J Neurophysiol
88: 107–116, 2002; 10.1152/jn.00679.2001.

Group I mGluRs Increase Excitability of Hippocampal CA1 Pyramidal Neurons by a PLC-Independent Mechanism

DAVID R. IRELAND AND WICKLIFFE C. ABRAHAM
Department of Psychology and the Neuroscience Research Centre, University of Otago, Dunedin, New Zealand

Received 14 August 2001; accepted in final form 19 February 2002

Ireland, David R. and Wickliffe C. Abraham. Group I mGluRs increase excitability of hippocampal CA1 pyramidal neurons by a PLC-independent mechanism. *J Neurophysiol* 88: 107–116, 2002; 10.1152/jn.00679.2001. Previous studies have implicated phospholipase C (PLC)-linked Group I metabotropic glutamate receptors (mGluRs) in regulating the excitability of hippocampal CA1 pyramidal neurons. We used intracellular recordings from rat hippocampal slices and specific antagonists to examine in more detail the mGluR receptor subtypes and signal transduction mechanisms underlying this effect. Application of the Group I mGluR agonist (RS)-3,5-dihydroxyphenylglycine (DHPG) suppressed slow- and medium-duration afterhyperpolarizations (s- and mAHP) and caused a consequent increase in cell excitability as well as a depolarization of the membrane and an increase in input resistance. Interestingly, with the exception of the suppression of the mAHP, these effects were persistent, and in the case of the sAHP lasting for more than 1 h of drug washout. Preincubation with the specific mGluR5 antagonist, 2-methyl-6-(phenylethynyl)-pyridine (MPEP), reduced but did not completely prevent the effects of DHPG. However, preincubation with both MPEP and the mGluR1 antagonist LY367385 completely prevented the DHPG-induced changes. These results demonstrate that the DHPG-induced changes are mediated partly by mGluR5 and partly by mGluR1. Because Group I mGluRs are linked to PLC via G-protein activation, we also investigated pathways downstream of PLC activation, using chelerythrine and cyclopiazonic acid to block protein kinase C (PKC) and inositol 1,4,5-trisphosphate-(IP_3)-activated Ca^{2+} stores, respectively. Neither inhibitor affected the DHPG-induced suppression of the sAHP or the increase in excitability nor did an inhibitor of PLC itself, U-73122. Taken together, these results argue that in CA1 pyramidal cells in the adult rat, DHPG activates mGluRs of both the mGluR5 and mGluR1 subtypes, causing a long-lasting suppression of the sAHP and a consequent persistent increase in excitability via a PLC-, PKC-, and IP_3-independent transduction pathway.

INTRODUCTION

In many types of neuron, cell excitability is modulated by the afterhyperpolarization (AHP) that follows a train of action potentials. In hippocampal pyramidal neurons, the AHP has three separable components: fast duration (fAHP), medium duration (mAHP), and slow duration (sAHP), each the result of activating different potassium conductances (Storm 1990). The fAHP contributes to action potential repolarization, the mAHP regulates firing frequency in a short train of action potentials or at the beginning of a longer train, and the sAHP modulates spike frequency adaptation in a long train (Storm 1990). Receptor activation by a variety of neurotransmitters, including glutamate (Charpak et al. 1990), can inhibit the sAHP, resulting in a decrease in spike frequency adaptation and a consequent increase in cell excitability.

The glutamate receptor-induced excitability change is mediated via activation of metabotropic glutamate receptors (mGluRs). The mGluR family consists of three groups, divided into eight subtypes, based on sequence homology, pharmacological sensitivity, and transduction mechanisms (Schoepp et al. 1999). In several cases, the subtypes can be further divided into multiple splice variants (Schoepp et al. 1999). Like glutamate, the Group I and II mGluR agonist aminocyclopentane-1,3-dicarboxylic acid (ACPD) suppresses the sAHP in CA1 and CA3 pyramidal neurons (Charpak et al. 1990; Desai and Conn 1991; Hu and Storm 1991) and dentate granule neurons (Abdul-Ghani et al. 1996a,b). In CA1 pyramidal neurons, the more selective Group I agonist 3,5-dihydroxyphenylglycine (DHPG) (Schoepp et al. 1994) duplicates the ACPD effect, implicating Group I mGluRs in the sAHP suppression (Davies et al. 1995; Gereau and Conn 1995). Suppression of the sAHP in CA1 with a partial agonist of mGluR5, (*S*)-(+)-2-(3′-carboxybicyclo[1.1.1]pentyl)-glycine, suggests that this effect is mediated by mGluR5 (Mannaioni et al. 1999), a conclusion supported by localization studies, which indicate that mGluR5 is the predominant or only subtype in CA1 pyramidal cells (Lujan et al. 1996; Shigimoto et al. 1997). However until recently, specific antagonists of mGluR subtypes have not been available, hampering confirmation of this result.

Downstream from mGluR activation, remarkably little is known about the signaling pathways that lead to changes in excitability and membrane parameters in hippocampal pyramidal neurons. Because Group I mGluRs couple to phosphoinositide hydrolysis (Abe et al. 1992; Aramori and Nakanishi 1992), mGluR-induced responses in CA1 are most likely mediated by one of the two second messengers downstream from activation of phospholipase C (PLC), 1,2-diacylglycerol (DAG), or inositol 1,4,5-trisphosphate (IP_3), which activate protein kinase C (PKC), and IP_3-sensitive Ca^{2+} stores respectively. This has been confirmed in part for mGluR-induced changes in excitability in dentate gyrus granule cells, where a G-protein-coupled mGluR, most probably mGluR1, couples to a pathway involving PLC, IP_3-activated Ca^{2+} stores, tyrosine kinase, and ryanodine-sensitive Ca^{2+} stores (Abdul-Ghani et

Address for reprint requests: D. R. Ireland, Dept. Psychology, University of Otago, P.O. Box 56, Dunedin, New Zealand (E-mail: direland@psy.otago.ac.nz).

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al. 1996a,b). In CA1 pyramidal neurons, however, no transduction pathway has yet been identified.

The present study used the Group I mGluR agonist, DHPG, to effect long-lasting changes in the excitability of pyramidal neurons and employed specific antagonists to investigate the receptor types and signal transduction mechanisms underlying those changes. This study stems from our previous observations that the mGluR-mediated increase in cell excitability is linked to the facilitation ("priming") of long-term potentiation (LTP) elicited by prior administration of Group I agonists (Cohen and Abraham 1996; Cohen et al. 1999). Our findings indicate that the DHPG-triggered membrane changes are in fact mediated by two subtypes of mGluR acting via PLC-independent mechanism(s) and that the pathways mediating the increased excitability can largely be dissociated from those mediating LTP priming.

METHODS

Tissue preparation

Transverse hippocampal slices (400 μm) were prepared from young adult male Sprague-Dawley rats (6–8 wk). All procedures were performed in accordance with New Zealand animal welfare legislation, and the experiments and procedures were approved by the University of Otago Committee on Ethics in the Care and Use of Laboratory Animals. Rats were anesthetized with ketamine (100 mg/kg ip) and decapitated, and the brain was quickly removed and cooled with ice-cold artificial cerebrospinal fluid (ACSF). The hippocampi were dissected free, and area CA3 was removed with a manual knife-cut to reduce potential hyperexcitability or slow-onset potentiation (Bortolotto and Collingridge 1993). Slices were transferred to a recording chamber and superfused (2 ml/min) with ACSF of the following composition (in mM): 124 NaCl, 3.2 KCl, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 2.5 $CaCl_2$, 1.3 $MgCl_2$, and 10 glucose (equilibrated with 95% O_2-5% CO_2). Before recording, slices were allowed to equilibrate for 2 h while the temperature was increased slowly to 32.5°C.

Data acquisition and analysis

Intracellular recording microelectrodes were pulled from borosilicate glass and filled with 2 M potassium acetate (resistances, 70–130 MΩ). Current-clamp recordings were made from CA1 pyramidal neurons using an Axoclamp 2A amplifier (Axon instruments) and pCLAMP 7.0 software (Axon instruments), and data were stored on a computer for off-line analysis using pCLAMP 7.0 software. The membrane potential of the impaled neuron was held constant throughout the experiment at −65 mV by manually adjusting the holding current. The following cell parameters were obtained during a typical experiment: sAHP amplitude and mAHP amplitude (when mAHP was distinctly present) measured at their peak amplitudes, number of action potentials fired by a depolarizing current pulse (0.5 nA, 250 ms), the amplitude and width at half-amplitude (half-width) of the first action potential in a train, input resistance (R_{in}), and the holding current required to hold the cell at −65 mV (I_{hold}). The sAHP and mAHP were induced by a train of four action potentials, each elicited by a separate depolarizing current pulse (2 ms, 3 nA, 5 ms interpulse interval). R_{in} was assessed by measuring the peak amplitude of the voltage change in response to a 250 ms, 0.2 nA hyperpolarizing current pulse. These parameters were obtained in a cyclical manner every 2 min throughout the course of the experiment. The acute effects of DHPG were quantified by averaging the last three data points of drug application. Persistent effects of DHPG following drug washout were quantified by averaging three data points at the time stated in the text. Statistical significance was determined by performing paired and unpaired Student's *t*-tests as appropriate at the $P < 0.05$ confidence level, and data are presented as mean ± SE.

Drugs and chemicals

All salts were obtained from BDH Chemicals (Poole, UK); (RS)-DHPG, 2-methyl-6-(phenylethynyl)-pyridine (MPEP), SIB 1757, LY367385 and LY341495 from Tocris Cookson (Bristol, UK); chelerythrine chloride, cyclopiazonic acid (CPA), U-73122 from Biomol Research Laboratories (Plymouth Meeting, PA); Go 6983 from Calbiochem. Drugs were dissolved in H_2O (DHPG, chelerythrine), 100 mM NaOH (LY367385, LY341495), or dimethyl sulfoxide (DMSO; CPA, Go 6983, MPEP, SIB 1757, U-73122) and diluted ≥500-fold to their final concentration in ACSF. Controls for those experiments where a DMSO-dissolved drug was applied prior to DHPG consist of application of DMSO alone prior to DHPG. Control cells were run interleaved with cells exposed to drugs.

RESULTS

Group I mGluR-specific agonist (RS)-DHPG induces a persistent increase in excitability

Application of DHPG (20 μM, 10 min) rapidly abolished the sAHP (−92 ± 3%, $n = 17$; Fig. 1*A*, Table 1). In the present experiments, the fAHP was not readily discernable; but in 40% of cells, a mAHP was measurable in addition to the sAHP, and this was also rapidly suppressed by DHPG (−76 ± 12%, $n = 11$; Fig. 1*B*, Table 1). While the mAHP recovered quickly to near baseline on washout of DHPG (−17 ± 5% at 30 min postwash), the suppression of the sAHP was persistent (−60 ± 6% at 30 min postwash). Even 60 min after commencing washout of DHPG, the sAHP was still depressed by −46 ± 6%. The sustained depression of the sAHP was not due to rundown of the cell because no deterioration of the sAHP was observed in control experiments run over the same length of time (Fig. 1*A*). The failure of the mAHP to completely recover to baseline can be attributed to contamination of the mAHP by the sAHP such that the persistent suppression of the sAHP prevented full recovery of the measured mAHP.

As a measure of cell excitability, we used the number of spikes fired during a 250-ms depolarizing current pulse. DHPG increased the number of spikes by 7.7 ± 0.7 ($n = 15$; Fig. 1*C*, Table 1). Following washout, the cells remained more excitable than prior to DHPG application with the number of spikes elevated by 3.7 ± 1.0 above baseline at 30 min after DHPG washout and nearly recovered by 60 min of washout (1.9 ± 1.2 above baseline; not significantly different from controls, $P > 0.1$). The observation that the time course of recovery of excitability during washout of DHPG lay between that of the sAHP and the mAHP probably reflects the fact that the number of spikes that are fired in response to a short depolarizing pulse of current is influenced by both s- and mAHP (Storm 1990). Application of DHPG also increased the input resistance (10 ± 3%, $n = 17$; Fig. 2*A*, Table 1) and depolarized the membrane (as inferred by a −0.10 ± 0.01 nA change in the holding current required to hold the membrane at approximately −65 mV; Fig. 2*B*, 2C, Table 1) as previously reported (Davies et al. 1995; Gereau and Conn 1995; Gereau et al. 1995). These effects were moderately persistent, with both parameters recovering to baseline after 60 min of washout. As previously reported for ACPD (Hu and Storm 1991; Wu and Barish 1999), application of DHPG produced a slight but measurable de-

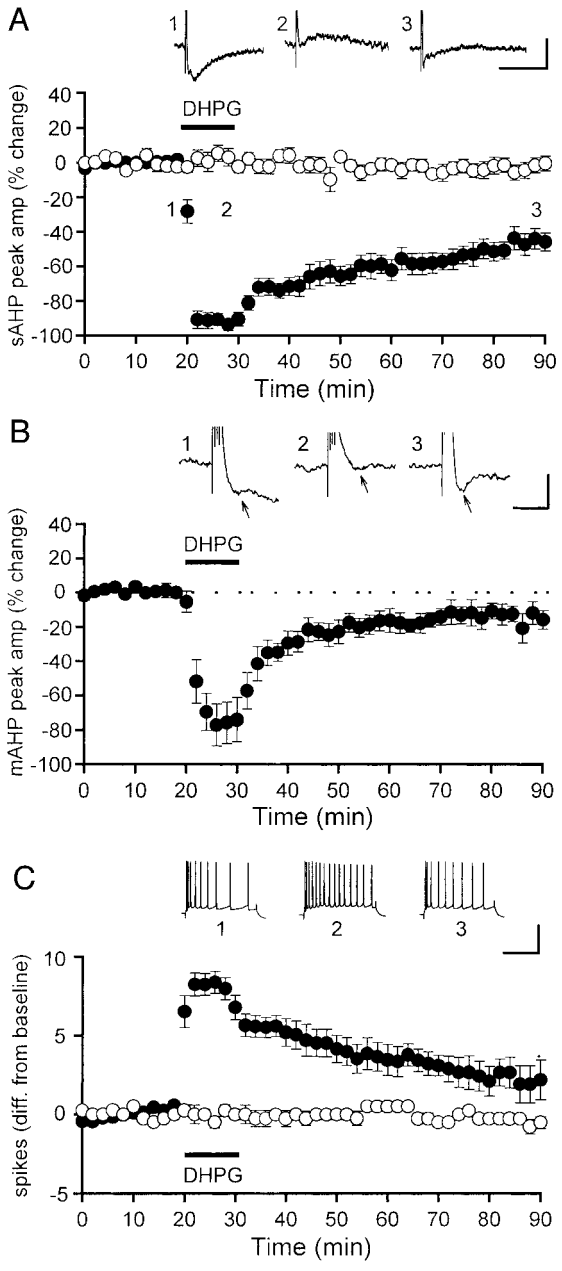

FIG. 1. (RS)-3,5-dihydroxyphenylglycine (DHPG) suppressed the slow and medium afterhyperpolarizations (s- and mAHPs) and caused an increase in excitability. *A*: peak amplitude of the sAHP in cells exposed to 20 μM DHPG for 10 min (●, $n = 17$) or in untreated cells (○, $n = 6$). *Inset waveforms* are single responses taken at the times indicated from a cell treated with DHPG. Action potentials have been truncated. Calibration bars: 5 mV, 1 s. In most cells, DHPG also induced or unmasked a depolarizing afterpotential such that the measured suppression of the s- and mAHP exceeded −100%. For such experiments, values exceeding −100% have been converted to −100%. *B*: peak amplitude of the mAHP in cells exposed to 20 μM DHPG for 10 min (●, $n = 11$). *Inset waveforms* are single responses taken from the same cell and at the same times as in *A*. mAHP is indicated in each case (↑) Calibration bars: 5 mV, 100 ms. *C*: number of action potentials in a train evoked by a 250-ms, 0.5-nA depolarizing pulse in cells exposed to 20 μM DHPG for 10 min (●, $n = 15$) or in untreated cells (○, $n = 6$). *Inset waveforms* are single responses taken from the same cell and at the same times as in *A*. Calibration bars: 20 mV, 100 ms.

crease in the amplitude of the action potential (−3 ± 1%, $n = 17$) and an increase in its half-width (+0.07 ± 0.01 ms difference from baseline). While DHPG-induced changes in the action potential, input resistance, and resting membrane potential would be expected to have some influence on excitability, it was sometimes not possible to reliably measure the effects of antagonists on the DHPG-induced changes in these parameters due to the high variability between cells and smaller numbers of cells within each drug group. Similarly, the mAHP was not always present in enough cells to reliably report the effects of some antagonists on this phase of the afterhyperpolarization.

mGluR subtypes that mediate DHPG-induced changes in the sAHP and excitability

Previous studies have suggested that mGluR5 receptors mediate the effects of DHPG on the sAHP and excitability in CA1 pyramidal neurons. To test this we used a specific antagonist of mGluR5, MPEP (Gasparini et al. 1999) at a saturating dose (10 μM). Surprisingly, preincubation of the slices with 10 μM MPEP only partly prevented the subsequent suppression of the sAHP and increase in excitability due to DHPG. In the presence of MPEP, DHPG suppressed the sAHP by -51 ± 12% ($n = 7$; Fig. 3*A*). This was a significantly smaller depression than that seen in the absence of MPEP ($P < 0.01$). The effect of DHPG was persistent with the sAHP still suppressed by −25 ± 8% 50 min after washout. The mAHP was also partially suppressed by DHPG in the presence of MPEP (−27 ± 8%, $n = 5$; Fig. 3*B*); this was a significantly smaller depression than in the absence of MPEP ($P < 0.01$). The number of spikes in a train was increased by DHPG by 1.8 ± 0.8 ($n = 6$; Fig. 3*C*) which was a significantly smaller increase than that seen in the absence of MPEP ($P < 0.001$), but this increase was not lasting, returning to baseline 10 min after commencement of washout. Interestingly, in the presence of MPEP, DHPG failed to evoke a significant change in the holding current required to hold the membrane potential constant ($P > 0.1$; Fig. 3*D*)

The effects of MPEP on the sAHP and excitability were extremely heterogeneous between cells (Fig. 5). This observation, taken together with the fact that 10 μM is a completely saturating dose (Gasparini et al. 1999) makes it appear unlikely that the partial effect of MPEP is attributable to incomplete

TABLE 1. *Effects of DHPG on cell parameters*

	Pre-DHPG	DHPG	30-min Wash	60-min Wash
sAHP amp, mV	−7.1 ± 0.3	−0.6 ± 0.2*	−2.8 ± 0.5*	−3.8 ± 0.5*
n	17	17	12	10
mAHP amp, mV	−4.6 ± 0.3	−1.3 ± 0.5*	−4.0 ± 0.4*	−3.9 ± 0.4*
n	11	11	11	10
No. of spikes	7.8 ± 0.6	15.5 ± 0.7*	10.9 ± 1.1*	9.4 ± 1.2
n	15	15	10	6
R_{in}, MΩ	61.1 ± 3.5	67.2 ± 4.1*	60.0 ± 5.2	58.8 ± 6.2
n	17	17	12	10
I_{hold}, nA	0.07 ± 0.02	−0.03 ± 0.02*	0.04 ± 0.03*	0.07 ± 0.03
n	17	17	12	10

Data are raw (non-normalized) values and are of the form: means ± SE. *n* are number of data sample. All data are from experiments performed in the absence of DMSO with the exception of the mAHP for which data from experiments performed in the presence and absence of DMSO have been pooled. sAHP, slow afterhyperpolarization; mAHP, medium afterhyperpolarization; R_{in}, input resistance; I_{hold}, holding current. * Indicates a significant difference from pre-DHPG (baseline) values ($P < 0.05$, paired Student's t-test).

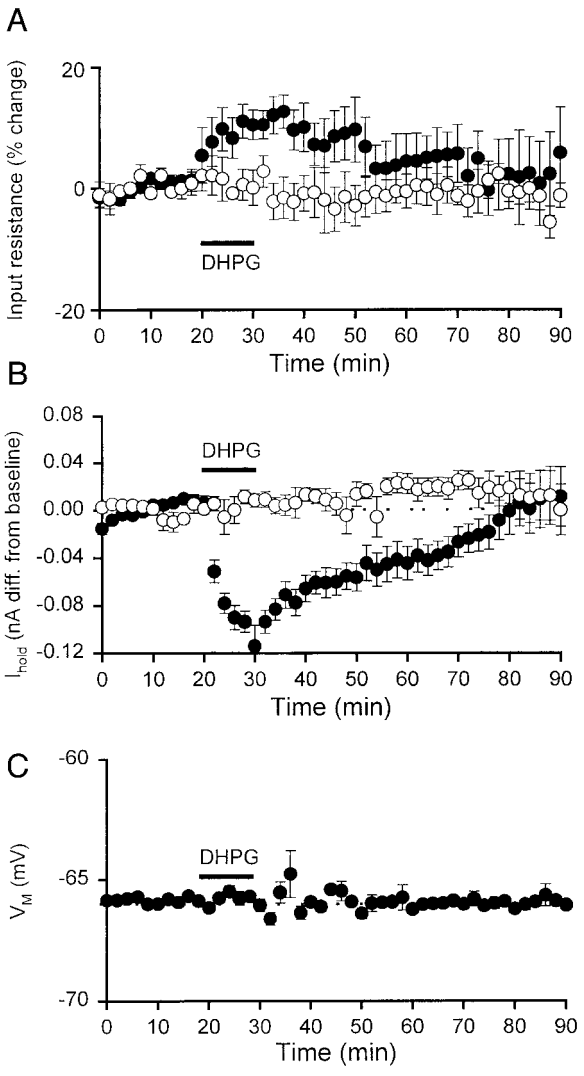

FIG. 2. DHPG caused an increase in input resistance and a change in holding current. *A*: input resistance as calculated from the voltage change in response to a 0.1-nA hyperpolarizing current step in cells exposed to 20 μM DHPG for 10 min (●, $n = 17$) or in untreated cells (○, $n = 6$). *B*: holding current required to maintain the membrane potential at approximately −65 mV in cells exposed to 20 μM DHPG for 10 min (●, $n = 17$) or in untreated cells (○ = 6). *C*: holding membrane potential in cells exposed to 20 μM DHPG for 10 min ($n = 17$), showing that manual current adjustment was effective in maintaining the membrane potential near −65 mV.

receptor blockade. However, to rule out this possibility, in two separate experiments, the concentration of MPEP was raised to 100 μM and the duration of application prior to DHPG was increased to 20 min. In these cells, the suppression of the sAHP due to DHPG was −72 and −89%, thus demonstrating that 100 μM MPEP is no more efficacious than 10 μM. As an additional check, an alternative mGluR5-specific antagonist, SIB 1757 (100 μM) was used. This antagonist also only partially blocked the DHPG-induced suppression of the sAHP ($n = 3$, data not shown).

Although localization studies have shown that mGluR5 is the predominant or only mGluR present in CA1 pyramidal cells, it is possible that the effect of DHPG on excitability is also mediated by mGluR1 because DHPG is an agonist of both mGluR5 and mGluR1 receptors. We therefore preincubated slices with LY367385 (300 μM), a selective competitive antagonist of mGluR1 (Clark et al. 1997) in addition to MPEP. Unexpectedly, the combination of MPEP and LY367385 blocked the DHPG-induced suppression of the sAHP (−9 ± 9%, $P > 0.1$) and the mAHP and prevented the DHPG-induced increase in the number of spikes in a train (Figs. 4, *A–C*, and 5). As with MPEP alone, MPEP plus LY367385 also prevented the DHPG-induced change in the holding current (Fig. 4*D*). The effects of blocking both mGluR5 and mGluR1 simultaneously were mimicked by the broad-spectrum mGluR antagonist, LY341495 (Kingston et al. 1998), which at 100 μM prevented suppression of the sAHP by DHPG (Fig. 5) as well as the increase in excitability and the DHPG-induced change in the holding current (data not shown).

These results demonstrate that the effects of DHPG on the sAHP, mAHP, and excitability are mediated by both mGluR5 and mGluR1, the contribution of each varying greatly from cell to cell. In contrast, the DHPG-induced change in holding current only appears to require activation of mGluR5.

Phosphoinositide hydrolysis signal transduction pathway not required for changes in the sAHP or excitability

Because Group I mGluRs are thought to be PLC-coupled receptors, it was expected that at least part of the response to DHPG would be mediated via one of the two branches of the phosphoinositide hydrolysis pathway downstream from PLC: stimulation of PKC by DAG or release of Ca^{2+} from stores by IP_3. We tested the former by incubating the slices in chelerythrine, a specific blocker of PKC. A high dose (10 μM) of chelerythrine applied for 20 min prior to DHPG application did not prevent the subsequent effects of DHPG on the sAHP and excitability (Fig. 6*A*). In the presence of chelerythrine, DHPG suppressed the sAHP (−91 ± 3%, $n = 4$) to the same extent as in the absence of chelerythrine ($P > 0.9$). The number of spikes in a train was increased by DHPG by 7.9 ± 1.4 ($n = 4$), which was also not significantly different to the increase seen in the absence of chelerythrine ($P > 0.9$). In the two cells in which a mAHP was distinguishable, chelerythrine did not affect the DHPG-induced depression (−100% depression in both cases). Because PKC is known to exist in multiple isoforms, only some of which are inhibited by chelerythrine, these results were confirmed by using an alternative PKC-inhibitor, Go 6983, at a dose (3 μM) 30 times higher than that previously shown to be effective in CA1 (Bortolotto and Collingridge 2000). Go 6983 had a similar lack of effect to chelerythrine on the DHPG-induced suppression of the sAHP and increase in excitability ($n = 3$, data not shown).

We also tested the alternative branch of the PLC transduction pathway by inhibiting IP_3-activated Ca^{2+} stores with CPA. Because CPA acts by preventing refilling of the stores, CPA (20 μM) (Seidler et al. 1989), was incubated for ≥30 min prior to application of DHPG to give the stores time to deplete. By itself CPA caused a small depression of the sAHP (−12 ± 3%, $n = 4$; data not shown) that may be due to a small contribution by Ca^{2+} stores toward activation of the Ca^{2+}-activated K^+ channels that underlie the sAHP, similar to that reported for ryanodine-sensitive Ca^{2+} stores in dentate granule neurons (Abdul-Ghani et al. 1996a). However, preincubation in CPA did not prevent the suppression of the sAHP by DHPG

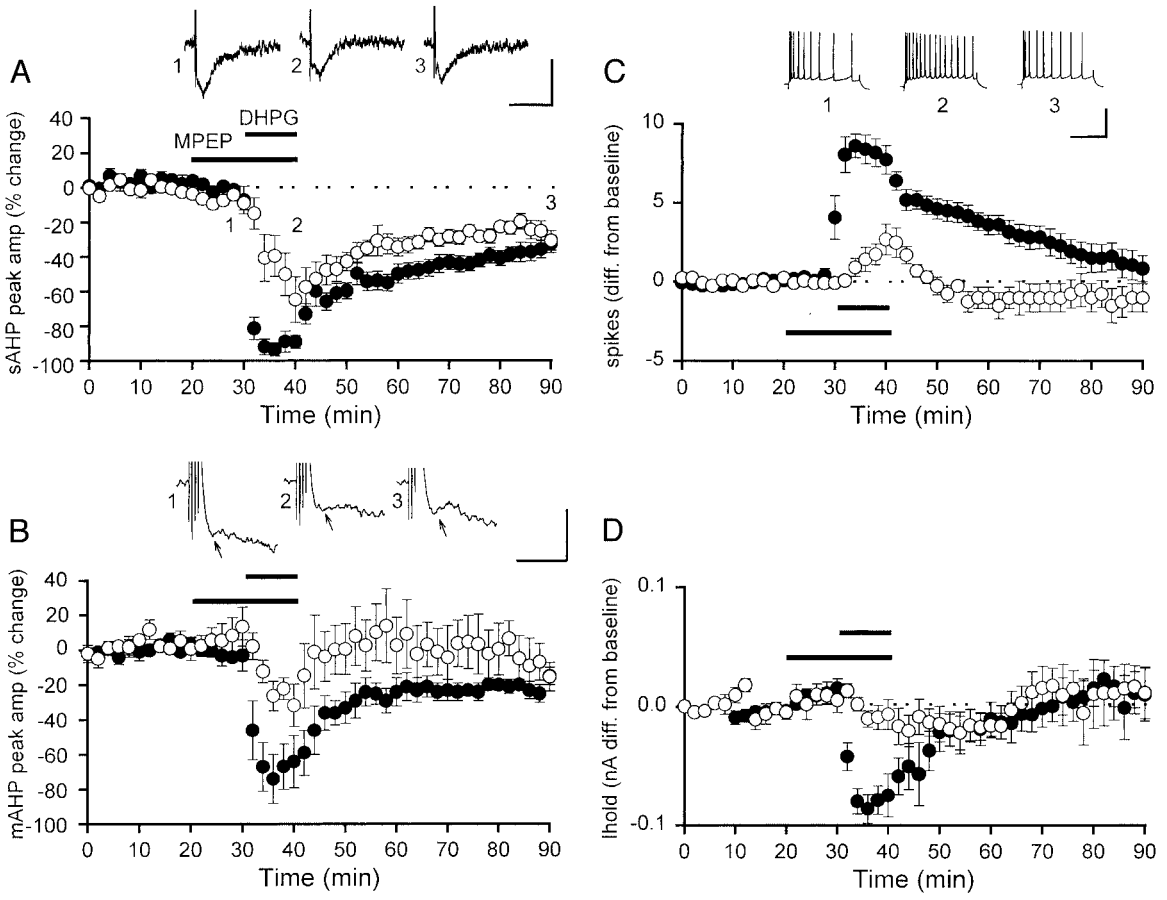

FIG. 3. mGluR5 antagonism partially prevented DHPG-induced changes. *A*: peak amplitude of the sAHP in cells exposed to 20 μM DHPG for 10 min in the presence (○, $n = 7$) or absence (●, $n = 10$) of 10 μM 2-methyl-6-(phenylethynyl)-pyridine (MPEP). *Inset waveforms* are single responses taken at the times indicated from a cell exposed to MPEP. Action potentials have been truncated. Calibration bars: 5 mV, 1 s. *B*: peak amplitude of the mAHP in cells exposed to 20 μM DHPG for 10 min in the presence (○, $n = 5$) or absence (●, $n = 7$) of 10 μM MPEP. *Inset waveforms* are single responses taken from the same cell and at the same times as in *A*. mAHP is indicated in each case (↑). Calibration bars: 5 mV, 100 ms. *C*: number of action potentials in a train evoked by a 250-ms, 0.5-nA depolarizing pulse in the same cells as in *A*. *Inset waveforms* are single responses taken from the same cell and at the same times as in *A*. Calibration bars: 20 mV, 100 ms. *D*: holding current required to maintain the membrane potential at approximately −65 mV in the same cells as in *A*.

(−98 ± 1%, $n = 4$; Fig. 6*B*), which was not significantly different to the suppression seen in the absence of CPA ($P > 0.2$). The increase in the number of spikes (8.9 ± 1.0 spikes above baseline, $n = 4$) caused by DHPG in the presence of CPA (Fig. 6*B*) was also not affected ($P > 0.5$). Due to the long incubation period for CPA, it was not possible to hold the cells in this experiment for a long washout period.

It is possible that both PKC and IP_3 pathways could independently mediate the complete suppression of the sAHP and increase in excitability. This could account for the failure of chelerythrine and CPA to prevent the DHPG-induced suppression of the sAHP and increase in excitability. To exclude this possibility, we blocked the phosphoinositide hydrolysis pathway at the level of PLC itself using U-73122 (Bleasdale and Fisher 1993). Incubation of U-73122 prior to DHPG application at a dose (10 μM) that has previously been effective in CA1 (Cohen et al. 1998) and for a longer duration than used in that study did not prevent the suppression of the sAHP by DHPG (−83 ± 7%, $n = 6$; Fig. 6*C*). This was not significantly different to the DHPG-induced suppression caused by DHPG alone ($P > 0.4$). Similarly, the increase in the number of spikes induced by DHPG in the presence of U-73122 (8.1 ± 1.1 above baseline, $n = 6$; Fig. 6*C*) was not affected ($P > 0.9$). Both of these effects exhibited the persistence seen in experiments with DHPG alone, with the sAHP suppressed by −41 ± 9% at 30 min after washout, and the number of spikes increased by 3.5 ± 1.0 above baseline at the same time. By 50 min after washout, the sAHP was still suppressed by −30 ± 4% while the number of spikes had recovered to near baseline (1.3 ± 1.2 above baseline). For the three cells in which a measurable mAHP was present, the DHPG-induced depression in the presence of U-73122 was −89 and −100% in two cells, but in one cell, U-731222 appeared to prevent the DHPG-induced suppression of the mAHP (−3% depression).

DISCUSSION

DHPG induces a persistent excitability increase

We have demonstrated that the changes induced in several membrane parameters by DHPG, in particular the suppression of the sAHP, are highly persistent following agonist washout. Although a persistent increase in excitability as a consequence

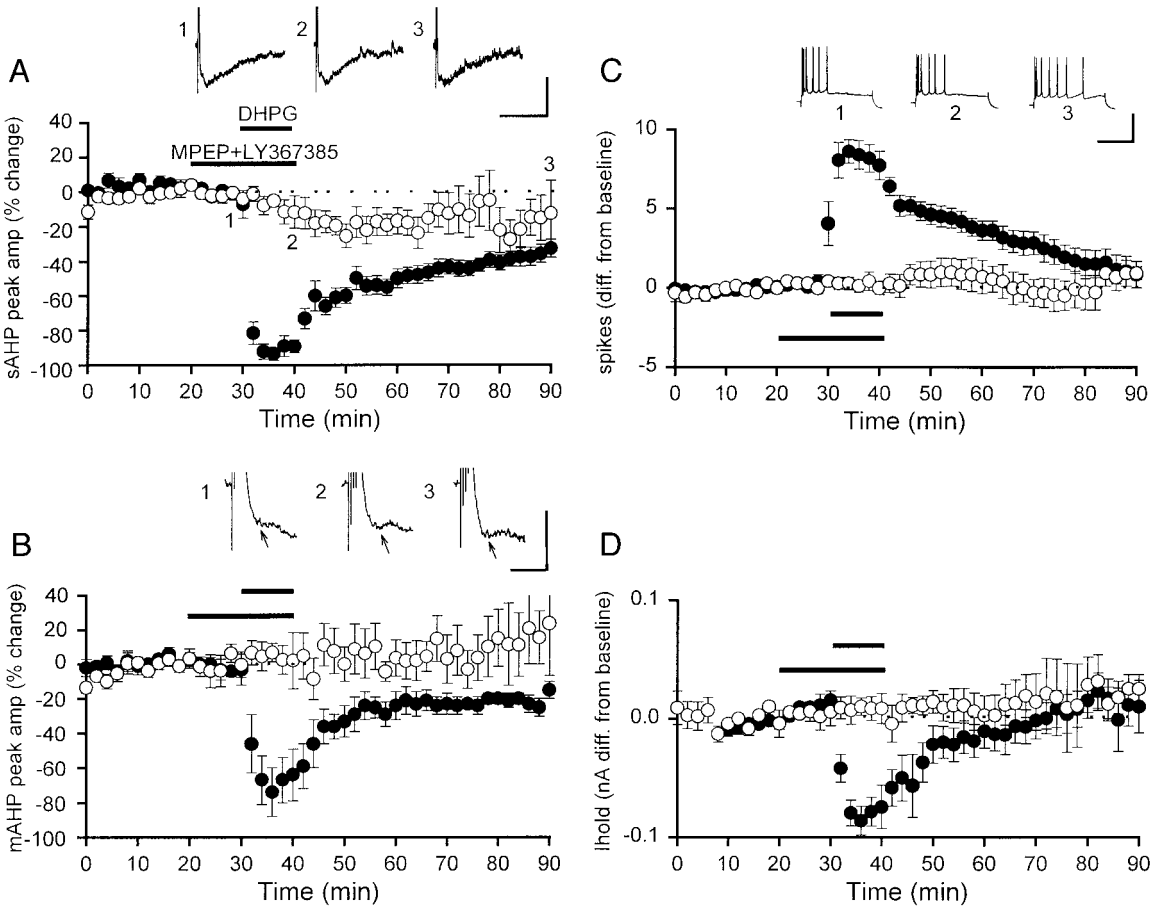

FIG. 4. Block of both mGluR5 plus mGluR1 fully prevented DHPG-induced changes. *A*: peak amplitude of the sAHP in cells exposed to 20 μM DHPG for 10 min in the presence (○, $n = 7$) or absence (●, $n = 10$) of 10 μM MPEP +300 μM LY367385. *Inset waveforms* are single responses taken at the times indicated from a cell treated with MPEP + LY367385. Action potentials have been truncated. Calibration bars: 5 mV, 1 s. *B*: peak amplitude of the mAHP in cells exposed to 20 μM DHPG for 10 min in the presence (○, $n = 6$) or absence (●, $n = 7$) of 10 μM MPEP +300 μM LY367385. *Inset waveforms* are single responses taken from the same cell and at the same times as in *A*. mAHP is indicated in each case (↑). Calibration bars: 5 mV, 100 ms. *C*: number of action potentials in a train evoked by a 250-ms, 0.5-nA depolarizing pulse in the same cells as in *A*. *Inset waveforms* are single responses taken from the same cell and at the same times as in *A*. Calibration bars: 20 mV, 100 ms. *D*: holding current required to maintain the membrane potential at approximately −65 mV in the same cells as in *A*.

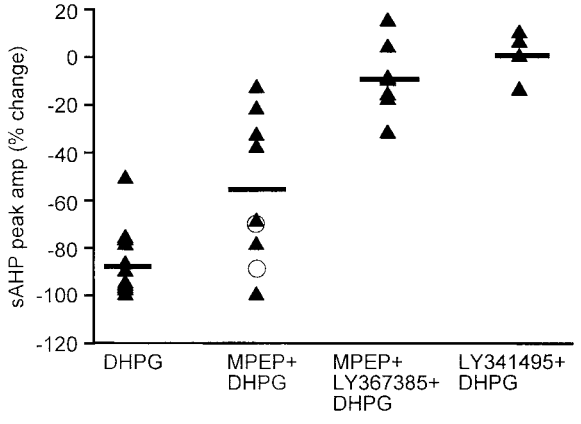

FIG. 5. Plot summarizing the effects of DHPG on the sAHP in the presence of mGluR antagonists. Each point represents the maximum suppression of the sAHP during a single experiment by DHPG alone ($n = 10$), DHPG in the presence of 10 μM MPEP ($n = 7$; ▲) and 100 μM MPEP ($n = 2$; ○), DHPG in the presence of 10 μM MPEP +300 μM LY367385 ($n = 7$), and DHPG in the presence of 100 μM LY341495 ($n = 4$). —, the average change in each case.

of depression of the sAHP has been previously observed in CA1 pyramidal neurons following application of the Group I and II agonist, ACPD (Cohen et al. 1999), other studies have found that DHPG causes only transient suppression of the sAHP (Gereau et al. 1995). The cause of this disparity is not clear, but it is possible that the duration of DHPG application in previous studies was insufficiently long for persistent effects to be induced. While it is conceivable that the persistence of the effects of DHPG observed in the present study is due to the action of residual DHPG, this seems improbable given that recovery of the different cell parameters during washout occurred with very different time courses. While the mAHP recovered rapidly to near baseline minutes after DHPG washout commenced, the holding current, input resistance, and cell excitability measures recovered more slowly over the course of 30–60 min, and the sAHP showed an even slower recovery function. In addition, the potentiating effect of DHPG on *N*-methyl-D-aspartate-induced depolarizations has been shown previously to wash out rapidly from hippocampal slices (Palmer et al. 1997). It therefore seems likely that differences in the down-

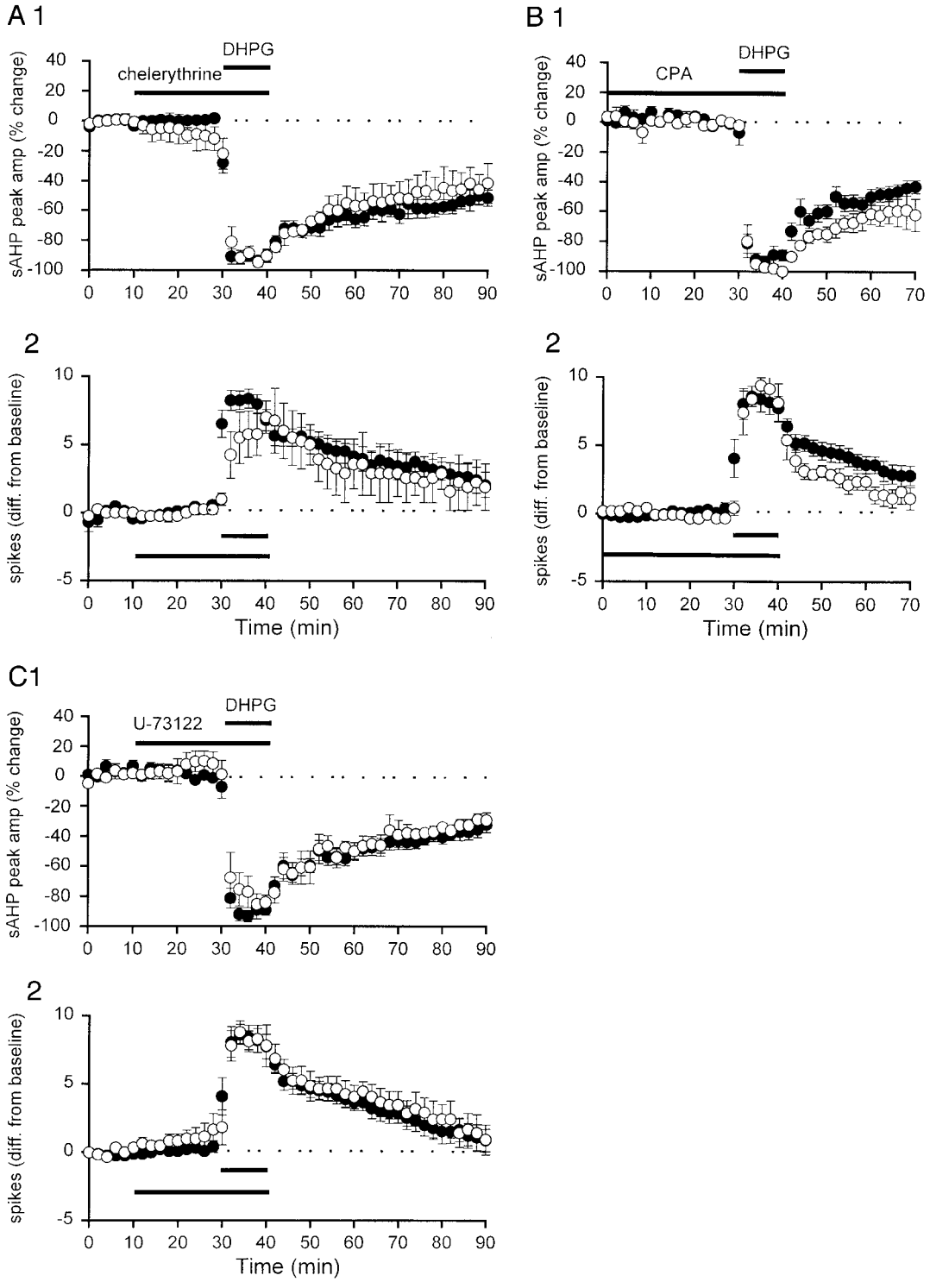

FIG. 6. DHPG-induced suppression of sAHP and increase in excitability is not dependent on phospholipase C. *A*: peak amplitude of the sAHP (*i*) and the number of action potentials in a train evoked by a 250-ms, 0.5-nA depolarizing pulse (*ii*) in cells exposed to 20 μM DHPG for 10 min in the presence (open circles, $n = 4$) or absence (filled circles, $n = 17$) of 10 μM chelerythrine (thin bar). *B*: peak amplitude of the sAHP (*i*), and the number of action potentials in a train evoked by a 250 ms, 0.5 nA depolarizing pulse (*ii*), in cells exposed to 20 μM DHPG for 10 min in the presence (open circles, $n = 4$) or absence (filled circles, $n = 10$) of 20 μM CPA. *C*: peak amplitude of the sAHP (*i*), and the number of action potentials in a train evoked by a 250 ms, 0.5 nA depolarizing pulse (*ii*), in cells exposed to 20 μM DHPG for 10 min in the presence (open circles, $n = 6$) or absence (filled circles, $n = 10$) of 10 μM U-73122.

stream signaling mechanisms responsible for these various effects accounts for the disparity in the time courses of recovery.

mGluR subtypes responsible for DHPG-induced changes

Of the two subtypes that make up Group I mGluRs, mGluR1 and mGluR5**,** localization studies have suggested that only the latter is present in the postsynaptic membrane in CA1 pyramidal neurons (Lujan et al. 1996; Shigimoto et al. 1997). This, together with an mGluR5 agonist study (Mannaione et al. 1999), provides support for the hypothesis that the DHPG-induced excitability increase is due to activation specifically of mGluR5 receptors. Further, in a recent publication that appeared while the present manuscript was in preparation, it has been shown that the mGluR5-specific antagonist MPEP can completely prevent the effects of DHPG on the sAHP (Mannaioni et al. 2001). In contrast, the results of the present study (using the same antagonists), demonstrate that both mGluR5 and mGluR1 contribute to the effects of DHPG on the sAHP and excitability. Because DHPG is also an agonist at mGluR1 receptors and mGluR1 appears to mediate the ACPD-induced suppression of the sAHP in dentate granule neurons (Abdul-Ghani et al. 1996a), it is perhaps not surprising that mGluR1 contributes to the DHPG effects. A further contrast is seen with the DHPG-induced depolarization, which in the present study was found to be mGluR5 dependent but which was previously reported to be exclusively mGluR1 dependent (Mannaioni et al. 2001). While the reason for these disparities is not known, the explanation may lie in the age of the rats used (2–3 wk in the case of Mannaioni et al. vs. 6–8 wk in the present study) because the expression of Group I mGluRs undergoes marked changes during postnatal development, both in terms of the types expressed and their location within the cell (Lopez-Bendito et al. 2001; Minakami et al. 1995; Romano et al. 1996).

An interesting feature of the present results is the extreme heterogeneity of the MPEP effect, which indicates that the relative contribution of each subtype to the observed excitability changes varies greatly between cells. This could be explained by differences in the levels of expression of mGluR5 and mGluR1 between cells or differences in the proportion of expressed receptors that couple to the transduction pathway responsible for suppression of the sAHP. The finding from localization studies that heterogeneous expression of mGluR5 receptors occurs in CA1 pyramidal neurons (Lujan et al. 1996, 1997) raises the possibility that differences between cells in the physical proximity of mGluR5 or mGluR1 receptors to transduction mechanisms or ion channels could determine the contribution of these receptors to the excitability increase.

Signal transduction pathways responsible for DHPG-induced changes

Because Group I mGluRs couple to the phosphoinositide hydrolysis transduction pathway, it is thought likely that the effects of DHPG on excitability are mediated via this pathway. We directly tested this idea by inhibiting the PKC and IP_3 branches of the PLC signaling pathway, and unexpectedly neither pathway is apparently involved. This was confirmed by blocking PLC itself. We therefore suggest that in CA1 pyramidal neurons mGluR5 and mGluR1 receptors are coupled to an alternative transduction pathway(s) that modulates excitability. There is already some evidence for PLC-independent actions of Group I mGluRs on excitability in other cell types. In CA3 pyramidal neurons, suppression of the fast AHP by DHPG has been shown in a preliminary study to be PLC independent (Bianchi et al. 2000), and in dentate granule cells, suppression of the sAHP by ACPD via Group I mGluRs is only partly dependent on PLC (Abdul-Ghani et al. 1996a). In addition, DHPG-induced long-term depression of field EPSPs in CA1 is independent of PKC and IP_3 pathways (Schnabel et al. 1999).

The question remains of what transduction pathway(s) mediates the DHPG-induced excitability changes in CA1. Recently, inhibition of G-protein activity with the endogenous G-protein regulatory protein, RGS4, has indicated that DHPG-induced suppression of the sAHP in CA1 is G-protein dependent (Saugstad et al. 1998), and it has been further postulated that the DHPG-induced changes in excitability are due to G_q linked to PLC (Saugstad et al. 1998) contrary to our results. However, although G_q has been shown to couple to PLC (Smrcka et al. 1991), each mGluR subtype is capable of coupling to multiple types of G protein (Akam et al. 1997; Hermans et al. 2000), raising the possibility that a G protein other than G_q mediates the effects of DHPG on excitability. Further, there is evidence that G_q itself can couple, directly or indirectly, to multiple transduction pathways. Alternative pathways might include PKA, Ca^{2+}/calmodulin-dependent protein kinase, protein kinase G, or tyrosine kinase. While we have shown that Ca^{2+} derived from IP_3-activated stores does not appear to play a part in the DHPG-induced changes, we cannot discount the possibility that Ca^{2+} release from ryanodine-sensitive stores is involved, as is the case for dentate granule neurons' response to ACPD (Abdul-Ghani et al. 1996a). A further possibility is that the mGluRs responsible for the DHPG-induced changes in excitability could directly modulate, via a G protein, the K^+ channels that underlie the sAHP (see Clapham 1994). Further work with specific antagonists of these and other potential signal transduction pathways will be required to elucidate the mechanisms that underlie the mGluR-mediated persistent excitability changes in CA1.

mGluRs and the priming of LTP

We have previously shown that both pharmacological activation of mGluRs with ACPD or DHPG and electrophysiological activation of glutamatergic afferents primes subsequent LTP in area CA1 (Cohen and Abraham 1996; Cohen et al. 1998; Raymond et al. 2000). Priming is a form of "metaplasticity" (Abraham and Bear 1996) whereby prior synaptic activity modulates subsequent synaptic plasticity. We have proposed that the excitability change induced by mGluR activation primes the initial induction of LTP, while mGluR-triggered protein synthesis facilitates the persistence of subsequent LTP (Cohen et al. 1999; Raymond et al. 2000). However, some of antagonists used in the present study (U-73122, chelerythrine) that failed to affect the DHPG-induced excitability changes have been shown to effectively block mGluR-induced priming of LTP (Bortolotto and Collingridge 2000; Cohen et al. 1998). Similarly, in preliminary studies, we have observed that protein

synthesis inhibitors that block priming (Raymond et al. 2000) nonetheless fail to block the DHPG-mediated excitability increase (unpublished observations). Thus although excitability changes induced by ACPD have been linked to LTP priming (Cohen et al. 1999), at least for DHPG, these effects appear to involve independent mechanisms. On the other hand, both the acute and persistent effects of mGluR activation on pyramidal cell excitability should have a profound influence on information processing and transfer through the hippocampus. Such cell-wide plasticity would interact with, and potentially amplify, any synapse-specific forms of plasticity that co-occur during bouts of intense neural activity.

We thank Dr. C. Raymond for helpful discussions.

This research was supported by a grant from the New Zealand Health Research Council.

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