Metabotropic Glutamate Receptors Trigger Homosynaptic Protein Synthesis to Prolong Long-Term Potentiation

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We investigated the mechanisms by which previous "priming" activation of group I metabotropic glutamate receptors (mGluRs) facilitates the persistence of long-term potentiation (LTP) in area CA1 of rat hippocampal slices. Priming of LTP was elicited by either pharmacological or synaptic activation of mGluRs before a weak tetanic stimulus that normally produced only a rapidly decaying phase of LTP that did not involve protein synthesis or mGluRs. Pharmacological priming of LTP persistence by a selective group I mGluR agonist was blocked by an inhibitor of group I mGluRs and by inhibitors of translation, but not by a transcriptional inhibitor. The same mGluR agonist increased ^{35}S-methionine incorporation into slice proteins. LTP could also be facilitated using a synaptic stimulation priming protocol, and this effect was similarly blocked by group I mGluR and protein synthesis inhibitors. Furthermore, using a two-pathway protocol, the synaptic priming of LTP was found to be input-specific. To test for the contribution of group I mGluRs and protein synthesis to LTP in nonprimed slices, a longer duration control tetanization protocol was used to elicit a more slowly decaying form of LTP than did the weak tetanus used in the previous experiments. The persistence of the LTP induced by this stronger tetanus was dependent on mGluR activation and protein synthesis but not on transcription. Together, these results suggest that mGluRs couple to nearby protein synthesis machinery to homosynaptically regulate an intermediate phase of LTP dependent on new proteins made from pre-existing mRNA.

Key words: LTP; mGluRs; metaplasticity; protein synthesis; synaptic plasticity; hippocampus

Long-term potentiation (LTP) is an input-specific and persistent increase in the strength of synaptic connections that is the major model mechanism for information storage in the brain (Bliss and Lømo, 1973; Bliss and Collingridge, 1993). Of critical importance for LTP as a memory mechanism is its long-term maintenance. In general, a distinction is made between a rapidly decaying early-phase LTP involving post-translational modification of proteins (Lovinger et al., 1987; Malinow et al., 1988) and a more persistent late-phase requiring gene transcription and new protein synthesis (Krug et al., 1984; Nguyen et al., 1994; Frey et al., 1996). However, there is some evidence for an intermediate phase of LTP dependent on translation but not transcription (Otani et al., 1989).

Many studies have reported that activation of metabotropic glutamate receptors (mGluRs) during a tetanus can promote the induction and, in particular, the persistence of LTP (Behnisch et al., 1991; Bashir et al., 1993). The proposed need for mGluR activation in LTP has been controversial, however, indicating that mGluR activation may not be necessary in all cases for durable LTP to occur (Chinestra et al., 1993; Thomas and O'Dell, 1995). One intriguing aspect of the mGluR contribution to LTP is that it can occur hours before a tetanus (Bortolotto et al., 1994). Similarly, we have shown that previous "priming" activation of group I mGluRs will transform a weak, decaying form of LTP into a more persistent form (Cohen and Abraham, 1996; Cohen et al., 1998). mGluR activation after tetanic stimulation can also facilitate LTP persistence (Manahan-Vaughan and Reymann, 1996).

The degree of LTP persistence is not directly coupled to the degree of initial potentiation (Abraham et al., 1993), and thus we have hypothesized that, whatever their contribution to the induction of LTP, mGluRs may separately regulate the persistence of LTP by coupling to protein synthesis mechanisms. This suggestion has a precedent in that mGluR-mediated epileptiform discharges in hippocampal slices have been shown to require protein synthesis (Merlin et al., 1998). Of particular interest, however, is the fact that protein synthesis machinery is located not only in somal regions of hippocampal neurons but also in dendrites and even dendritic spines (Steward, 1997). Accordingly, mGluRs are well placed to trigger transcription-independent *de novo* protein synthesis in spatially restricted synaptic sites. In confirmation of this, activation of mGluRs can trigger protein synthesis in hippocampal synaptoneurosome preparations, which contain no transcriptional machinery (Weiler and Greenough, 1993; Weiler et al., 1997).

Here, we provide evidence that the priming of LTP persistence in area CA1 of the hippocampus by previous pharmacological or synaptic activation of mGluRs occurs by a homosynaptic protein synthesis-dependent, but transcription-independent, mechanism. Furthermore, we identified a phase of nonprimed LTP that also entails mGluR activation and transcription-independent protein synthesis. Together, these findings suggest that local protein synthesis, triggered by group I mGluRs, allows for a long-lasting and

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input-specific phase of LTP without complex macromolecular trafficking.

Parts of this work have been published previously in abstract form (Raymond and Abraham, 1998).

MATERIALS AND METHODS

Electrophysiology. Hippocampal slices (400 μm) were prepared from young adult male (200–300 gm) Sprague Dawley rats, as described previously (Cohen et al., 1998). Slices were submerged in a brain slice chamber and preincubated for at least 2 hr in a continuous flow (2–3 ml/min) of artificial CSF (ACSF) [containing (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 D-glucose, equilibrated with 95% O_2–5% CO_2] at 32.5°C. Extracellular synaptic potentials were recorded from stratum radiatum in area CA1 using glass microelectrodes (1–3 MΩ) filled with 2 M NaCl. Baseline synaptic responses were evoked by stimulation of the Schaffer collateral–commissural pathway at 0.033 Hz (diphasic pulses, 0.1 msec half-wave duration) with a 50 μm tungsten monopolar electrode. The stimulation intensity was adjusted to elicit field EPSPs at population spike threshold as observed in the dendritic field recording. This corresponded to a field EPSP of approximately two-thirds maximum amplitude (the baseline potential was typically between 1.5 and 2.0 mV in amplitude). In most cases, a single stimulating electrode was used. In the heterosynaptic priming experiments, one electrode was placed on either side of, and equidistant from, the recording electrode to stimulate independent inputs to the same neuronal population. LTP was induced by theta-burst stimulation (TBS), consisting of trains of 10 × 100 Hz bursts (five diphasic pulses per burst) with a 200 msec interburst interval, at the test pulse intensity. Thus, 2 TBS consisted of two trains of TBS, each train separated by 30 sec, and 0.5 TBS equaled half a train, or five bursts only. Initial slopes of EPSPs were measured off-line and expressed as percentage change from baseline, calculated as the average of the last 15 min of baseline recordings. Slices that had baseline values that varied >10% over the 30 min baseline period were discarded from the analysis. Two-tailed Student's *t* tests were performed to determine significance at the 95% confidence level, unless otherwise stated. Data are presented as group means ± SEM.

Drugs and reagents. Reagents were obtained from the following vendors: all salts from BDH Chemicals (Poole, UK); (*R,S*)-3,5-dihydroxyphenylglycine (DHPG) and (*R,S*)-1-aminoindan-1,5,dicarboxylic acid (AIDA) from Tocris Cookson (Bristol, UK); D-2-amino-phosphonopentanoic acid (AP-5) from Research Biochemicals (Natick, MA); and cycloheximide, actinomycin-D (Act-D), and emetine from Sigma (St. Louis, MO). Drugs were dissolved in 100 mM NaOH (AIDA, D-AP-5), dH_2O (DHPG, emetine, cycloheximide), or DMSO (Act-D) and diluted at least 500-fold to their final concentration in ASCF.

^{35}S-Methionine incorporation. To measure the extent of ^{35}S-methionine (^{35}S-Met) incorporation into new proteins, four groups of four randomly assigned hippocampal slices, prepared as above, were placed in culture dishes containing 5 ml of ACSF and preincubated for 1 hr in a humidified carbogen atmosphere at 32.5°C. In groups 3 and 4, 5 μl of emetine (final concentration of 20 μM) was added. After a 30 min incubation, 67 μl of ^{35}S-methionine (final concentration of 2 μCi/ml) was added to all four groups. Five minutes later, 5 μl of DHPG (final concentration of 20 μM) was added to groups 2 and 4. After a final 30 min incubation, 100 μl of unlabeled methionine (final concentration of 0.1 mg/ml) was added to each dish to compete out further incorporation of radiolabel. The four slices in each group were pooled, and the protein was extracted in 1 ml of 10% TCA–0.1% methionine and centrifuged (12,000 rpm) for 15 min at 4°C. The pellet was washed three times in 5% TCA–0.05% methionine and centrifuged (12,000 rpm) at 4°C between each wash. The pellet was resolubilized in 1 M NaOH (500 μl) and incubated overnight at 37°C. The next day, the extract was neutralized with 1 M HCl (500 μl), and ^{35}S-met incorporation was measured on an LKB-Wallac (Gaithersburg, MD) Liquid Scintillation Counter in 6 ml of Starscint (Packard, Meridian, CT). This procedure was replicated using five different batches of 16 slices.

RESULTS

DHPG primes LTP through *de novo* protein synthesis from existing mRNA

We have shown previously that LTP induction and persistence can be primed by previous activation of group I mGluRs by the selective agonist DHPG (Cohen et al., 1998). To replicate this finding in the present experiments, slices were administered DHPG (20 μM) in the bathing medium for a 10 min period, beginning 30 min before a weak tetanus, i.e., 0.5 TBS. DHPG treatment by itself produced a mild long-lasting synaptic depression that persisted after drug washout, as has been reported previously (Palmer et al., 1997; Cohen et al., 1998). More importantly, DHPG increased the induction and persistence of subsequent LTP (40 ± 6%; $n = 7$; measured 60 min after tetanus) relative to untreated control slices (19 ± 1%; $n = 6$; $p < 0.05$) (Fig. 1*A*).

The use of the priming paradigm provides the ability to temporally separate mGluR activation from tetanic stimulation, thus facilitating investigations of the mechanisms downstream of mGluR activation that may be important for LTP. Because we hypothesized that mGluR activation primes LTP at least in part by triggering *de novo* protein synthesis, we tested the effects of two protein synthesis inhibitors on this phenomenon. Inhibition of protein synthesis by emetine (20 μM) or cycloheximide (60 μM), given for 10 min before and during the mGluR priming period, did not affect the initial induction of subsequent LTP but caused it to decay rapidly to control levels within 1 hr after tetanus (emetine, 17 ± 9%; $n = 4$; $p < 0.05$; cycloheximide, 22 ± 4%; $n = 4$; $p < 0.05$) (Fig. 1*B*). Neither drug had any effect on the nonprimed LTP induced by 0.5 TBS (data not shown). To assess whether DHPG acted by triggering *de novo* protein synthesis rather than simply priming translation mechanisms engaged by the subsequent tetanus, emetine was added at one of two times after DHPG priming. When emetine was applied beginning 10 min before, during, and for 10 min after the tetanus, the priming of LTP was again blocked (16 ± 3%; $n = 4$; $p < 0.05$; data not shown). However, when the timing of emetine application was further delayed so as to occur for the 20 min immediately after LTP induction, it had no effect on the maintenance of primed LTP (Fig. 1*C*). This stands in contrast to its effectiveness after a stronger (nonprimed) tetanus that produces a more persistent form of LTP than the 0.5 TBS used here (compare with Fig. 4*C*). This time course over which emetine blocks the priming effect suggests that DHPG triggers a rapid synthesis of proteins before the induction of LTP that then interacts with events triggered by the tetanus to promote the persistence of the induced LTP.

In contrast to the ability of protein synthesis inhibitors to block the DHPG-induced priming of LTP persistence, the transcriptional inhibitor actinomycin-D, delivered for 20 min before and during DHPG application at a dose (40 μM) and previously used to block the late phase of LTP (Nguyen et al., 1994; Nguyen and Kandel, 1997), had no effect on the priming of LTP (41 ± 4%; $n = 5$) (Fig. 1*D*). This result indicates that DHPG was triggering *de novo* synthesis of critical proteins from existing mRNA rather than from new transcripts.

As a more direct test for mGluR-triggered protein synthesis, we performed experiments that measured the incorporation of radiolabeled ^{35}S-Met into newly synthesized protein in hippocampal slices, as described in Materials and Methods. DHPG caused a small but significant increase in total ^{35}S-Met incorporation measured 30 min after drug application (17 ± 3%; $n = 5$ pooled samples of four slices each; $p < 0.05$; paired *t* test) (Fig. 2). Preincubation of slices with emetine reduced the increase in ^{35}S-Met incorporation by 78 ± 15% ($n = 5$).

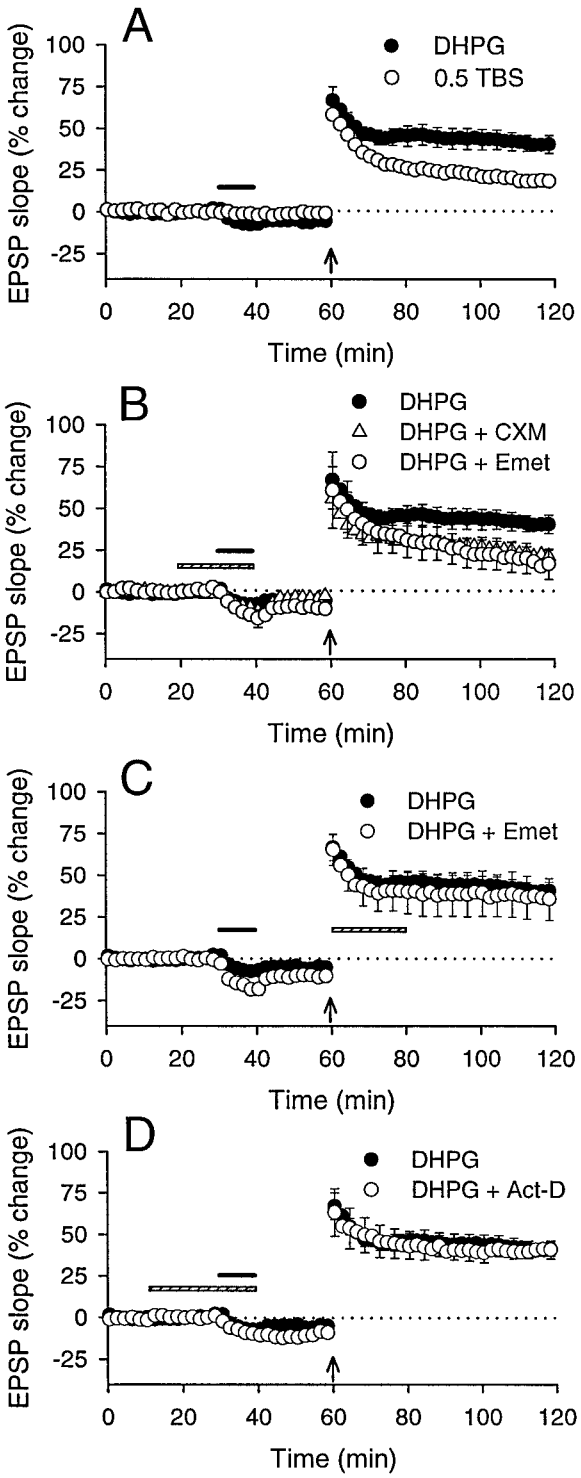

Figure 1. Mechanisms of DHPG-induced priming of LTP in CA1 slices. *A*, After 1 hr of baseline recording, control slices were administered 0.5 TBS (*arrow*), which induced a decaying form of LTP. A 10 min priming application of DHPG (20 μM; *dark bar*) mildly depressed synaptic transmission but significantly enhanced the persistence of subsequent LTP. *B*, Delivery of either emetine (20 μM, 20 min; *Emet*) or cycloheximide (60 μM, 20 min; *CXM*, *striped bar*) before and during the DHPG priming period (*dark bar*) prevented the enhancement of LTP persistence. *C*, Emetine (20 μM, 20 min; *striped bar*) given immediately after 0.5 TBS had no effect on primed LTP (compare with *B*). *D*, Actinomycin-D (40 μM, 30 min; *Act-D*, *striped bar*), given before and during the DHPG priming period (*dark bar*), had no effect on the enhancement of LTP by DHPG. The slices presented in this figure were run in an interleaved manner.

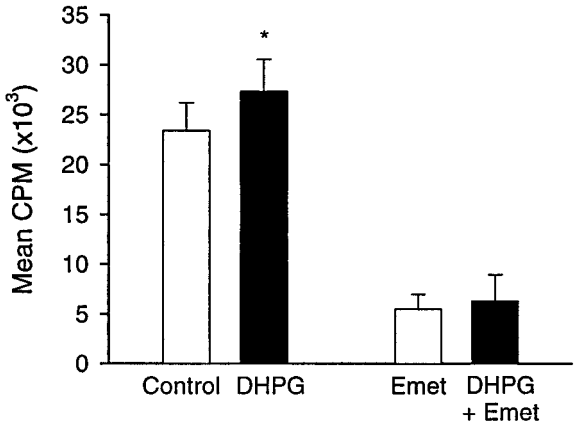

Figure 2. Summary histogram of ^{35}S-methionine incorporation into hippocampal slice proteins. A 30 min incubation with DHPG (20 μM) caused a significant increase in ^{35}S-methionine incorporation measured by scintillation counting. Preincubation of slices with emetine (20 μM, 30 min; *Emet*) reduced incorporation by 78%, confirming the efficacy of emetine in blocking protein synthesis, and blocked the DHPG-induced increase. Each of the five replications involved four pooled slices (randomly assigned) per treatment group. ($^*p < 0.05$, significant difference from control; paired *t* test).

Mechanism of LTP priming by mGluRs activated by synaptically released glutamate

Can LTP be primed by mGluRs activated by synaptically released glutamate? To test this, we primed slices by synaptically activating mGluRs through the delivery of 2 TBS but in the presence of the NMDA receptor antagonist AP-5 (50 μM) to prevent LTP induction. The AP-5 was then washed out for 20 min before the delivery of a weak LTP-inducing stimulus (1 TBS). Despite the AP-5 treatment, the priming tetanus caused a small, slowly developing potentiation that probably reflects an NMDA receptor-independent form of LTP (Grover and Teyler, 1990). As predicted, however, synaptically priming in this way significantly enhanced subsequent LTP compared with AP-5-treated controls given the same weaker tetanus (control, 14 ± 5% measured 60 min after tetanus; $n = 5$; primed, 36 ± 7%; $n = 5$; $p < 0.05$) (Fig. 3*A*). To determine whether this priming effect relied on the activation of mGluRs and protein synthesis, either the group I mGluR antagonist AIDA (500 μM) (Moroni et al., 1997), which we have shown previously to block the DHPG-induced priming of LTP (Cohen et al., 1998), or emetine was applied before and during the priming stimulation. Neither drug affected the small NMDA receptor-independent potentiation elicited by the priming stimulation in the presence of AP-5, suggesting a lack of effect on voltage-dependent calcium channel function. However, AIDA completely blocked the enhancement of subsequent LTP (13 ± 3%; $n = 4$; $p < 0.05$) (Fig. 3*B*), as did 20 μM emetine (15 ± 3%; $n = 4$; $p < 0.05$) (Fig. 3*C*). These results support the hypothesis that synaptic priming is mediated by group I mGluR activation and that, as for pharmacologically primed LTP, protein synthesis is critical for this enhancement of LTP.

It was notable in these synaptic priming experiments that there was a stronger facilitation of LTP at early time points after the induction of LTP than that observed for the pharmacological priming paradigm (compare Figs. 3*A*, 1*A*). This may reflect the release of other neurotransmitters in addition to glutamate, such as noradrenaline, which is capable of priming the initial LTP induction but not its persistence (as opposed to mGluRs, which

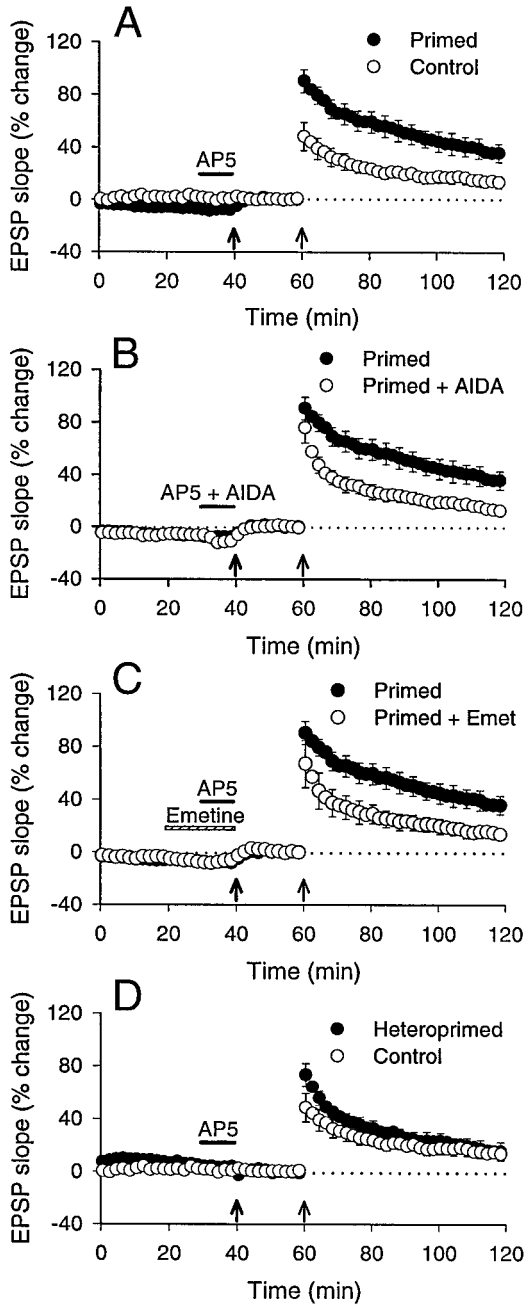

Figure 3. Synaptically released glutamate primes LTP by an mGluR-mediated mechanism. *A*, In controls, 1 TBS (*light arrow*) delivered 20 min after AP-5 (50 μM, 10 min; *dark bar*) wash induced a decaying form of LTP. Priming stimulation consisting of 2 TBS (*dark arrow*) in the presence of AP-5 resulted in a small, NMDA receptor-independent LTP that stabilized during AP-5 washout. LTP induced by 1 TBS subsequent to the priming stimulation was significantly enhanced. *B*, The group I mGluR antagonist AIDA (500 μM; *dark bar*), in combination with AP-5 during the priming stimulation, prevented the facilitation of LTP. *C*, Application of emetine (20 μM; *Emet*, *striped bar*) during the priming stimulation blocked the priming of LTP. *D*, To test for synapse specificity of the priming effect, two independent pathways to the same population of pyramidal cells were used. Path 1 (data not shown) received the 2 TBS priming stimulation (*dark arrow*) in the presence of AP-5 (*dark bar*). When 1 TBS was delivered to path 2 20 min later (*light arrow*), there was only a mild facilitation of the initial induction of LTP, which rapidly decayed to control levels. The control data from *A* are presented for comparison. These data demonstrate the input-specific nature of the mGluR and protein synthesis regulation of LTP. The slices *A–C* were run in an interleaved manner.

prime both features of LTP) (Cohen et al., 1999). Such release of other neuromodulators may account for the residual early facilitation still remaining in the AIDA-treated slices (Fig. 3*B*) compared with control values (Fig. 3*A*).

In the experiments above, the synaptic priming protocol was used to facilitate the induction of LTP homosynaptically, i.e., for the same synapses that were primed. The critical proteins, however, could in principle have been either synthesized at, or transported to, nonprimed synapses and thus been capable of modifying LTP heterosynaptically (Frey and Morris, 1997). To address this question, we delivered 2 TBS plus AP-5 to one pathway and, after a 20 min wash, induced LTP on a second, independent pathway terminating in the same dendritic zone using the weaker 1 TBS protocol. Although the initial induction of LTP on the second pathway was facilitated compared with controls, its persistence was not (16 ± 6%; $n = 4$) (Fig. 3*D*). These data are consistent with the interpretation that the proteins synthesized in response to the priming protocol are active only at the primed synapses. This finding, together with the rapidity of the priming effect (<20 min), suggests that these proteins are synthesized by polyribosomes located close to the synaptically activated mGluRs.

Contributions by mGluRs and protein synthesis to nonprimed LTP

We were interested in determining whether mGluR-triggered protein synthesis might also play a role in conventional, nonprimed LTP. As mentioned in the introductory remarks, it has been controversial whether mGluRs play any vital role in the induction or persistence of nonprimed LTP. The necessity of mGluR activation for LTP, however, may depend on the nature of the tetanic stimulus (Wilsch et al., 1998). Accordingly, we chose to use for these experiments the same tetanic stimulus (2 TBS) that successfully primed LTP above but without concurrent application of AP-5. The 2 TBS protocol produced an LTP that was nearly identical in magnitude to that observed after 0.5 TBS in DHPG-primed slices over 2 hr after tetanus (Fig. 4*A*), confirming that it may be a suitable stimulus for activating mGluRs and triggering protein synthesis.

The LTP induced by 2 TBS was robust yet slowly decaying (25 ± 3% at 2 hr after TBS; $n = 6$) (Fig. 4*B*). The persistence of this LTP over 2 hr was dependent on mGluR activation because AIDA, delivered for 10 min before and during the TBS, caused the LTP to decay significantly more rapidly (12 ± 2%; $n = 4$; $p < 0.05$) (Fig. 4*B*). Consistent with previous studies of late-phase LTP after TBS (Nguyen and Kandel, 1997), the persistence of LTP induced by 2 TBS was dependent on the synthesis of new proteins. Thus, emetine (20 μM), when applied for 20 min beginning immediately after tetanus, caused LTP to decay more rapidly than controls (14 ± 4%; $n = 8$; $p < 0.05$) (Fig. 4*C*). However, unlike the late phase of LTP induced by stronger, repeated trains of stimulation, the maintenance of 2 TBS-induced LTP was not affected by 40 μM actinomycin-D given before, during, and after the tetanus (25 ± 5%; $n = 5$) (Fig. 4*D*). These results show that *de novo* protein synthesis, triggered apparently by group I mGluR activation, is required to maintain the nonprimed LTP induced by a moderate stimulus and that such synthesis is programmed from pre-existing mRNA.

Comparison of drug effects across different LTP paradigms

Figure 5*A* summarizes and compares the profile of drug effects on LTP, measured at the end of the 1–2 hr post-tetanus recording

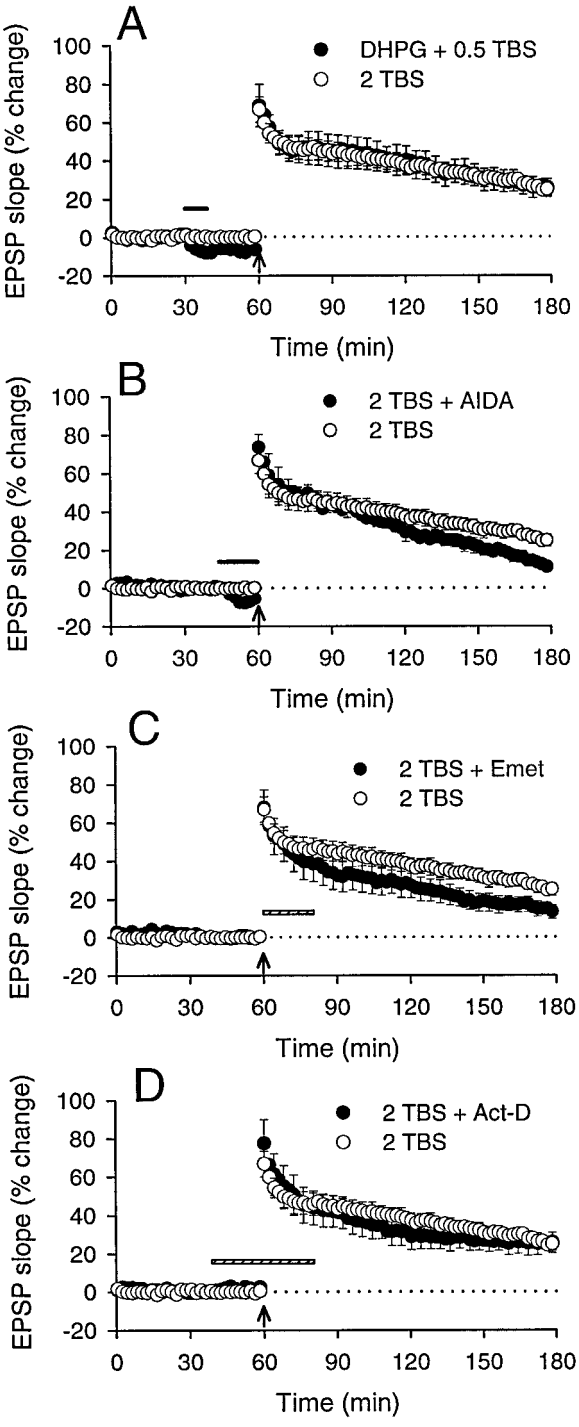

Figure 4. Role of mGluR-triggered protein synthesis in nonprimed LTP. *A*, The delivery of 2 TBS (*arrow*) in nonprimed slices (*open circles*) gave a moderate degree of LTP induction and persistence similar to that observed previously in DHPG-primed slices (data from Fig. 1*A* replotted for comparison purposes; *filled circles*). *B*, The control level of LTP for 120 min after 2 TBS is plotted (*open circles*; same slices as in *A*). Note that LTP is still decremental. Block of group I mGluRs with AIDA (500 μM, 10 min before and during the tetanus; *dark bar*) significantly reduced the persistence of LTP. *C*, Emetine (20 μM, 20 min; *Emet*, *striped bar*), applied immediately after TBS to avoid any possible effects on the initial induction mechanisms of LTP, also caused a more rapid decay of LTP over the 2 hr period. *D*, In contrast to emetine, actinomycin-D (40 μM; *Act-D*, *striped bar*) applied for 20 min before and after 2 TBS had no effect on LTP persistence. The slices used for this figure were run in an interleaved manner.

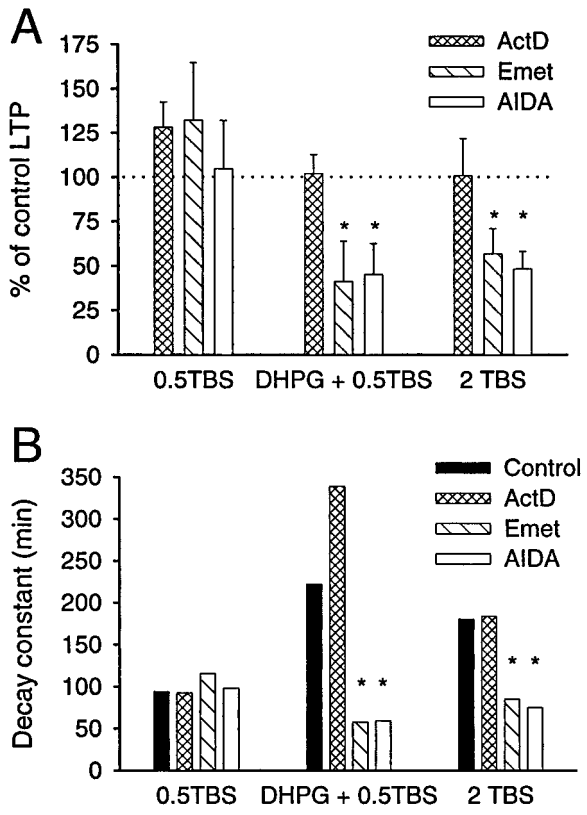

Figure 5. Summary histogram showing the effects of inhibiting transcription, translation, and group I mGluR activation on three LTP paradigms. *A*, The degree of LTP is represented as a percentage of their respective paradigm controls, measured at a fixed time after tetanus (1 hr time point for the 0.5 TBS groups, and 2 hr after tetanus for the 2 TBS groups). LTP induced by 0.5 TBS was unaffected by any of the drug treatments, thus reflecting the early protein synthesis-independent phase of LTP dependent on post-translational modifications. In contrast, both DHPG-primed LTP and LTP induced by 2 TBS were reduced by emetine and AIDA. Thus, both of these groups appear to engage similar mechanisms that govern the persistence of LTP, i.e., transcription-independent protein synthesis triggered by group I mGluRs. *B*, To control for differences in the level of induction, the persistence of LTP was taken as the rate constant of decay for the second, slower exponential function used in a double-exponential fit to the post-LTP data (Cohen and Abraham, 1996). Although statistics were performed on the rate constant data, for clarity reasons the data are plotted as the time constant of decay in minutes (i.e., the inverse of the rate constant). The drug profile of effects on decay rate for each tetanization condition was similar to that observed for LTP measured at a fixed value, as shown in *A*. These data confirm a role for mGluRs and protein synthesis in LTP persistence mechanisms, independent of any effects on LTP induction. (*$p < 0.05$, significant difference from control; Student's *t* test). *Act-D*, Actinomycin-D; *Emet*, emetine.

period, across three experimental paradigms described above. The degree of LTP for each drug group is expressed as a percent of the control LTP to normalize for different levels of LTP induction across the three paradigms. LTP induced by 0.5 TBS was not affected by either AIDA, emetine, or actinomycin-D. These findings are in contrast to those for DHPG-primed LTP (0.5 TBS) and nonprimed 2 TBS-induced LTP, which shared identical response profiles to each drug; namely, AIDA and emetine both inhibited LTP persistence, whereas actinomycin-D had no effect.

The above data represent snapshots of LTP, taken at a particular time after tetanization. Because such values depend on the initial degree of LTP induction, even assuming a constant rate of

decay, some of the drug effects may relate to altered LTP induction rather than its persistence. To obtain a more complete picture of LTP persistence, the post-tetanization data points for each slice presented in Figure 5*A* were fit with the sum of two negative exponentials, and the rate constant of decay for the second, slower exponential was obtained and used as a measure of LTP persistence (Cohen and Abraham, 1996). Data from two slices were discarded because fits could not be made. The remaining rate constant values for each drug group were then compared statistically with their respective control values. For clarity, the inverse of the mean rate constant for each group was calculated and plotted in Figure 5*B* as the decay time constant, expressed in minutes. In accord with the data presented in Figure 5*A*, none of drugs affected LTP persistence in the 0.5 TBS condition, with all groups showing time constants of decay of ~100 min. The two control groups given either DHPG priming plus 0.5 TBS or nonprimed 2 TBS both showed a more persistent form of LTP than that after 0.5 TBS, with a time constant of decay close to 200 min. In both of these experimental paradigms, LTP persistence was significantly reduced by AIDA and emetine back to the level seen in those slices given 0.5 TBS alone. Actinomycin-D, however, had no significant effect on LTP persistence in these experiments.

DISCUSSION

The present data are consistent with the hypothesis that activation of group I mGluRs triggers *de novo* protein synthesis from existing mRNA and thereby promotes the persistence of LTP. Interestingly, this protein synthesis can be initiated well before the tetanus and still affect LTP. Thus, the activated synapses can lie in an LTP-primed state, without significant overt evidence of the priming event. This effect is a classic example of "metaplasticity" (Abraham and Bear, 1996; Abraham and Tate, 1997), in that the LTP response to a weak tetanus by primed synapses is much different (in this case, more persistent) than it otherwise would have been.

Local protein synthesis and LTP

The new proteins important for the mGluR-mediated enhancement of LTP appear to have been synthesized near the activated synapses, although we cannot completely rule out a role for somal translation. First, the effects were blocked by translational but not transcriptional inhibitors, and the translational inhibitor was effective only within a relatively narrow time window after mGluR stimulation. More importantly, however, the newly synthesized proteins promoted LTP stability in an input-specific manner, as demonstrated by the synaptic priming experiments (Fig. 4). In combination, these characteristics suggest that at least some of the necessary proteins are synthesized in close proximity to the sites of mGluR activation. These conclusions are in accord with the finding that protein synthesis is stimulated by group I mGluRs in synaptoneurosomes (Weiler and Greenough, 1993). The input specificity of the present effect contrasts markedly with the robust heterosynaptic interactions that characterize the "synaptic tag" mechanism underlying the late phase of LTP (Frey and Morris, 1997, 1998). In this latter case, tetanized synapses are able to sequester proteins translated elsewhere in the neuron, allowing an otherwise decremental potentiation to be stabilized by heterosynaptic activity.

There is considerable evidence that dendritic protein synthesis does occur in hippocampal neurons (Steward, 1994, 1997). Early indications for local protein synthesis of proteins came from visualization of synapse-associated polyribosomes in the dendrites of dentate granule cells (Steward and Levy, 1982). Subsequently, both morphological and biochemical studies have revealed a full complement of functional protein synthesis machinery in the dendrites of many different neuronal types (Tiedge and Brosius, 1996; Torre and Steward, 1996; Gardiol et al., 1999). The other important finding has been the localization of mRNA in neurites of cultured hippocampal neurons (Bruckenstein et al., 1990; Kleiman et al., 1993) and, more importantly, at the base of dendritic spines in rat hippocampus (Martone et al., 1996). To date, mRNAs encoding over 20 proteins have been isolated near dendritic spines, including some with known or inferred roles in LTP, such as αCaMkinase II (αCaMKII), growth-associated protein 43, tyrosine receptor kinase B, cAMP response element-binding protein, activity-related cytoskeletal protein, and several glutamate receptor subunits (Steward, 1997; Tongiorgi et al., 1997; Gao, 1998).

A role for synaptically located protein synthesis has already been proposed to underlie various forms of synaptic plasticity, such as neurotrophin-induced synaptic potentiation in the hippocampus (Kang and Schuman, 1996), long-term facilitation at the *Aplysia* sensory to motor neuron synapse (Ghirardi et al., 1995), and long-term depression at cerebellar synapses (Linden, 1996). In addition, our laboratory has shown previously that a phase of dentate gyrus LTP in anesthetized rats may involve transcription-independent protein synthesis (Otani et al., 1989). Thus, protein synthesis in localized dendritic or synaptic areas may be a general feature of long-term synaptic plasticity. Because the hippocampal LTP experiments have not been performed in reduced synaptic preparations (Linden, 1996), the possibility remains that the critical proteins are translated from existing somal mRNA and transported down the dendrites to activated synaptic sites. In this latter model, however, the issue of how newly synthesized proteins can be targeted to the correct synaptic sites in so short a time frame is highly problematic (Schuman, 1997). Locally synthesized proteins, in contrast, provide a means for rapid synapse-specific stabilization of LTP, without requiring complex macromolecular trafficking.

mGluRs and LTP

There has been considerable controversy surrounding the extent to which mGluRs play a role in hippocampal LTP. It is clear from a number of studies that mGluRs are not always essential for normal LTP induction and persistence (Selig et al., 1995; Thomas and O'Dell, 1995), raising the possibility that they play a more modulatory role (Ben-Ari and Anikstejn, 1995). In accord with this concept, we and others have observed that mGluRs contribute to LTP only for certain tetanization conditions. For example, in the present experiments, AIDA did not affect the decremental LTP elicited by a weak tetanus (0.5 TBS). We interpret this finding as reflecting a relatively poor activation of mGluRs by synaptically released glutamate. It has been shown for mossy fiber synapses in CA3, for example, that activation of mGluRs is use-dependent, being considerably magnified during the course of high-frequency stimulation (Scanziani et al., 1997). This is probably because of a use-dependent increase in glutamate concentration in the synaptic cleft. Thus, we consider it likely that an insufficient glutamate concentration is established during 0.5 TBS in the present experiments to activate group I mGluRs, which are located peripherally on the postsynaptic density (Lujan et al., 1996). This can be rectified if the mGluRs are activated pharmacologically by DHPG or if the glutamate concentration is raised by using longer duration tetanic stimulation (2 TBS).

A separate study has shown that too strong a tetanus reduces the dependency of LTP on mGluR activation (Wilsch et al., 1998). This result was interpreted as reflecting activation of alternative mechanisms that elevate calcium postsynaptically, thus occluding any contribution made by mGluRs. Alternatively, this may reflect redundant mechanisms for triggering the necessary synthesis of proteins. Together, these studies indicate that mGluRs play a critical role in LTP formation only under certain conditions. We propose that one major contribution made by mGluRs under conditions of moderate activation is the triggering of dendritic protein synthesis, perhaps through protein kinase C (PKC)-mediated phosphorylation of translation initiation factors, such as the eIF4E subunit of the mRNA cap binding complex eIF4F (Sonenburg, 1996). In accord with this hypothesis, we have observed that PKC inhibitors block the priming of LTP by DHPG (C. R. Raymond and W. C. Abraham, unpublished observations).

An mGluR-dependent intermediate phase of LTP

Typically, LTP persistence is divided into two distinct phases, referred to as early LTP and late LTP. In this model, early LTP is solely dependent on post-translational modifications, whereas late LTP is dependent on both new transcription and translation, respectively (Matthies, 1989, Nguyen et al., 1994). On the other hand, an analysis of dentate gyrus LTP persistence in freely moving animals revealed three families of exponential decay curves [termed LTP1, 2, and 3 (Abraham and Otani, 1991 after Racine et al., 1983)], and the suggestion was made that there may be a protein synthesis-dependent, but transcription-independent, intermediate phase of LTP (i.e., LTP2). This hypothesis was supported by the observations that a form of LTP in anesthetized animals was blocked by anisomycin but not actinomycin-D (Otani et al., 1989) and that the intermediate phase of LTP persistence was associated with little or no activation of immediate early genes (Abraham et al., 1993).

The present study demonstrates that such an intermediate stage of LTP also exists in CA1 slices. The dependence of this phase on protein synthesis stands in contrast to another recently described intermediate form of LTP that is independent of protein synthesis but regulated by protein kinase A and calcineurin (Winder et al., 1998). Both forms of intermediate LTP, however, appear to be masked when a full-blown transcription-dependent late phase of LTP is induced. In the mGluR- and protein synthesis-dependent intermediate phase, this occlusion could be attributable to a similarity in the proteins being made from the new and existing mRNA or, alternatively, an inhibition of the mGluR-stimulated cascade when a stronger tetanization is used and a greater calcium concentration is established. Protein synthesis in some systems can be inhibited by the calcium-dependent phosphorylation of the α-subunit of initiation factor eIF2 (Rowlands et al., 1988). This process would be expected to compete with the PKC-dependent facilitation of protein synthesis described above.

Conclusions

Overall, our data provide firm evidence for an intermediate phase of LTP in area CA1 of the hippocampus that is dependent on mGluR-triggered protein synthesis from pre-existing mRNA. The involvement of transcription-independent protein synthesis in this phase of LTP suggests that mRNAs constitutively present in the dendrites encode proteins that promote moderate duration LTP. For example, an increase in the sensitivity of AMPA receptors by αCaMKII phosphorylation has been proposed as one of the mechanisms of early LTP expression (Bliss and Collingridge, 1993). The mRNA for αCaMKII is constitutively present in dendrites (Steward, 1997; Gao, 1998), and its local translation is subject to activity-dependent control (Wu et al., 1998). Newly synthesized αCaMKII may thus act to augment and prolong the action of the constitutively expressed kinase, or it may act at a different subsynaptic location. On the other hand, the synthesis of proteins required for longer-term maintenance of LTP (requiring, for example, structural modifications) is transcriptionally controlled, hence the critical requirement for a period of transcription to express this late phase.

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