHPG on paired-pulse facilitation of fEPSPs in either the presence of DHPG or following washout (n = 9, data not shown). Although this argues against a presynaptic locus for the depression induced by DHPG, studies at other synapses where mGluR agonists inhibit synaptic transmission through presynaptic effects have also failed to find consistent effects on paired-pulse facilitation (Barnes-Davies and Forsythe 1995). Thus as an additional test we examined whether NMDA, as well as AMPA, receptor-mediated synaptic currents were depressed by DHPG. If DHPG inhibits synaptic transmission through a presynaptic inhibition of transmitter release, then one prediction is that both the NMDA and AMPA receptor-mediated components of the postsynaptic excitory currents recorded in CA1 pyramidal cells should be depressed. Consistent with this we found that a 5-min application of 100 μM DHPG induced a nearly identical and persistent depression of both AMPA and NMDA receptor-mediated EPSCs in voltage-clamped pyramidal cells (Fig. 6).

**Discussion**

Similar to previous observations in rat hippocampal slices (Palmer et al. 1997), our results show that DHPG, but not the broader spectrum mGluR agonist ACPD, induces a mGluR-dependent and NMDR-independent form of LTD of synaptic transmission in the CA1 and CA3 regions of the mouse hippocampus. Moreover, as has been observed in the rat hippocampus (Fitzjohn et al. 1998), the persistent depression of synaptic transmission induced by DHPG could be reversed by application of the mGluR antagonist LY341495, suggesting that activation of mGluR is not only required for the induction of DHPG-induced LTD but also for its expression. One possible interpretation of this second finding is that the persistent depression of synaptic transmission induced by DHPG is simply due to poor washout of the agonist from the slice. Two of our observations suggest that this is unlikely. First, after the expression of DHPG-induced LTD had been blocked with LY341495, LTD was reestablished when the antagonist was washed from the recording chamber. This suggests that DHPG-induced LTD is not simply due to activation of mGluRs by persistently bound DHPG because displacing DHPG from the receptors with LY341495 should facilitate DHPG washout and block both the expression and maintenance of the depression. Second, following co-application of DHPG and LY341495, synaptic transmission was not depressed when both compounds were washed from the recording chamber (Fig. 2) as would be expected if DHPG remained in the slice long after washing with drug-free ACSF. Together, these findings are more consistent with the recent suggestion that DHPG induces an “autopotentiation” of mGluRs (Merlin and Wong 1997; Schnabel et al. 2001). In this model, activation of mGluRs with DHPG induces a lasting change in mGluRs that can now persistently activate mGluRs and depress synaptic transmission. The mechanisms that might underlie such a process are currently unknown. Recent results showing that inhibitors of the calcium/calmodulin-dependent protein kinase CamKII (Schnabel et al. 1999b) and protein phosphatases (Schnabel et al. 2001) can modulate DHPG-induced LTD
suggest, however, that changes in protein phosphorylation have an important role.

While the induction of mGluR-dependent forms of LTD by low-frequency patterns of synaptic stimulation occurs in the postsynaptic CA1 pyramidal cells, presynaptic changes in transmitter release are responsible, at least in part, for the expression of this form of LTD (Bolshakov and Siegelbaum 1994; Oliet et al. 1997). Consistent with these findings, our results suggest that the persistent depression of synaptic transmission induced by pharmacological activation of group I mGluRs with DHPG is dependent on postsynaptic GTP-dependent signaling pathways but is also strongly affected by experimental manipulations (altered \([Ca^{2+}]_o\), and 4-AP) that affect presynaptic \(Ca^{2+}\) influx. Although the dramatic effects of changes in \([Ca^{2+}]_o\) on the depression induced by DHPG are consistent with the notion that DHPG depresses transmission via effects on presynaptic \(Ca^{2+}\) signaling, changes in the concentration of extracellular divalent cations will also strongly affect neuronal excitability. Because under some conditions the ability of DHPG to depress synaptic transmission can be facilitated by manipulations that enhance neuronal excitability (Palmer et al. 1997), an alternative explanation is that elevating \([Ca^{2+}]_o\) inhibits the DHPG-induced depression of synaptic transmission by decreasing neuronal excitability while reducing \([Ca^{2+}]_o\) facilitates the effects of DHPG by enhancing excitability. Two of our findings suggest, however, that this is not the case. First, the addition of a low concentration of \(Cd^{2+}\) to the high-\(Ca^{2+}\) ACSF restored the ability of DHPG to induce a significant inhibition of synaptic transmission in high-\(Ca^{2+}\) ACSF even though \(Cd^{2+}\) should depress excitability even further. Second, the finding that 4-AP inhibits the ability of DHPG to depress synaptic transmission also seems more consistent with the interpretation that changes in \([Ca^{2+}]_o\) modify the ability of DHPG to depress synaptic transmission through effects on \(Ca^{2+}\) signaling. According to Eq. 1, if both the initial and persistent depression induced by DHPG arise from an inhibition of presynaptic \(Ca^{2+}\) signaling, then broadening the duration of presynaptic action potentials with 4-AP should have an effect similar to increasing \(i_{Ca}\) by elevating \([Ca^{2+}]_o\), i.e., the depression induced by DHPG should be inhibited in 4-AP-treated slices. On the other hand, if changing \([Ca^{2+}]_o\) regulates the ability of DHPG to depress synaptic transmission through changes in excitability, then the enhanced excitability due to 4-AP should have an effect similar to bathing slices in low-\(Ca^{2+}\) ACSF, i.e., the depression induced by DHPG should be enhanced in 4-AP-treated slices. Therefore 4-AP should have opposite effects on the DHPG-induced depression depending on whether changing \([Ca^{2+}]_o\) regulates the effects of DHPG on synaptic transmission by modulating \(Ca^{2+}\) signaling or by altering neuronal excitability. Our results showing that 4-AP blocks the depression induced by DHPG thus suggest that changes in excitability are unlikely to account for the effects of varying \([Ca^{2+}]_o\) on the DHPG-induced depression of synaptic transmission. Moreover, the ability of DHPG to depress synaptic transmission in 4-AP-treated slices when \([Ca^{2+}]_o\) is reduced to 0.5 mM supports the notion that 4-AP antagonizes the effects of DHPG by increasing \(Ca^{2+}\) channel \(P_\text{Ca}\) and also indicates that 4-AP does not inhibit the effects of DHPG under normal conditions by acting as a nonselective inhibitor of group I mGluR signaling.

While our results are consistent with the notion that DHPG depresses synaptic transmission by inhibiting presynaptic \(Ca^{2+}\) signaling, changes in \([Ca^{2+}]_o\) will also alter \(i_{Ca}\) of both voltage-activated and ligand-gated \(Ca^{2+}\) channels (such as the NMDA receptor) in the postsynaptic cell. Likewise, bath application of 4-AP will also inhibit postsynaptic \(K^+\) channels and thus could enhance \(Ca^{2+}\) entry into the postsynaptic cell through voltage-activated as well as NMDA receptor ion channels. Our results therefore do not rule out the possibility that 4-AP and changes in \([Ca^{2+}]_o\) modulate the effects of DHPG by altering postsynaptic, rather than presynaptic, \(Ca^{2+}\) signaling. We found, however, that both the initial and persistent inhibition induced by DHPG were not affected in cells where the electrode-filling solution contained \(Cs^+\) and TEA at concentrations that should block postsynaptic \(K^+\) channels (Chen and Wong 1992; Velumian et al. 1993). Given the experimental limitations of the different \(K^+\) channel blockers used in our experiments, we were unable to directly compare how the ability of DHPG to depress synaptic transmission was altered when the same compound was used to block both pre- and postsynaptic \(K^+\) channels versus postsynaptic channels alone. It is important to note, however, that the combination of postsynaptic \(Cs^+\) and TEA used in our experiments will strongly block postsynaptic A-type \(K^+\) channels that are most sensitive to extracellular 4-AP (Brown et al. 1990; Chen and Wong 1992; Storm 1990; Velumian et al. 1993). Thus while inhibiting both pre- and postsynaptic \(K^+\) channels with bath applied 4-AP antagonizes the effects of DHPG, blocking postsynaptic \(K^+\) channels alone has no effect. This indicates that the ability of 4-AP to block the effects of DHPG on synaptic transmission can be attributed to an inhibition of presynaptic \(K^+\) channels, a result consistent with the hypothesis that DHPG inhibits excitatory synaptic transmission through effects on presynaptic calcium signaling. Because responses elicited by direct activation of postsynaptic NMDA receptors with exogenous agonists are enhanced by mGluR activation (Aniksztejn et al. 1991; Fitzjohn et al. 1996; Mannaieni et al. 2001), our finding that DHPG induces a persistent depression of NMDA, as well as AMPA, receptor-mediated components of EPSCs in CA1 pyramidal cells also supports a presynaptic locus for the effects of DHPG on synaptic transmission.

In our experiments we found that both the initial depression of synaptic transmission seen in the presence of DHPG and the persistent depression of synaptic transmission that remained following DHPG washout were similarly affected by postsynaptic GDPβS, extracellular 4-AP, and changes in \([Ca^{2+}]_o\). Because our results suggest that the DHPG-induced inhibition of synaptic transmission may be due to presynaptic changes that occur following activation of postsynaptic mGluRs, it seems likely that both phases of the synaptic depression involve some form of retrograde signaling. One candidate retrograde messenger is arachidonic acid, which is thought to be involved in the induction of mGluR-dependent forms of LTD by synaptic stimulation (Bolshakov and Siegelbaum 1995) and can inhibit high-voltage-activated \(Ca^{2+}\) channels in hippocampal neurons (Keyser and Alger 1990). Endocannabinoids represent another possible candidate because activation of postsynaptic mGluRs in cerebellar Purkinje cells inhibits release from climbing fibers in a cannabinoid receptor-dependent manner (Maejima et al. 2001). Our results suggest, however, that if a diffusible retrograde messenger is involved in DHPG-
induced LTD, it must act in a highly spatially restricted manner because loading single postsynaptic CA1 pyramidal cells with GDPβS blocked the effects of bath applied DHPG. Interestingly, mGluR-dependent LTD induced by both synaptic stimulation and DHPG is inhibited when postsynaptic protein synthesis is blocked (Huber et al. 2000). Together with this finding our results suggest that a form of retrograde signaling involving postsynaptic protein synthesis-dependent processes that regulate presynaptic function may also be involved in mGluR-dependent forms of LTD.

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