Cellular and Molecular Neurobiology, Vol. 23, No. 2, April 2003 (© 2003)

The Mechanism of Presynaptic Long-Term Depression Mediated by Group I Metabotropic Glutamate Receptors

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Received November 25, 2002; accepted December 18, 2002

SUMMARY

1. Metabotropic glutamate receptors (mGluRs) are known to play a role in synaptic plasticity. In a study of rat hippocampal brain slices, we find that a brief perfusion of a group I mGluR agonist, (S)-3,5-dihydroxyphenylglycine (DHPG), induced a robust long-term depression (DHPG-LTD) in area CA1.

2. The action was accompanied by an enhancement of the paired-pulse facilitation (PPF) ratio.

3. At the same time DHPG enhanced ionophoretic responses to alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA), kainic acid (KA), and *N*-methyl-D-aspartate (NMDA) in CA1 pyramidal neurons. This was only partially reversed by washing.

4. These observations indicate that DHPG exerts two opposing actions, suppression of the synaptic transmission and facilitation of postsynaptic responses. However, the presynaptic action dominates, since the net effect of monosynaptic activation is a reduction of response.

5. Perfusion of DHPG reduced three calcium-dependent responses in CA3 pyramidal neurons, which are presynaptic to CA1 neurons. These are calcium spike width and amplitude, after-hyperpolarization (AHP), and spike frequency adaptation (SFA).

6. These results suggest that the DHPG-LTD results from modulation of the presynaptic calcium currents by group l mGluRs.

KEY WORDS: hippocampus; metabotropic glutamate receptors; calcium; presynaptic; ionophoretic responses; DHPG; AMPA; NMDA; kainate.

LIST OF ABBREVIATIONS

GABA	γ-Aminobutyric acid
AHP	After-hyperpolarization
AMPA	Alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid
CNQX	6-Cyano-7-nitroquinoxaline-2,3-dione
DHPG-LTD	DHPG-induced LTD
D-AP5	D(−)-2-Amino-5-phosphonopentanoic acid

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0272-4340/03/0400-0187/0

DL-AP5	DL-2-Amino-5-phosphonopentanoic acid
fEPSP	Field excitatory postsynaptic potential
IPSP	Inhibitory postsynaptic potential
iGluR	Ionotropic glutamate receptor
KA	Kainic acid
LFS	Low frequency stimulation
LTP	Long-term potentiation
LTD	Long-term depression
mEPSP	Miniature excitatory postsynaptic potential
mIPSP	Miniature inhibitory postsynaptic potential
mGluR	Metabotropic glutamate receptor
NMDA	*N*-methyl-D-aspartate
PPF	Paired-pulse facilitation
s-DHPG	(S)-3,5-dihydroxyphenylglycine
SFA	Spike frequency adaptation
TEA	Tetraethyl ammonium
TTX	Tetrodotoxin

INTRODUCTION

L-Glutamate is the predominant fast excitatory neurotransmitter in the mammalian central nervous system, but also acts through metabotropic glutamate receptors (mGluRs) to exert more long-lasting actions (Anwyl, 1999; Bortolotto *et al*., 1999; Riedel and Reymann, 1996). The mGluR family consists of eight known subtypes that are further categorized into three groups by their pharmacological properties, homology of their amino acid sequences, coupled second messengers, and effectors (Conn and Pin, 1997). Group I receptors are positively coupled via G_q/G_{11}-like G-proteins to inositol phosphate hydrolysis, while groups II and III receptors are negatively coupled via G_i/G_o-like G-proteins to adenylyl cyclase. Recent studies suggest a role of mGluRs in various forms of synaptic plasticity that are thought to be part of the molecular basis for learning and memory, including long-term potentiation (LTP), depotentiation, and stimulation-induced long-term depression (LTD). The characteristics of mGluRs make them ideal candidates for translating transient activity into long-lasting memory.

Pharmacological (Balschun *et al*., 1999; Manahan, 1997) and transgenic (Aiba *et al*., 1994; Conquet *et al*., 1994) studies have shown that group I mGluR agonists induce a LTD of synaptic transmission in CA1 (Overstreet *et al*., 1997; Palmer *et al*., 1997) and dentate gyrus (Camodeca *et al*., 1999; OMara *et al*., 1995) of rat hippocampus and other brain areas (Conquet *et al*., 1994; Kano and Kato, 1987; Kato, 1993). However, the mechanism of this action is poorly understood. We have performed a series of experiments in hippocampal brain slices using a group I mGluR agonist, DHPG, to investigate the mechanisms of action of group I mGluR-LTD. A preliminary report has been presented (Tan *et al*., 1999).

METHODS

Young adult male Wistar rats (150–200 g) were euthanized by cervical dislocation. The brain was removed and semitransverse hippocampal slices (450 μM thick) were prepared on a vibratome (OTS 3000, FHC, Inc.) in ice-cold modified Krebs-Ringer solution (in mM: sucrose 212.5; KCl 3.5; KH_2PO_4 1.2; $MgSO_4$ 1.3; $CaCl_2$ 2.4; $NaHCO_3$ 26; glucose 10) saturated with 95% O_2 and 5% CO_2. Slices were then incubated in oxygenated 95% O_2 and 5% CO_2 normal Ringer (125 mM NaCl instead of 212.5 mM sucrose) for 1–8 h at 34°C before recording. After the recovery period slices were transferred one at a time to a submersion chamber for recording. Some experiments were performed in low Ca^{2+} (0.5 mM) and high Mg^{2+} (5 mM) Krebs-Ringer solution.

Field excitatory postsynaptic potentials (fEPSPs) were recorded in the stratum radiatum of area CA1 in response to Schaffer collateral pathway stimulation by a monopolar stimulating electrode (FHC). Extracellular recording electrodes were pulled from thin-wall glas capillaries (o.d. 1.2 mm, i.d. 0.9 mm, FHC) and filled with normal Ringer's solution (tip 25 μM, with impedance of less than 2 MΩ). Paired pulses with a interpulse interval of 35-ms interval were delivered via a stimulus isolator (A360, World Precision Instruments), driven by a PulseMaster A300 at a frequency of 0.017 Hz. The pulse was 0.05–0.20 nA, 50-μs duration, adjusted so that half of the maximal EPSP was elicited as by the first pulse (the baseline response). Simultaneous intra- and extracellular recordings were obtained from the CA1 region and the fEPSPs were utilized for EPSP analysis. Since in some experiments the fEPSP was abolished by a manipulation (such as application of TTX) and slope estimation was not possible, amplitude was utilized for analysis in all experiments. S-DHPG (25–50 μM) and in some experiments 50–100 μM rs-DHPG (Tocris) were used. 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX), D(−)-2-amino-5-phosphonopentanoic acid (D-AP5), and DL-AP5 were obtained from Tocris. All other chemicals were obtained from Sigma, unless specified.

Conventional intracellular recordings were made from CA3 or CA1 pyramidal neurons using thick-wall (1.2-mm o.d., 0.6-mm i.d., FHC) borosilicate glass electrodes filled with 3 M potassium acetate (pH 7.3; impedance 40–85 MΩ). Three-barreled ionophoretic electrodes were pulled from thin-wall glass (1.2-mm o.d., 0.9-mm i.d., FHC) and filled with alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA), kainic acid (KA), and *N*-methyl-D-aspartate (NMDA) (from Tocris, all in distilled water, pH 7.2–7.4), respectively. γ-Aminobutyric acid (GABA) (from Tocris) was used at a concentration of 0.5 mM (pH about 3.5 in distilled water) for ionophoretic application. The intracellular electrode was connected to a bridge amplifier (Neurodata IR183)/bath electrode circuit and the ionophoretic electrode connected to a Medical Systems BH-2 unit. Depolarizing or hyperpolarizing currents were injected into the cell to test the passive membrane properties of the cell. The ionophoretic currents (positive for GABA and negative for AMPA, KA, and NMDA) were 5–35 nA (1-s duration) and passed to each barrel at an autocycle with 30-s delay. The autocycle was in the solution at least 5 min prior to positioning the ionophoretic electrode adjacent to the recording electrode.

Retaining currents of 0–2 nA with appropriate polarity were used on the drug barrels.

Data were collected with the Whole Cell Analysis Program (John Dempster) and/or a Gould 2400S chart recorder for off-line analysis. Values are reported as mean ± SEM. The student's *t* test was used for statistical analysis and a *P* value of less than 0.01 was accepted as being significant.

RESULTS

DHPG-LTD in Area CA1

Brief bath perfusion of DHPG induced a progressive depression of the CA1 fEPSP elicited by stimulation of the Schaffer collateral pathway, accompanied by an increase in PPF. Figure 1(A) shows these actions from one experiment while Fig. 1(B) shows pooled results from nine slices. With a 5-min bath perfusion the fEPSP fell to about 30.1 ± 5.2% of baseline. On washout there was a relatively rapid recovery of about half of the decrement but a maintained depression to 66.6 ± 7.7% of the initial response. The depression of the evoked monosynaptic synaptic response was accompanied by a significant increase in the PPF ratio (110.9 ± 4.5%).

Effects of DHPG on Postsynaptic Neurons

PPF is a presynaptic phenomena (Harris and Cotman, 1983; Katz and Miledi, 1968; Kombian *et al*., 1996; Leung and Fu, 1994; Schulz *et al*., 1994) and there is a marked increase in the PPF ratio during DHPG-LTD. These observations may indicate a presynaptic locus for DHPG-LTD. To test determine whether the suppression of synaptic transmission is due to pre-or postsynaptic mechanisms, the effects of DHPG were tested on responses to ionophoretically applied glutamate agonists at postsynaptic CA1 neurons. In these studies, the presynaptic neurotransmitter release is mimicked by a multibarrel microelectrode loaded with different ionotropic glutamate receptor (iGluR) agonists. In agreement with previous reports (Bleakman *et al*., 1992; Harvey and Collingridge, 1993; O'Connor *et al*., 1994), DHPG reversibly enhanced (did not depress) the ionophoretic responses to AMPA, KA, and NMDA, an action only partially reversed by washing (Fig. 2). Together with results shown in Fig. 1, these observations indicate that DHPG exerts two opposing actions, suppression of transmitter release but facilitation of postsynaptic responses. However, the presynaptic action dominates, since the net effect is a reduction of synaptic responses. Four of 10 intracellular recordings were done in the presence of NiCl(1 mM), but the potentiation of postsynaptic responses under these circumstances was not altered. These results may indicate that mGluRs potentiate the iGluRs by direct coupling to the G-protein or by some other calcium-independent mechanism(s).

We also examined the effects of DHPG on membrane conductance and membrane potential, which may also influence synaptic transmission by altering the driving force for ions. In line with other reports, brief perfusion of DHPG caused a depolarization of membrane potential (Fig. 3) and a decrease in membrane conductance in CA1 (Fig. 4) and CA3 (data not shown) neurons. These effects could not be

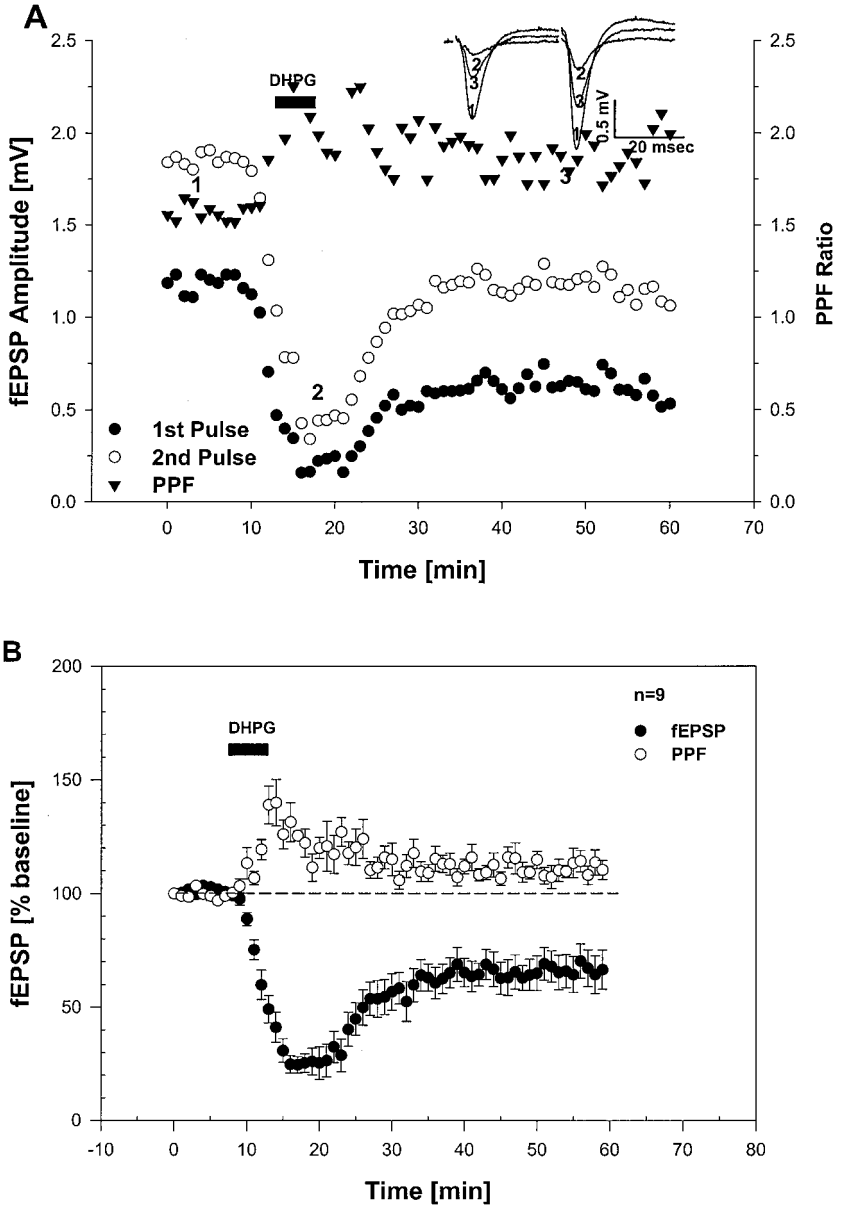

Fig. 1. Long-term depression induced by DHPG at the Schaffer collateral-CA1 synapses in the rat hippocampus. Data are only taken from extracellular recordings (simultaneously intracellular recordings were also made from most slices). A representative example (A) and pooled data (B) show the effect of brief perfusion (indicated by the horizontal bar) of 20–50 μM DHPG on the EPSP and PPF ratio. Paired-pulses were delivered every minute. (A) showsthe amplitude of EPSPs elicited by the paired-pulses, with the stimuli delivered at a 35-ms interval. The PPF ratio was obtained by dividing the fEPSP from the 2nd pulse by that of the 1st. (B) shows the pooled data from nine slices, two of which totally recovered after a 60-min wash. Both the EPSP (elicited by the first pluse) and PPF ratio were normalized to % baseline (usually the value from the first record).

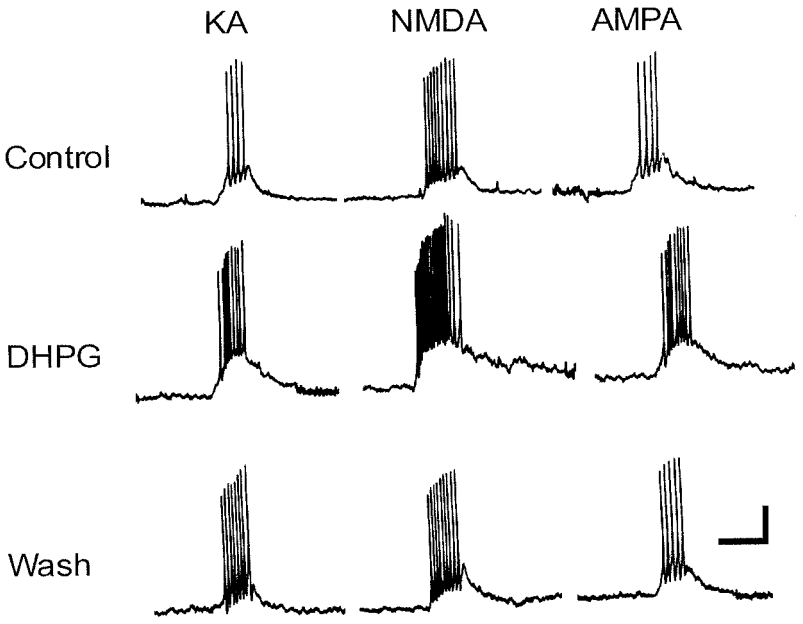

Fig. 2. DHPG (50 μM) potentiates the ionophoretic responses to both NMDA and non-NMDA agonists. Kainic acid (KA), NMDA, and AMPA were applied at 25 nA in 1-s pulses. The upper responses are controls. In the middle traces DHPG was perfused at a concentration of 50 μM. The lower traces are response obtained after a 30 wash with control Krebs-Ringer. The potentiation was only partially reversible in this cell. Calibration, 10 mV (vertical bar) and 10 s (horizontal bar).

reversed on washing in most neurons. Both of these actions should result in a reduction in synaptic response at the postsynaptic sites. Yet we still observe an increase in postsynaptic responses with ionophoretic application of agonists.

We further explored this issue by cutting off the cell bodies in the CA1 area (the CA3 region was also cut off in half of the slices), and recording extracellularly from apical dendrites. This manipulation should reduce the number of mGluRs and consequently reduce the postsynaptic responses. The responses from these slices were

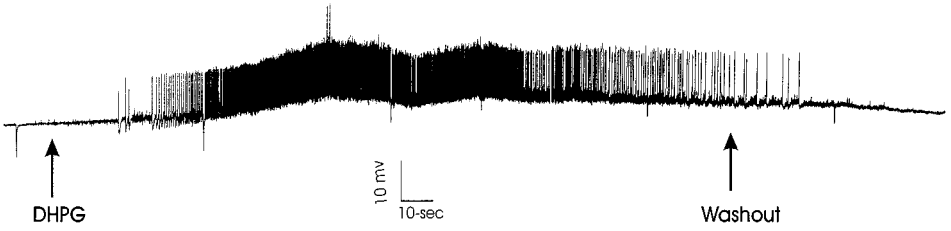

Fig. 3. The effects of DHPG on membrane potential and membrane noise. An intracellular recording was made from one CA1 neuron from the experiment in Fig. 1. A brief perfusion of DHPG (50 μM) causes a membrane depolarization and noise enhancement. In at least half of the slices these effects are not totally reversible.

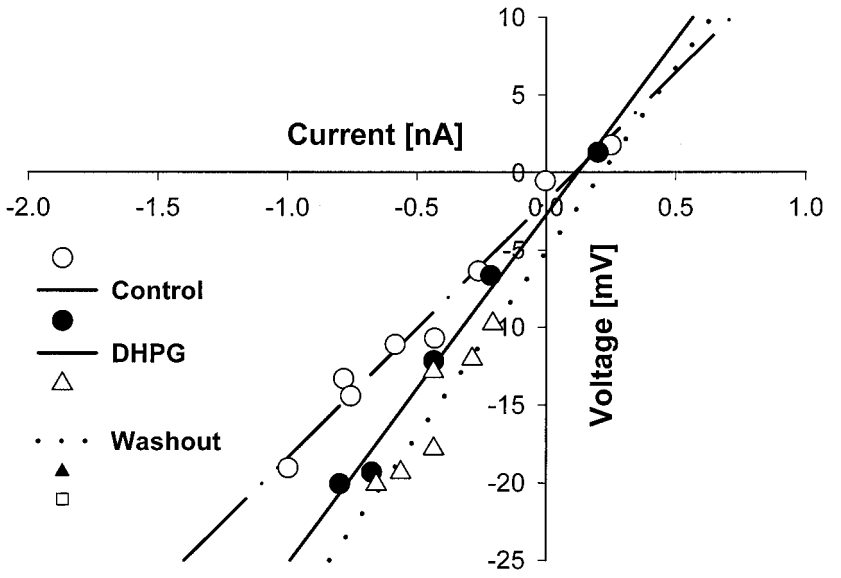

Fig. 4. The effect of DHPG on the membrane input resistance in CA1 neurons. The voltage–current curves (with the regression lines) were plotted before (open circles and dashed lines), during (solid circles and solid line), and 15 min after wash (open triangles and dotted line) of DHPG. Four cells were washed for 15 min and six cells were examined every 10 min after wash for 60 min. In most cells this effects did not fully recover.

small and the stimulating intensity required to elicit the response was at least twice that used normally. The pooled data are shown in Fig. 5. DHPG induced an acute depression ($54.1 \pm 14.1\%$) that was greatly reduced compared with normal slices ($30.1 \pm 5.2\%$); however, there was no significant change in the amplitude of DHPH-LTD ($68.5 \pm 9.9\%$ vs. $66.6 \pm 7.7\%$ in control) and the PPF ratio ($123.7 \pm 8.2\%$ vs. $110.9 \pm 4.5\%$ in control) compared to normal slices. This evidence is consistent with the conclusion that the DHPG-induced alteration in membrane potential and conductance has no effect on DHPG-LTD.

Effects of DHPG on Three Calcium-Dependent Responses in Presynaptic Neurons

We have explored possible mechanisms for reduction in transmitter release by recording intracellularly from the CA3 pyramidal neurons, which are presynaptic to the CA1 neurons. There is evidence that group I mGluR agonists inhibit Ca^{2+} channels (Lester and Jahr, 1990; Lovinger and McCool, 1995; McCool *et al.*, 1998; Netzeband *et al.*, 1997; Sayer, 1998; Swartz and Bean, 1992; Takahashi *et al.*, 1996). To test whether a reduced presynaptic Ca^{2+} current is responsible for the synaptic depression, we examined the effects of DHPG on three calcium-dependent responses in CA3 cells: width and amplitude of the calcium spike, the spike afterhyperpolarization (AHP), and spike frequency adaptation (SFA). In hippocampal pyramidal and other neurons, a Ca^{2+}-dependent potassium current underlies the AHP (Horn and McAfee, 1979; McAfee and Yarowsky, 1979), which is important

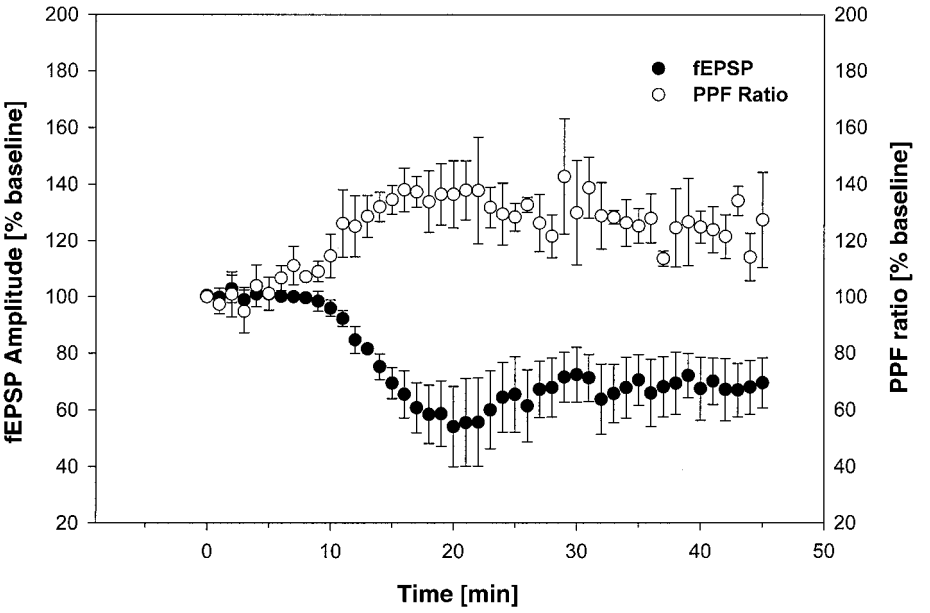

Fig. 5. DHPG-induced LTD and PPF enhancement in absence of cell bodies. CA1 pyramidal neuron cell bodies were cut off and recordings obtained from the apical dendrites. Data is the average of seven slices, in which the CA3 area was also cut off. The amplitude of the LTD was 68.5 ± 9.9% of baseline and PPF 123.7 ± 8.2% of baseline.

in regulating the SFA (Madison and Nicoll, 1984). Obviously we can record only from the cell bodies, but it is resonable to assume that Ca^{2+}-dependent events in the presynaptic terminal will also be reflected in the cell body.

In slices incubated with tetrodotoxin (TTX) and tetraethyl ammonium (TEA), DHPG caused a marked depression of the amplitude and width of the calcium spike measured 30 min after washout of DHPG (Fig. 6(a)). As shown in Fig. 6(b) and (c), the spike AHP and SFA were also markedly diminished after application of DHPG and recovered only partially after 60-min wash. A number of potassium currents, including the leak current, I_{leak} (Charpak *et al.*, 1990; Guerineau *et al.*, 1994), voltage-dependent current, I_M (Charpak *et al.*, 1990), and the calcium-dependent AHP, I_{AHP} (Baskys, 1992; Charpak *et al.*, 1990; Gereau and Conn, 1995a) are inhibited by group I mGluR agonists. It is not possible from our experiments to determine whether these effects are due only to direct inhibition of calcium entry or to blockade of both the Ca^{2+} and K^+ currents. However, the calcium spikes studies clearly show that calcium currents are reduced after perfusion of DHPG.

All three Ca^{2+}-dependent potentials were also reversibly attenuated by application of the calcium antagonist nickel (1-mM NiCl in Krebs-Ringer, data not shown).

DHPG-LTD Is Not Identical to Stimulation-Induced LTD

Stimulation-induced LTD is saturable; thus we performed occlusion experiments to examine whether DHPG-LTD results from the same mechanism(s) as

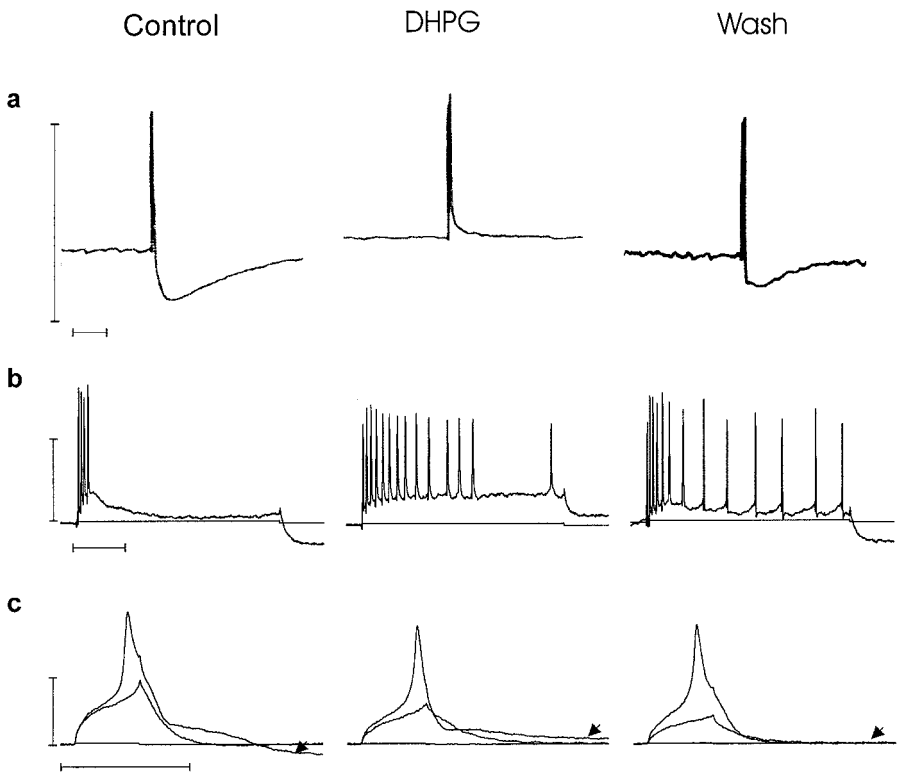

Fig. 6. Effects of DHPG on calcium-dependent responses. (a) Spike after hyperpolarization (AHP), elicited by 4–5 action potentials triggered by depolarizing current (50 ms, 2 nA) before, after 5-min perfusion, and after 60-min wash of DHPG. (b) Spike frequency adaptation (SFA), studied by application of depolarizing current of 8 nA for 400 ms, before during a 5-min perfusion, and after 60-min wash of DHPG. (c) Ca^{2+} spikes, recorded from a CA3 pyramidal neuron (Vm = −65 mV) by application of a depolarizing current of 10 nA for 50 ms, in a slice incubated in TTX (1 μM) and TEA (5 mM). The recordings were taken before application (left), at the end of a 5-min application of DHPG (center), and after a 30-min wash (right). Seven of eight neurons studied show similar results, while one was unaffected. Note that the AHP recovered only partially. Calibration: vertical bar is 40 mV; horizontal bar is 100 ms in (a) and (b), 0.5 s in (c). One of seven cells fully recovered for (a) and (b).

conventional stimulation-induced LTD. The results are shown in Fig. 7(A) and (B). Maximal LTD was induced by 2–4 stimulations at 5 Hz for 3 min. DHPG was then applied after induction of the maximal LTD. DHPG still induced a significant additional synaptic depression on top of the saturated stimulation-induced LTD. The amplitude of the DHPG-LTD was similar as in control slices, as shown in Fig. 1. The magnitude of PPF change was also similar.

We performed further experiments to test whether or not DHPG-LTD is NMDA dependent. Stimulation-induced LTD is thought to be a postsynaptic action and is NMDA dependent in hippocampal CA1 and other brain region (Dodt *et al.*, 1999), although some report that there is another type of stimulation-induced LTD that is mGluR dependent (Bolshakov and Siegelbaum, 1994; Oliet *et al.*, 1997). Figure 8(A)

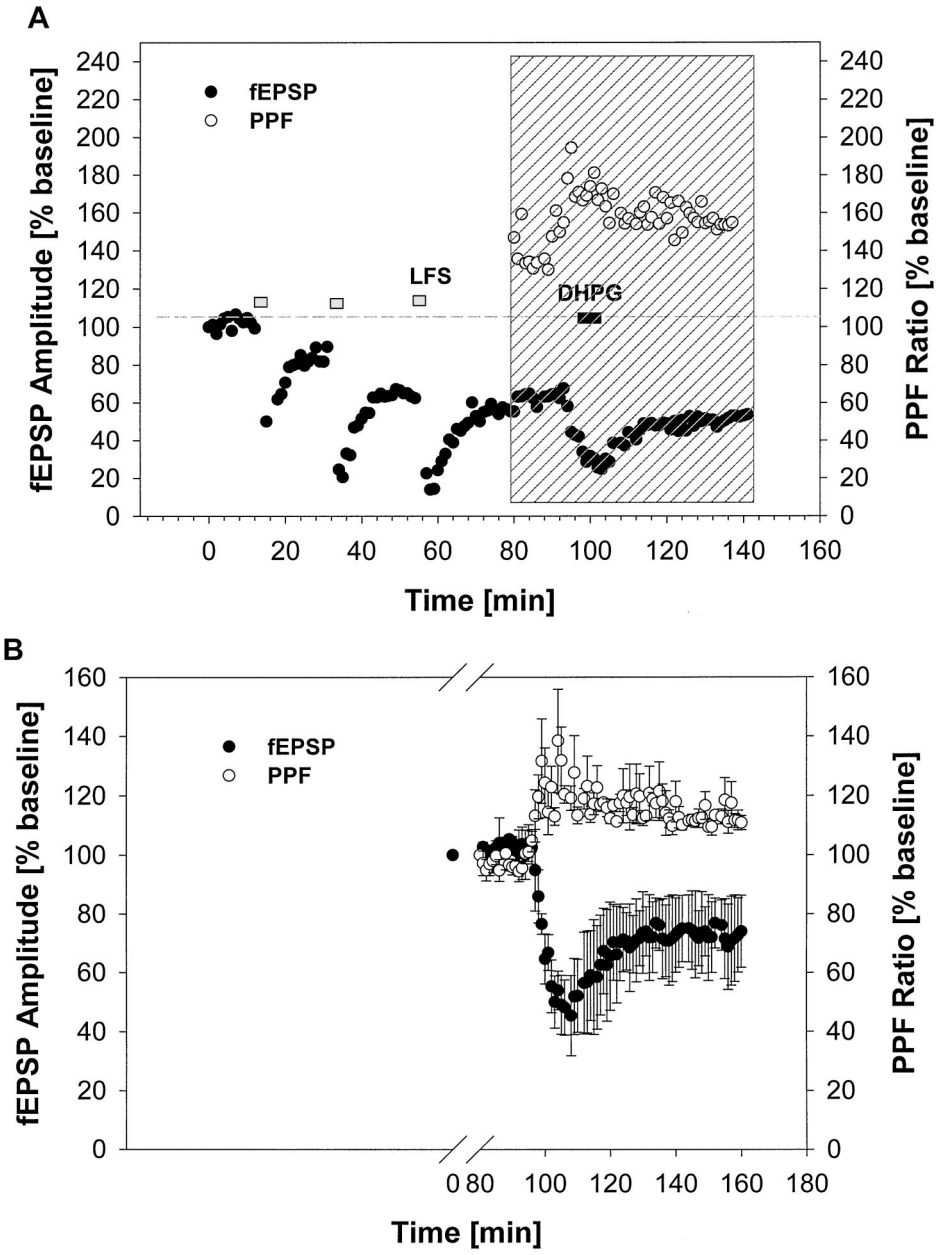

Fig. 7. Stimulation-induced LTD and DHPG-LTD result from different mechanisms. (A) Results from one experiment show that after maximal LTD was induced by three application of stimulation at 5 Hz for 3 min, perfusion of DHPG resulted in additional LTD. (B) Pooled data from six slices showing that DHPG-LTD (72.6 ± 12.3) is not reduced when applied on top of a saturated stimulus-induced LTD. Data were taken from 10 min before perfusion of DHPG (framed box in (A)).

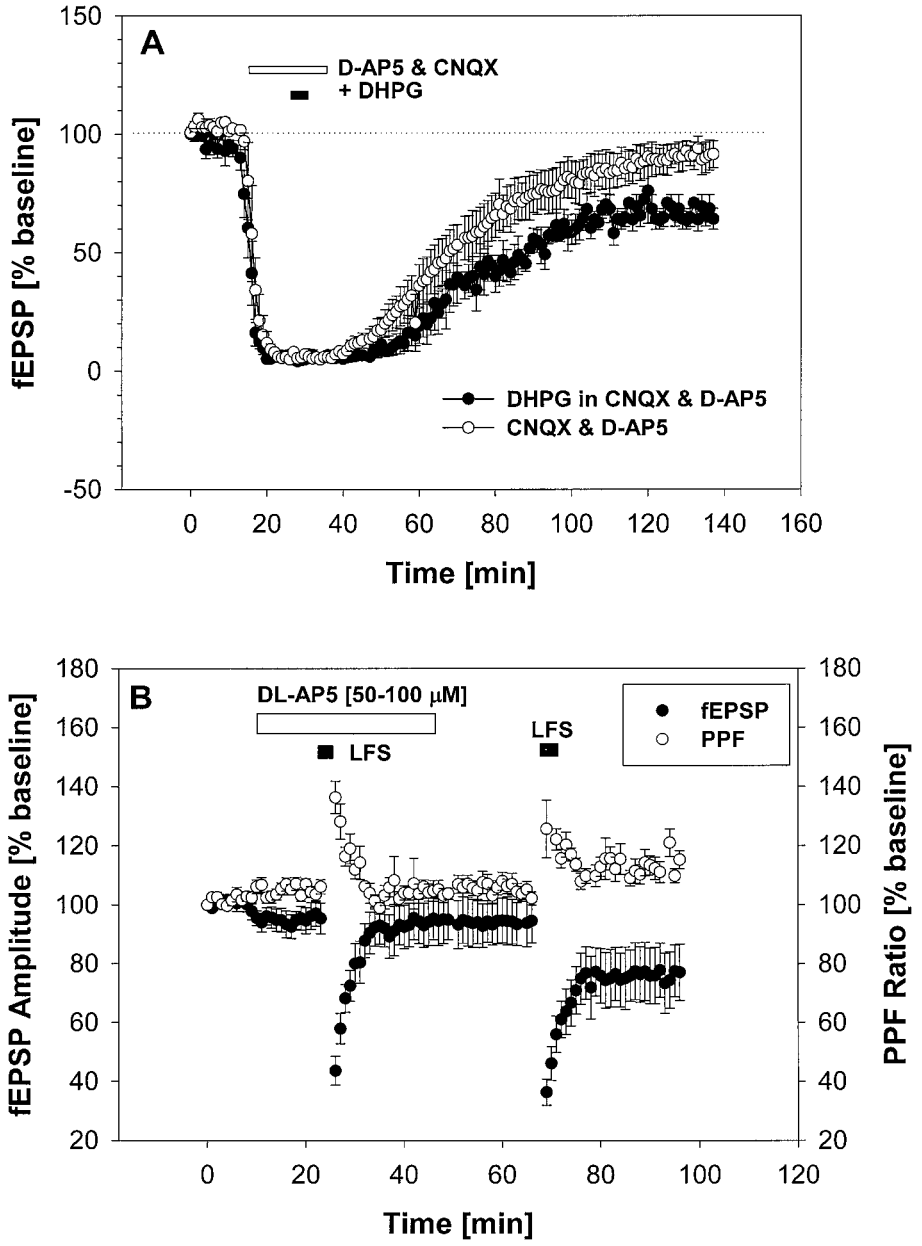

Fig. 8. DHPG-LTD is independent of NMDA receptors. (A) Perfusion of 20-μM CNQX and 25 or 50-μM D-AP5 did not prevent induction of DHPG-LTD (66.5 ± 4.5), which can be observed after washout of the ionotropic receptor antagonists (average of four slices). When DHPG was applied 10 min after CNQX and D-AP5, the fEPSP did not fully recovered after wash (91.1 ± 5.5, $n = 5$). (B) LFS (5 Hz for 3 min) failed to induce LTD when DL-AP5 was applied about 10 min before and 15 min after LFS. A robust LTD (78.3 ± 10.1) was induced by LFS after wash of D-AP5.

shows that application of CNQX and DL-AP5, which together totally block the fEPSP, has no effect on DHPG-LTD. In these experiments, DHPG was applied after the fEPSP was completely abolished by CNQX and DL-AP5. The perfusion of the iGluR antagonists continued for 5 min after DHPG washout. In control slices, the fEPSP recovered almost completely, but in slices perfused with DHPG there is a significant and maintained reduction in fEPSP amplitude after washing out D-AP5 and CNQX. These results indicate that, unlike stimulation-induced LTD, DHPG-LTD is independent of iGluRs, including NMDA receptors.

As shown in Fig. 8(B), the stimulation-induced LTD in our preparation is solely NMDA dependent, since it can be blocked by NMDA antagonists. LFS (5 Hz for 3 min) failed to induce LTD in presence of 50–100-μM DL-AP5 but elicited a robust LTD after washout of DL-AP5. Another receptor blocker, CPP (10 μM), and one channel blocker, MK 801 (10 μM), also blocked the stimulation-induced LTD (data not shown). The dependence of DHPG-LTD on extracellular Ca^{2+} was also tested. When low Ca^{2+} and high Mg^{2+} Krebs-Ringer was applied, the evoked EPSP was totally abolished. Application of DHPG in this Ringer's still induced a robust LTD ($72.6 \pm 12.3\%$) with an amplitude as in control ($66.6 \pm 7.7\%$) (see Fig. 9). These findings, together with the lack of effect of iGluR antagonists, indicate that NMDA and subsequent Ca^{2+} increase are not necessary for DHPG-LTD.

DISCUSSION

There are two distinct forms of LTD, which can coexist at the same synapse (Oliet *et al*., 1997). Most stimulation-induced LTD results from a modest Ca^{2+} rise in the postsynaptic membrane secondary to NMDA channel activation invoked by depolarization. However, there is another distinct form of LTD induced by activation of group I mGluRs (Fitzjohn *et al*., 1998; Manahan, 1997; Otani and Connor, 1998). Some authors report induction of mGluR-LTD following low frequency stimulation. However, in our study low frequency stimulation yielded NMDA-LTP, since it was blocked by NMDA antagonists. Occlusion experiments provide strong evidence that DHPG-LTD and stimulation-induced LTD utilize different mechanisms. In NMDA-LTD, the postsynaptic depolarization and calcium increase through NMDA channels are essential for induction of LTD. Our data demonstrate that this is not the case for DHPG-LTD, since CNQX and D-AP5, which would prevent calcium rise at postsynaptic sites through NMDA channels, did not block the response.

Some immunochemical studies have reported that group I mGluRs exist exclusively at postsynaptic sites in hippocampus (Lujan *et al*., 1996; Shigemoto *et al*., 1997), yet there are also data implying the existence of mGluR at the presynaptic terminal (Rodriguez-Moreno *et al*., 1998). Our observations are consistent with previous reports (Baskys and Malenka, 1991; Desai *et al*., 1992; Gereau and Conn, 1995b) that group I mGluRs reduce excitatory synaptic transmission in hippocampal CA1 via a presynaptic action. This conclusion is based on analysis of miniature EPSCs, observations on PPF (Gereau and Conn, 1995b), and the results from this study, all of which provide strong support for the conclusion that the mGluRs exist on presynaptic terminals. This is not to deny that mGluRs also exist on the postsynaptic membrane. Our evidence that DHPG increases the response to ionophoretic

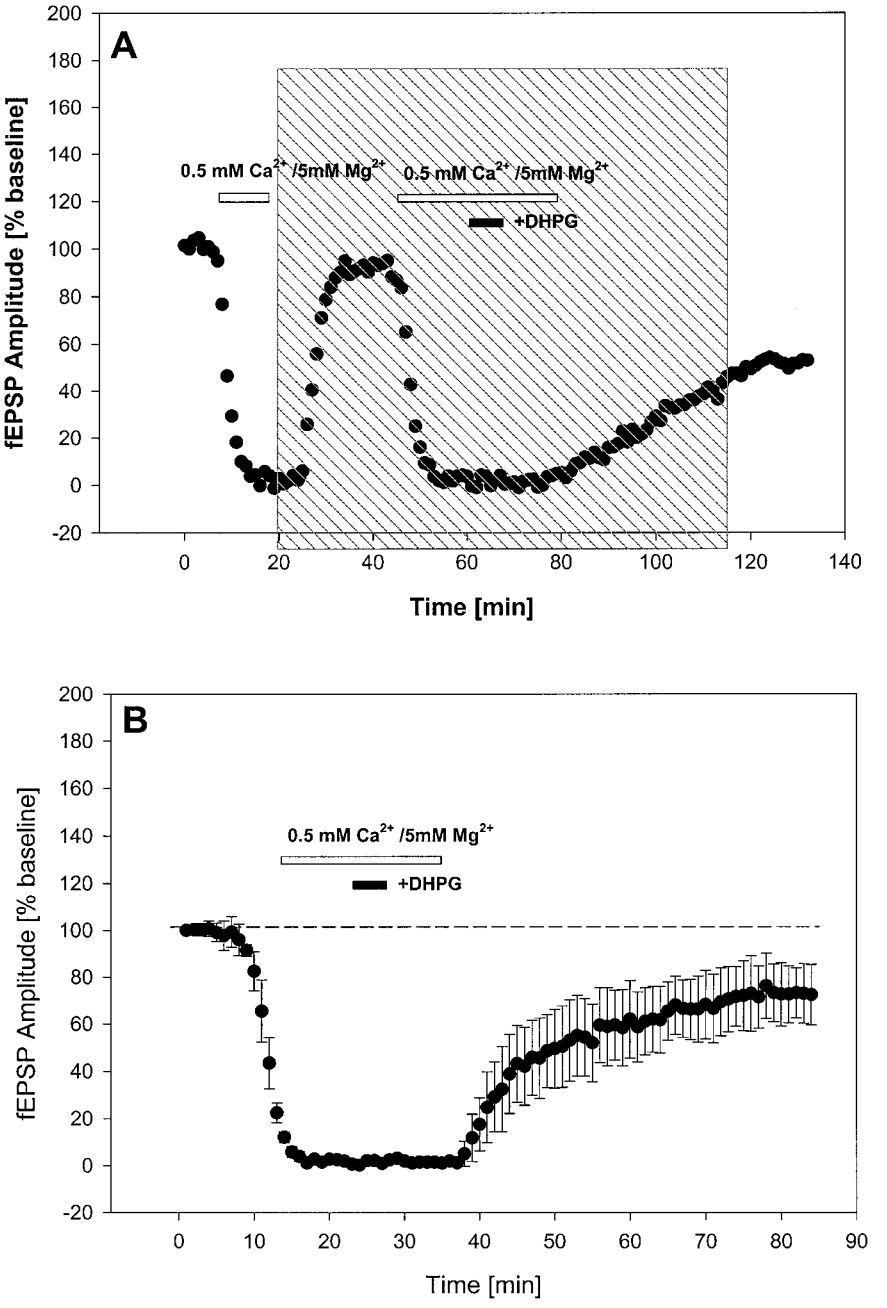

Fig. 9. DHPG induces LTD in low Ca^{2+} and high Mg^{2+} Ringer's. (A) A single experiment shows that the fEPSP was quickly abolished by low Ca^{2+} (0.5 mM) and high Mg^{2+} (5 mM) Krebs-Ringer, and recovered 5 min after perfusion with normal Krebs-Ringer. When DHPG was applied 10 min before and 10 min after low Ca^{2+} and high Mg^{2+} Ringer solution, LTD was induced. (B) Pooled data ($n = 5$) from the boxed frame in (A) shows the DHPG induces LTD (72.7 ± 13.4) when applied in the background of low Ca^{2+} and high Mg^{2+} Ringer.

application of glutamate receptor agonists, and alters membrane potential and membrane conductance is consistent with an effect on postsynaptic mGluRs. However, the presynaptic effects dominate in our studies.

Recently there have been reports that group I mGluR induced LTD is due to a postsynaptic mechanism (Huber *et al*., 2000; Snyder *et al*., 2001). Our results do not rule out the possibility that the DHPG action is postsynaptic, but leads to the generation of retrograde messengers, which in turn result in a presynaptic depression of transmitter release. Several retrograde messengers could have such actions. Arachidonic acid has been proposed to play a role in plasticity, and it can be formed during the activation of mGluRs. Nitric oxide (Boulton *et al*., 1994; Daniel *et al*., 1993; Kimura *et al*., 1998; Malen and Chapman, 1997; Schuman and Madison, 1994) and adenosine (Manzoni *et al*., 1994) are other two possible candidates that might mediate the depression of synaptic transmission. Both could be generated by NMDA receptor activation. Since our results and those of others have shown that activation of mGluRs can potentiate the NMDA response, this is a reasonable possibility. Further study is necessary to determine whether the mechanisms responsible for decreased transmitter release are entirely presynaptic or presynaptic but mediated by a retrograde messenger from the postsynaptic membrane.

Activation of group I mGluR has been shown to decrease voltage-dependent calcium currents in a variety of neurons (Lester and Jahr, 1990; Sahara and Westbrook, 1993; Stefani *et al*. 1994; Swartz *et al*., 1993; Swartz and Bean, 1992). It also has been shown that about 90% of hippocampal pyramidal cells in culture express ACPD-induced inhibition of voltage-gated calcium channels (Sahara and Westbrook, 1993). We have been able to record only from the cell body, not the terminals, of the presynaptic neuron, but the alteration in PPF and the clear demonstration of mGluR action on calcium currents in the cell body are most consistent with the conclusion that there are mGluRs located on presynaptic terminals of CA3 pyramidal neurons which reduce transmitter release by reducing calcium entry.

We conclude that DHPG induces LTD through a reduction of presynaptic calcium influx. This mechanism is distinct from that of stimulation-induced LTD, and may play an important role in preventing excitotoxic effects of glutamate action.

ACKNOWLEDGMENT

This study was supported by NIEHS P42 ESO4913 to DOC.

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