Long-Lasting Increase in Cellular Excitability Associated With the Priming of LTP Induction in Rat Hippocampus

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Cohen, Akiva S., Christine M. Coussens, Clarke R. Raymond, and Wickliffe C. Abraham. Long-lasting increase in cellular excitability associated with the priming of LTP induction in rat hippocampus. J. Neurophysiol. 82: 3139–3148, 1999. The mechanisms underlying the facilitation (priming) of long-term potentiation (LTP) by prior activation of metabotropic glutamate receptors (mGluRs) were investigated in area CA1 of rat hippocampal slices. In particular, we focused on whether a long-lasting increase in postsynaptic excitability could account for the facilitated LTP. Administration of the mGluR agonist 1S,3R-1-amino-1,3-cyclopentanedicarboxylic acid (ACPD) produced rapid decreases in the amplitude of both the slow spike afterhyperpolarization (AHPslow) and spike frequency adaptation recorded intracellularly from CA1 pyramidal cells. These changes persisted after drug washout, showing only a slow decay over 20 min. ACPD also caused a leftward shift of the field EPSP-population spike relation and an overall increase in population spike amplitude, but this effect was not as persistent as the intracellularly measured alterations in cell excitability. ACPD-treated cells showed increased spike discharges during LTP-inducing tetanic stimulation, and the amplitude of the AHPslow was negatively correlated with the degree of initial LTP induction. The β-adrenergic agonist isoproterenol also caused excitability changes as recorded intracellularly, whereas in extracellular experiments it weakly primed the induction but not the persistence of LTP. ACPD primed both LTP measures. Isoproterenol administration during the tetanus occluded the priming effect of ACPD on initial LTP induction but not its effect on LTP persistence. We conclude that the persistent excitability changes elicited by ACPD contributes to the priming of LTP induction but that other ACPD-triggered mechanisms must account for the facilitated persistence of LTP in the priming paradigm.

INTRODUCTION

The ability to produce activity-dependent synaptic plasticity, such as long-term potentiation (LTP) and long-term depression (LTD) is not a fixed property of individual synapses but rather a highly regulated function. External signals such as hormones and modulatory neurotransmitters that affect cellular excitability or signalling in biochemical pathways can exert a profound influence on the degree of synaptic plasticity induced by a particular stimulus protocol. Such changes in cellular or synaptic “state” can be induced either directly, by activation of postsynaptic receptors, or indirectly by modulating the activity of afferent principal cells and interneurons.

Another factor contributing to the synaptic state, and pertinent to synaptic plasticity, is the history of pre- and postsynaptic activity. Prior synaptic activity has been shown in some cases to inhibit subsequent LTP and facilitate LTD, or in other cases to facilitate LTP (see Abraham and Tate 1997 for a review). This family of effects has been collectively referred to as “metaplasticity” (Abraham 1996; Abraham and Bear 1996). An early report of facilitated LTP showed that prior activation of metabotropic glutamate receptors (mGluRs) can set a “molecular switch” that enables the establishment of a late phase of LTP by subsequent tetanization (Bortolotto et al. 1994). Although the existence of this mGluR-mediated molecular switch is controversial (Thomas and O’Dell 1995), we recently confirmed that transient activation of mGluRs by the agonist (1S,3R)-1-amino-1,3-cyclopentanedicarboxylic acid (ACPD), which by itself had no long-lasting effect on the slope of the field excitatory postsynaptic potential (fEPSP), nonetheless facilitated the induction and stability of LTP induced by a weak tetanus delivered 20–60 min later (Cohen and Abraham 1996). This LTP “priming” effect did not require coactivation of α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) or N-methyl-d-aspartate (NMDA) receptors, but was blocked by the mGluR antagonist (+)-2-amino-3-phosphonopropionic acid (L-AP3).

Whereas the mechanism underlying the ACPD-induced priming effect has yet to be elucidated, protein kinases may be involved because the general kinase inhibitor K-252b blocked the setting of the molecular switch (Bortolotto et al. 1994). mGluRs belong to the large superfamily of G-protein coupled receptors that activate several biochemical cascades, but a recent study from our lab has limited the potential mechanisms to those activated by Group I mGluRs that couple to phospholipase C (PLC) (Cohen et al. 1998). Activation of this PLC pathway leads to the liberation of the second messengers inositol trisphosphate (IP3) and diacylglycerol following hydrolysis of phosphatidylinositol. It also increases basal cAMP accumulation by an L-AP3–sensitive mechanism (Winder and Conn 1992). Thus both protein kinase C (PKC) and protein kinase A (PKA) are putative candidate kinases for mediating the priming of LTP by mGluR activation.

One consistent downstream effect of both PKA and PKC activation is a general increase in hippocampal pyramidal cell excitability, evidenced by resting membrane depolarization, an increase in input resistance (Rm), a decrease in several potassium conductances, including the transient A current and the slow spike afterhyperpolarization (AHPslow), and a decrease in spike frequency adaptation, i.e., the decrease in spike firing rate during a prolonged depolarizing current pulse (Hoffman and Johnston 1998; Nicoll 1988). These changes can be engendered by activation of a variety of G-protein coupled receptors including mGluRs of the Group I type (Charpak et al. 1990;
Desai and Conn 1991; Gereau and Conn 1995; Goh and Ballyk 1993), muscarinic (Cole and Nicoll 1983), β-adrenergic (Dunnwiddie et al. 1992; Madison and Nicoll 1986), and dopamine receptors (Gribkoff and Ash 1984; Pedarzani and Storm 1995). In the case of β-adrenergic receptor activation, the increased cell excitability decays very slowly after drug washout and it has been suggested that such changes might promote the subsequent induction of LTP, which is a voltage-dependent process (Dunnwiddie et al. 1992). If the mGluR-mediated excitability changes are equally long-lasting, it is possible that they could contribute to the priming of LTP by ACPD.

The present study was undertaken to investigate whether the same moderate dose of ACPD that primes LTP (Cohen and Abraham 1996) also produces a persistent aftereffect on postsynaptic cell excitability that would account for its facilitation of subsequent LTP. The effects of ACPD on cell excitability and the induction of LTP were compared with those generated by the β-adrenergic agonist isoproterenol. Our findings indicate that increased postsynaptic excitability can account for part of the LTP facilitation effect elicited by mGluR priming, i.e., the enhanced initial induction, but not for the enhanced persistence of LTP. A preliminary report of some of these results has appeared (Cohen and Abraham 1997).

METHODOLOGY

Tissue preparation

Transverse hippocampal slices (400 µm thick) were prepared from young adult male Sprague-Dawley rats (2-3 mo old, 200-300g) as previously described (Kerr and Abraham 1995). All procedures were performed in accord 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 either anesthetized with halothane or injected with ketamine (100 mg/kg, i.p.) and decapitated. The brain was quickly removed and the hippocampi were dissected free. To reduce potential hyperexcitability, area CA3 was routinely removed by a manual knife cut. This procedure also prevents the slow-onset potentiation that can otherwise arise following mGluR activation (Bortolotti and Collingridge 1993). Slices were transferred to a slice chamber and allowed to equilibrate for 2 h while being continually superfused (2-3 ml/min) by an artificial cerebrospinal fluid (ACSF) with the following composition (in mM) 124 NaCl, 3.2 KCl, 1.25 NaH2PO4, 26.0 NaHCO3, 2.5 CaCl2, 1.3 MgCl2, and 10 glucose (equilibrated with 95% O2-5% CO2 at 32.5°C).

Recording and data analysis

Intracellular recording microelectrodes were fabricated from borosilicate glass on a Flaming/Brown model P-97 micropipette puller (Sutter Instruments). The electrodes had resistances ranging from 60–100 MΩ when filled with 4 M potassium acetate. Standard current clamp recordings were made with an Axoclamp-2A amplifier (Axon Instruments). Hippocampal CA1 pyramidal neurons were impaled in the bridge mode, and when the impalement stabilized, the recording configuration was switched to the discontinuous current clamp mode (sample rate about 5 KHz, filtered at 3 KHz). Current and voltage signals were stored on a microcomputer for offline analysis.

The input resistance (Rin) of the neuron was monitored by measuring the amplitude of the voltage transient in response to a 0.5 nA hyperpolarizing current step (100 ms duration). AHPslow was induced by a train of four action potentials and elicited by four separate depolarizing current pulses (3 nA, 2 ms duration, 5 ms interpulse interval). Adaptation of spike firing was tested using a 0.5 nA depolarizing current step (250 ms duration). EPSPs were evoked by stimulation of the Schaffer collateral/commissural pathway in area CA1 by a 75 µm monopolar teflon-coated stainless steel electrode (100 µs pulse duration). Analysis of the intracellular electrophysiological data were performed with pClamp 6.0 software (Axon Instruments). Neurons were accepted for study if they had a stable resting membrane potential (<−60 mV) throughout the experiment, an action potential overshoot >15 mV, an RM >25 MΩ, and showed typical properties of pyramidal cells such as spike frequency adaptation and AHPslow.

The following measures were routinely obtained throughout the recording period at 2–4 min intervals: Rin, the holding current required to keep the resting membrane potential at about −70 mV, the number of spikes per depolarizing current pulse, peak amplitude of the AHPslow and EPSP slope. It should be noted that the holding current was manually adjusted throughout the experiment to keep the resting membrane potential constant (nominally at −70 mV). The resting membrane potential was later corrected for the tip potential recorded after exiting from the cell, and the actual resting membrane potential was found to vary between −65 mV and −72 mV.

In some experiments, EPSPs and population spikes were recorded extracellularly in the stratum radiatum and stratum pyramidale, respectively. Recordings were made with glass microelectrodes (1–2 MΩ) filled with 2 M NaCl. LTP (for both intracellular and extracellular experiments) was induced by theta-burst stimulation (TBS), which consisted of 5 × 100 Hz bursts (5 diphasic pulses/burst, 200 ms interburst interval). During intracellular recordings, TBS was given at a stimulus intensity that was raised to just above threshold for postsynaptic action potentials. For extracellular recordings, TBS was given at baseline stimulation strength. Responses were followed for 30 min posttetanus in intracellular experiments and for 60 min in extracellular experiments. The slope of the EPSP was expressed as percent change from baseline values. LTP was measured as the mean of the last 5 min of values during the posttetanus recording period. Student’s t-tests were performed to determine significance at the P < 0.05 confidence level, and data are presented as group means ± SE.

To examine the effects of mGluR activation on the field EPSP-population spike (E-S) relationship, mini-input/output (I/O) curves were obtained by continuously rotating the stimulus strength (100 µS duration, 0.67 Hz) across four amplitudes that during the baseline recording period generated population spikes that were just at threshold, or were 0.5, 1.0, and 1.5–2.0 mV in amplitude. Recordings were made for 30 min before and after a 10 min application of ACPD.

RESULTS

mGluR activation causes a long-lasting increase in postsynaptic excitability

Putative CA1 pyramidal cells were impaled with sharp microelectrodes and a 10 min bath application of ACPD (20 µM) was used to produce several changes in postsynaptic membrane parameters, as previously reported (see INTRODUCTION). These changes included membrane depolarization (as inferred by the
amount of holding current required to keep the cell at about −70 mV), an increase in input resistance, a reduction in spike frequency adaptation, a decrease in the peak amplitude and duration of the AHPslow, and a decrease in the EPSP slope. Many of these changes however, occurred in a variable fashion with only the depression of the AHPslow (−83 ± 6%, n = 10, P < 0.001) and the reduction in spike frequency adaptation (6.7 ± 1.5 additional spikes per current pulse, n = 5, P < 0.01) reaching statistical significance (Fig. 1, A–D). Control cells exposed to a sham solution change did not show such changes (AHPslow: −16 ± 4%, n = 5; spike adaptation: 0.4 ± 0.3 spikes, n = 5; both measures P < 0.01 compared with ACPD-treated cells).

It was of particular interest in these experiments to see whether the ACPD-induced increase in cell excitability was persistent enough to account for the previously reported LTP priming effect by this same dose of ACPD (Cohen and Abraham 1996). Thus recordings were continued for ≥20 min washout period in normal ACSF following ACPD application, a washout time during which priming of LTP is robustly observed. The ACPD-treated slices showed only a partial recovery over the first 20 min of washout, with both the AHPslow (−54 ± 7%, n = 10) and spike frequency adaptation (3.9 ± 1.1 spikes) still significantly reduced at this time point (P < 0.05 compared with control cells; Fig. 1, A–D). In two cells with stable impalements, the AHPslow remained reduced by 45 and 50%, respectively, 30–45 min post-ACPD. These persistent effects were unlikely to be caused by a rundown of the relevant Ca2+ or K+ channels because control cells held for 20 min postsham treatment showed no deterioration of the response over this time period (n = 5; Fig. 1, A and C).

**mGluR activation produces short-lasting E-S potentiation**

One common way to test for changes in cell excitability is to examine the E-S ratio over a range of stimulus intensities. An increase in postsynaptic excitability is often expressed as an increase in the E-S ratio, termed E-S potentiation. To assess whether ACPD would cause E-S potentiation in our CA1 mini-slices, as was reported for CA3-intact slices (Breakwell et al. 1996), field potential recordings were made in both stratum radiatum to record the fEPSP. Single pulse stimuli were continuously rotated across four strengths for 30 min before and for 30 min following a 10 min application of the broad-spectrum metabotropic receptor agonist, ACPD (20 μM). This dose and postdrug time period are effective parameters for priming LTP (Cohen and Abraham 1996). Synaptic stimulation was halted during the period of drug administration. The fEPSPs and population spikes were normalized to the maximum response obtained during the baseline recording and I/O curves constructed from averages of the potentials at each stimulus strength. In the first 1–6 min post-ACPD there was a depression of the fEPSPs and a significant facilitation of the population spikes [1-way analysis of variance (ANOVA), P < 0.05], thus producing a marked reduction in the population spike threshold and an overall shift to the left of I/O curve (n = 10, Fig. 2). This effect was not entirely caused by a decrease in

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**FIG. 1.** 1S,3R-aminocyclopentane dicarboxylic acid (ACPD) causes a long-lasting depression of both the slow spike afterhyperpolarization (AHPslow) and spike frequency adaptation. A: ACPD (20 μM, 10 min, ○) depressed the normalized AHPslow peak amplitude recorded in CA1 hippocampal pyramidal neurons by 83 ± 6% (n = 10). AHPslow was still depressed by 54 ± 7% 20-min post-ACPD application. The AHPslow was unaffected by switching to a control artificial cerebrospinal fluid (ACSF) solution (n = 5; ○). B: sample AHPslow waveforms for an ACPD-treated neuron taken before drug (control), 8 min into drug application, and after 20 min of wash. † † †: membrane potential at which the cell was held during the experiment. This cell was held at −66 mV and had an Rm of 45 MΩ. The AHPslow was induced by a train of four action potentials evoked by depolarizing current pulses (3 nA, 2 ms, 200 Hz). Spike amplitudes in the present and all subsequent waveforms are truncated for graphic purposes. Calibration bar: 10 mV, 250 ms. C: average change in the number of action potentials per 250 ms depolarizing current pulse (0.5 nA) vs. time, plotted for the same two groups of cells as illustrated in A (although only 5 of 10 ACPD-treated neurons were tested on this measure). D: sample traces for an ACPD-treated neuron given depolarizing current steps (0.5 nA, 250 ms) just before the drug (control), 8 min into drug application, and after 20 min of wash. Note for both C and D the long-lasting increase in cell excitability observed for the ACPD-treated slices. The cell in D was held at −68 mV and had an Rm of 60 mΩ. Calibration bars: 25 mV, 100 ms.
It is reasonable to hypothesize that the excitability increase produced by ACPD and recorded intracellularly may be an important mechanism contributing to the priming of LTP by this mGluR agonist (Cohen and Abraham 1996). To test this hypothesis more directly however, we first addressed whether priming of LTP could be observed for intracellularly recorded EPSPs. Four of the ACPD-treated cells described above in Fig. 2 were given mild TBS (5 bursts) 20-min post-ACPD application and the amount of LTP generated was compared with that obtained in five new control cells. The stimulus intensity during the tetanus was increased for all cells so that a single test shock was just above threshold for consistently firing an action potential. The magnitude of response change measured 5-min post-TBS was significantly greater in the ACPD group (107 ± 20%) relative to the control group (32 ± 10%; P < 0.01; Fig. 3A). LTP, measured 25–30 min post-TBS, also occurred robustly in the ACPD group (35 ± 11%) but was absent in the control group (−2 ± 7%; P < 0.01; Fig. 3A). Thus intracellularly recorded EPSPs showed an mGluR-mediated priming effect similar to that previously observed for field EPSPs (Cohen and Abraham 1996).

We then addressed whether the ACPD-induced change in membrane parameters in these cells related to the facilitated LTP induction by carrying out two further analyses. First, the number of action potentials generated during the LTP-inducing TBS was compared between the primed and control cells. As predicted by the ACPD-induced reduction in spike frequency adaptation, there was a significant increase in the total number of action potentials occurring during the TBS for the ACPD-treated cells (15.8 ± 3.0 spikes, n = 4) relative to the control cells (9.2 ± 0.7 spikes, n = 5; P < 0.05; Fig. 3B and C). Second, we considered whether the amplitude of the AHPslow measured just before the TBS, was predictive of the amount of LTP. A linear regression analysis indicated that there was a statistically significant correlation between the degree of potentiation 5 min post-TBS and the amplitude of the pretetanus AHPslow (r = −0.80; P < 0.01; Fig. 3D). The correlation between the degree of LTP at 25–30 min posttetanus and the amplitude of the AHPslow was weaker (r = −0.62, P < 0.08).

### β-adrenergic receptor activation increases cell excitability and primes LTP induction

If the ACPD-induced increase in neuronal excitability contributes to the LTP priming effect, it follows that activation of other neurotransmitter receptors known to heighten pyramidal cell excitability should also prime LTP. Therefore we investigated whether activation of β-adrenergic receptors, which has been reported to result in a persistent decrease in the AHPslow and a persistent increase in cell excitability, measured as a long-lasting increase in population spike amplitude (Dunwiddie et al. 1992), would prime LTP. For these experiments, it was essential to first confirm the ability of the β-adrenergic receptor agonist isoproterenol to induce persistent changes in membrane parameters consistent with an increase in cell excitability. A 10 min application of 0.5 μM isoproterenol strongly depressed the AHPslow (−63 ± 14%, n = 7; P < 0.01) and reduced spike frequency adaptation (4.4 ± 1.1 spikes per 250 ms current pulse, n = 7, P < 0.01; Fig. 4A and B). Isoproterenol also caused membrane depolarization and an increase in Rin, but as for ACPD these changes were variable and not statistically significant (data not shown). Four cells were held for ≥20 min after drug washout, and whereas the drug-induced depression of both the AHPslow and spike frequency adaptation dissipated during this period, they were still reduced compared with baseline values.

### Relation between heightened cell excitability and priming of LTP

It is reasonable to hypothesize that the excitability increase produced by ACPD and recorded intracellularly may be an important mechanism contributing to the priming of LTP by this mGluR agonist (Cohen and Abraham 1996). To test this hypothesis more directly however, we first addressed whether
AHP slow: $-39 \pm 14\%$; $P < 0.05$; spike adaptation: $2.5 \pm 1.3$; n.s.; Fig. 4, C and D).

Having confirmed that $\beta$-adrenergic activation leads to persistent membrane changes consistent with an increase in pyramidal cell excitability, we used field potential recordings to test whether the same dose of isoproterenol would prime LTP. In correspondence with the protocols used for the ACPD experiments, 0.5 $\mu$M isoproterenol was bath-applied for 10 min and 0.5 TBS was administered 20 min after drug washout. The initial induction of LTP (taken as an average of 6 data points around 5 min post-TBS) was weakly enhanced by isoproterenol ($56 \pm 10\%$, $n = 6$) compared with a group of interleaved control slices ($33 \pm 7\%$, $n = 6$; $P < 0.05$, one-tailed Student’s $t$-test; Fig. 5, A and B). The degree of LTP measured at 60 min post-TBS for isoproterenol treated slices ($27 \pm 6\%$) was larger than, but not significantly different from, that for control slices ($20 \pm 4\%$). To characterize the persistence of LTP in these slices independently of the initial induction, a decay curve for each slice was fit by the sum of two negative exponential functions, and the decay time constant ($\tau = 1/rate$) for the second, slower exponential was extracted as an estimate of LTP persistence. There was no significant difference between the two groups on this measure, indicating that although isoproterenol enhanced the initial induction of LTP, this was not associated with a concomitant increase in its stability (Fig. 5, B and C). These findings contrast with the effects of ACPD, which enhanced both the initial induction and the persistence of LTP (Fig. 5, D and E), confirming our previous report (Cohen and Abraham 1996).

Partial occlusion of ACPD-induced LTP priming by isoproterenol

We predicted that if isoproterenol and ACPD both facilitate the level of initial LTP induction through common mechanisms, such as enhanced postsynaptic cell excitability, isoproterenol treatment should occlude the ability of ACPD to prime the induction of LTP. To test this, the LTP generated in isoproterenol-treated slices was compared against that for slices treated with both isoproterenol and ACPD and against control slices. In these experiments, ACPD was administered 20 min before TBS, whereas isoproterenol was applied for 10 min before and during the TBS to elicit maximal excitability changes at the time of the TBS. Because isoproterenol did not facilitate LTP persistence (Fig. 5C), we predicted that it would not occlude ACPD’s facilitation of the decay time constant.

In control slices, 0.5 TBS elicited a typical moderate induction of a decaying form of LTP (5 min: $43 \pm 6\%$; 60 min: $18 \pm 3\%$, $n = 8$). Isoproterenol (0.5 $\mu$M) given during the tetanus, whereas having no effect on baseline synaptic transmission, produced a substantial increase in LTP induction (5 min: $72 \pm 11\%$, $n = 9$; $P < 0.05$ compared with controls; Fig. 6, A and B). In contrast, but as expected, the second exponential decay constant of this isoproterenol-enhanced LTP was not affected (isoproterenol $\tau = 80.2$ min; control $\tau = 82.5$ min; Fig. 6C). Slices primed with ACPD and then treated with isoproterenol during the TBS showed no additional LTP induction (5 min: $74 \pm 7\%$, $n = 7$) beyond that seen in slices treated with isoproterenol alone (Fig. 6, A and B). However, the slices primed with ACPD did show a decay constant ($\tau = 145.0$ min)
significantly greater than either control slices or slices treated with isoproterenol alone ($P < 0.05$ for both comparisons). The occlusion of the ACPD-induced facilitation of LTP induction by isoproterenol supports the view that common mechanisms triggered by the two receptor agonists are responsible for the priming of LTP induction. Some other mechanism unique to the ACPD treatment appears to be responsible for facilitating LTP persistence.

DISCUSSION

Activation of mGluRs produces a persistent increase in cellular excitability

In our experiments, ACPD produced an acute depression of synaptic responses accompanied by an increase in postsynaptic cellular excitability, confirming many previous studies of the effects of mGluR activation on the electrophysiological properties of hippocampal neurons (e.g., Charpak et al. 1990; Desai and Conn 1991). In this study, two measures of postsynaptic excitability, spike frequency adaptation and the amplitude of the AHP$_{slow}$, were persistently reduced by ACPD. These persistent changes were not observed in some previous studies, probably because of the use of a different ACPD isomer (1S,3S-ACPD) which is not as an effective agonist at Group I mGluRs (Gereau and Conn 1994), the use of a lower concentration of the active isomer (Hu and Storm 1991), or the application of the ACPD for a shorter length of time than that used here (Nouranifar et al. 1998). The fact that the membrane depolarization, $R_{in}$, and EPSP slope changes were not elicited reliably enough to reach statistical significance probably reflects our use of a relatively low dose of ACPD (20 μM). Because the excitability increases that were observed might be expected to facilitate the induction of LTP, and thereby contribute to the priming of LTP by ACPD (Cohen and Abraham 1996), their persistence was followed for $\approx 20$ min and up to 45 min following ACPD washout. We confirmed that the changes in these two parameters did indeed last long enough to serve as candidate mechanisms underpinning LTP priming. It is interesting to note that the present findings are similar to those of a previous study which found that a large dose of quisqualate (100 μM), a potent agonist of the Group I mGluRs, caused a reduction of the AHP$_{slow}$ and a decrease in positive holding current lasting for $\approx 60$ min following drug washout (Baskys 1992). Selective activation of AMPA receptors, which are also activated by quisqualate, did not affect the AHP confirming a role of mGluRs in mediating the quisqualate effect. In accordance with these observations, we have observed that selective activation of AMPA receptors does not prime LTP (Cohen et al. 1998).

It could be hypothesized that the prolonged excitability changes were because of incomplete washout of the drug. However, there are several good reasons why this was not the case. First, the acute effects of ACPD on the EPSP slope washed out extremely quickly on return to drug-free solution.
whereas the depression of the AHP slow and spike frequency adaptation were observed to last up to 45 min after drug washout. The rapid recovery of the EPSP depression is consistent with our use of submerged slices and a high flow rate (2–3 ml/min) for the superfusion bathing medium. Second, we have shown previously that application of an mGluR antagonist during ACPD delivery, but not shortly after, blocked the priming of LTP (Cohen and Abraham 1996). Similar results have been reported by Bortolotto et al. (1994). These findings clearly demonstrate an efficient washout of the drug. Finally, it is noteworthy that agonists of other neurotransmitter receptors, such as $\beta$-adrenergic receptors (Dunwiddie et al. 1992; present results), dopamine receptors (Gribkoff and Ashe 1984) and histamine receptors (Selbach et al. 1997) also have been reported to exert long-lasting effects on cellular excitability. Thus the prolonged excitability increase induced by mGluR activation may be characteristic of the responsible potassium channels (e.g., $I_{\text{AHP}}$, $I_{\text{M}}$, and/or $I_{\text{A}}$), for which there appears to be a slow return to the baseline state following the presumed phosphorylation events that lead to their initial down-regulation.

An increase in cell excitability can also be detected, in principle, as an E-S potentiation in field potential recordings. Indeed, in the present experiments ACPD induced a significant potentiation of the population spike and E-S relation, but these effects largely decayed over the 20–30 min wash period. Thus the E-S potentiation did not correlate well with the long-lasting decreases in the AHP$_{\text{slow}}$ and spike frequency adaptation or with the priming of LTP. The E-S potentiation, therefore may be caused by other actions of ACPD such as reduced GABAergic inhibition or a increased input resistance and membrane depolarization.

Superficially, our E-S findings conflict with those of Bortolotto et al.
well et al. (1996), who demonstrated that transient ACPD application led to a long-lasting E-S potentiation accompanied by a potentiation of the fEPSP. This potentiation, however, developed slowly and was prevented by NMDA receptor antagonists and removal of area CA3. These features suggest that such ACPD-induced E-S potentiation is induced by hyperactivity among the CA3 afferents to area CA1 (Chinestra et al. 1994). Our findings indicated that there is no significant long-lasting E-S potentiation caused by ACPD application in isolated CA1 slices that lack area CA3 and provide further evidence that ACPD is cleared from the slices during the wash period.

**mGluR-mediated changes in cellular excitability and the facilitation of LTP**

The primary rationale for the present experiments was to investigate possible mechanisms underlying the priming of LTP by prior synaptic or pharmacological activation of mGluRs (Bortolotto et al. 1994; Christie et al. 1995; Cohen and Abraham 1996). Because the priming stimulation generally did not affect synaptic transmission directly, we reasoned that changes in cell excitability not apparent in field potential recordings might underlie the facilitation of LTP. Indeed, our data confirm that activation of mGluRs leads to an increased cellular excitability that correlates in at least three ways with the facilitation of LTP induction. First, both effects are induced by pharmacological activation of mGluRs. Second, the excitability changes are persistent enough to span the period of time between the priming stimulus and the subsequent induction of LTP, although it was not feasible to hold the cells long enough to determine whether the excitability changes recover over the same 1–3 h period as does the LTP priming effect (Cohen and Abraham 1996). Finally, we found a significant correlation between the degree of AHPslow suppression and the degree of LTP initially induced by the tetanus. Taken together, these findings indicate that raised postsynaptic excitability is an important regulator of LTP induction, confirming previous studies showing that suppression of the AHPslow by either pharmacological or tetanic synaptic activation leads to facilitated LTP (Blitzer et al. 1995; Sah and Bekkers 1996). The latter study, however, concentrated on the role that activation of noradrenergic receptors may play in modulating concurrently induced LTP. In contrast, the present study emphasizes the role that mGluRs, as well as β-adrenergic receptors, play in regulating cell excitability and LTP over periods of time well after the mGluR activation. Thus these experiments have identified one set of potential “metaplasticity” (Abraham and Bear 1996) mechanisms that may mediate priming of LTP (Cohen and Abraham 1996) and the mGluR-controlled “molecular switch” proposed by Bortolotto et al. (1994).

**β-adrenergic priming of LTP**

If enhanced postsynaptic membrane excitability induced by mGluR activation is important for the associated priming of LTP, one would expect that excitability increases elicited by other means should prime LTP. We chose to use activation of β-adrenergic receptors to test this hypothesis, because the agonist isoproterenol is known to decrease the AHPslow and increase excitability in hippocampal pyramidal cells (Madison and Nicoll 1986). Furthermore, these effects have been reported to outlast the period of drug application for ≥45 min, and it has been proposed that there may be a corresponding persistently enhanced ability to elicit LTP (Dunwiddie et al. 1992). In the present experiments, we confirmed that a low dose of isoproterenol produces a large and durable increase in pyramidal cell excitability and that this altered state LTP was more readily induced. However, the increase in excitability and the priming of LTP appeared to be slightly weaker for isoproterenol than for ACPD. More importantly, we found that the decay of LTP remained unaffected by isoproterenol, in contrast to the more prolonged LTP following ACPD exposure.

To test whether β-adrenergic receptor and mGluR activation prime at least the initial induction of LTP through common mechanisms, we undertook an occlusion experiment, using ACPD as a priming stimulus while applying isoproterenol during the tetanus to maximize its effects. It is notable that under this protocol isoproterenol alone induced a pronounced facilitation of LTP induction, as previously reported (Sah and Bekkers 1996), but still without effect on the persistence of LTP. Whereas isoproterenol successfully did occlude the ability of ACPD to further increase LTP induction, it did not prevent ACPD from slowing the decay of the isoproterenol-enhanced LTP.

Our interpretation of the above experiments is that the mGluR-mediated priming of LTP has at least two components; enhanced initial induction and facilitated persistence. Isoproterenol shares the ability to prime LTP induction, and we propose that a facilitation of postsynaptic pyramidal cell excitability is the common mechanism operating for both receptor systems. Other shared effects such as upregulated NMDA receptor function or downregulated synaptic inhibition could in principle be common mechanisms facilitating LTP, but these two possibilities have been ruled out as being involved in the mGluR priming effect (Cohen and Abraham 1996). One prediction arising from the above interpretation is that other neurotransmitters which elicit a persistent increase in pyramidal cell excitability, such as histamine (Selbach et al. 1997), should also be able to prime the induction of LTP. We previously have reported that the muscarinic agonist carbachol, which is known to increase pyramidal cell excitability through a PKC pathway (Cole and Nicoll 1983; Worley et al. 1987), can prime LTP to a similar extent as isoproterenol (Cohen et al. 1998). It is not known in this case, however, whether carbachol's membrane effects are long-lasting enough to account for the LTP priming effect.

**Mechanisms downstream of mGluR activation for facilitating LTP induction**

Group I mGluR activation is transduced by diverse second-messenger pathways beginning with the activation of PLC, which leads to the liberation of IP3 and activation of PKC by diacylglycerol and elevation of cAMP concentrations. Either pathway is in principle capable of mediating increases in postsynaptic excitability via final common regulation of potassium channels, such as the AHPslow (Nicoll 1988). It has been suggested that ACPD, at a higher dose (100 μM) than that used in this study, produces an increase in pyramidal cell excitability through stimulation of adenyl cyclase and increased activation of protein kinase A by cAMP (Goh and Ballyk 1993).
This finding is consistent with studies showing that activation of other receptors positively coupled to the cAMP signaling cascade can also induce long-lasting changes in pyramidal cell excitability (Dunwiddie et al. 1992; Gribkoff and Ashe 1984; Pedarzani and Storm 1995) and that Group I mGluRs and β-adrenergic receptors can work synergistically to produce this same effect (Gereau and Conn 1994; Gereau et al. 1995). Alternatively, it recently has been reported that the ACPD-induced inhibition of the AHP, in dentate granule cells is mediated by tyrosine kinases activated following release of Ca^{2+} from intracellular stores (Abdul-Ghani et al. 1996). Thus further work is required to determine the relative roles of PKA, PKC and tyrosine kinases in mediating the effects observed here.

**mGluR activation and the persistence of LTP**

Whereas the initial induction of LTP relies on posttranslational mechanisms, the later phases of LTP require de novo protein synthesis. It has been suggested that mGluR activation can play an important role in establishing these later phases (Behnisch et al. 1991; Bortolotto et al. 1994; Manahan-Vaughan and Reymann 1996). It is not obvious, however, that isoproterenol did not promote LTP persistence, despite profoundly increasing cell excitability. We propose, therefore, that mGluRs activate a privileged alternative signaling pathway that modulates LTP persistence. One possibility is the coupling of mGluR activation to dendritic protein synthesis machinery (Weiler and Greenough 1993; Weiler et al. 1996). Indeed, other studies from our laboratory have shown that the ACPD-induced priming of LTP persistence is blocked by protein synthesis inhibitors (Raymond et al. submitted). However, identifying the signaling pathways leading to the protein synthesis and the critical proteins themselves requires much further exploration.

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