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Two Distinct Forms of Long-Term Depression Coexist in CA1 Hippocampal Pyramidal Cells

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Summary

Two distinct forms of long-term depression (LTD), one dependent on the activation of NMDA receptors (NMDARs) and the other dependent on the activation of metabotropic glutamate receptors (mGluRs), are shown to coexist in CA1 hippocampal pyramidal cells of juvenile (11–35 day-old) rats. Both forms were pathway specific and required membrane depolarization and a rise in postsynaptic Ca^{2+}. mGluR-LTD, but not NMDAR-LTD, required the activation of T-type Ca^{2+} channels, group 1 mGluRs, and protein kinase C, while NMDAR-LTD, but not mGluR-LTD, required protein phosphatase activity. NMDAR-LTD was associated with a decrease in the size of quantal excitatory postsynaptic currents, whereas for mGluR-LTD there was no change in quantal size, but a large decrease in the frequency of events. NMDAR-LTD, but not mGluR-LTD, reversed NMDAR-dependent long-term potentiation, and NMDAR-LTD was unaffected by prior saturation of mGluR-LTD. These findings indicate that NMDAR-LTD and mGluR-LTD are mechanistically distinct forms of synaptic plasticity.

Introduction

Use-dependent changes in synaptic strength are thought to play an important role in learning and memory. Most attention has been given to long-term potentiation (LTP), in which brief high frequency stimulation results in a long lasting increase in synaptic strength (Bliss and Collingridge, 1993; Larkman and Jack, 1995; Nicoll and Malenka, 1995). However, it is now well established that prolonged lower frequency stimulation can cause a long lasting depression in synaptic strength referred to as long-term depression (LTD; Linden, 1994; Bear and Malenka, 1994; Bear and Abraham, 1996). Previous studies in the CA1 region of the hippocampus (Dudek and Bear, 1992; Mulkey and Malenka, 1992) found that LTD, similar to LTP, was dependent on the activation of NMDA receptors (NMDAR-LTD). Subsequently, studies have appeared reporting that LTD, both in the CA1 region (Stanton et al., 1991; Bolshakov and Siegelbaum, 1994; Yang et al., 1994) and in other regions, including the cerebellum (Linden et al., 1991; Aiba et al., 1994; Conquet et al., 1994; Shigemoto et al., 1994), the visual cortex (Kato, 1993; Hensch and Stryker, 1996), the striatum (Calabresi et al., 1992), and the CA3 region of the hippocampus (Kobayashi et al., 1996), depends on the activation of metabotropic glutamate receptors (mGluRs). In addition, depotentiation, in which low frequency stimulation of potentiated synapses reverses LTP, has been reported to be blocked either by NMDAR antagonists (Fujii et al., 1991; O'Dell and Kandel, 1994) or by an mGluR antagonist (Bashir and Collingridge, 1994).

These varied findings raise the question of whether there are several distinct forms of LTD which involve distinct induction and expression mechanisms. Alternatively, it is possible that, depending on a large number of factors such as the pattern of synaptic activation or the level of inhibition, there are different means by which the same signal transduction cascades can be activated, resulting, at least in some cases, in LTD which utilizes essentially the same induction and expression mechanisms even though the initial triggering events are clearly different (e.g., NMDARs versus mGluRs). The CA1 region of the hippocampus is a particularly attractive place to address this issue because the Schaffer collateral–CA1 pyramidal cell synapse is one of the best understood synapses in the mammalian brain and exhibits both NMDAR-LTD (Dudek and Bear, 1992; Mulkey and Malenka, 1992) and, at least in neonatal (3–8 day-old) rats, mGluR-LTD (Bolshakov and Siegelbaum, 1994; but see Selig et al., 1995). Here, we show in slices from juvenile (11–35 day-old) rats that NMDAR-LTD and mGluR-LTD coexist. We describe the conditions required to activate the two forms of LTD and investigate the mechanisms involved in their induction and expression.

Results

mGluR-LTD Is Present in Neonatal and Juvenile Hippocampal Neurons

Using field potential recording, we first tested for the existence of an NMDAR-independent, mGluR-dependent form of LTD in the CA1 region of neonatal (6–7 day-old) hippocampal slices. For these experiments, the slices were continuously bathed in a solution containing the NMDAR antagonist D-AP5 (100 μM). As shown in Figure 1A, a 5 Hz, 3 min stimulation given in the presence of the mGluR antagonist MCPG (500 μM) caused only a transient depression in synaptic transmission, lasting about 10 min. If, however, the identical tetanus is repeated in the same slices in the absence of the mGluR antagonist (but still in the presence of D-AP5), a lasting depression of synaptic strength occurred ($-32.6\% \pm 7.1\%$, $n = 5$), thus confirming previous results with whole-cell recording from neonatal slices (Bolshakov and Siegelbaum, 1994). Since previous studies in older rats reported a sensitivity of LTD to D-AP5 (Dudek and Bear, 1992; Mulkey and Malenka, 1992), the possibility exists that a developmental switch in the mechanism underlying LTD might occur. We therefore repeated, in slices from juvenile (11–35 day-old) rats, the exact same

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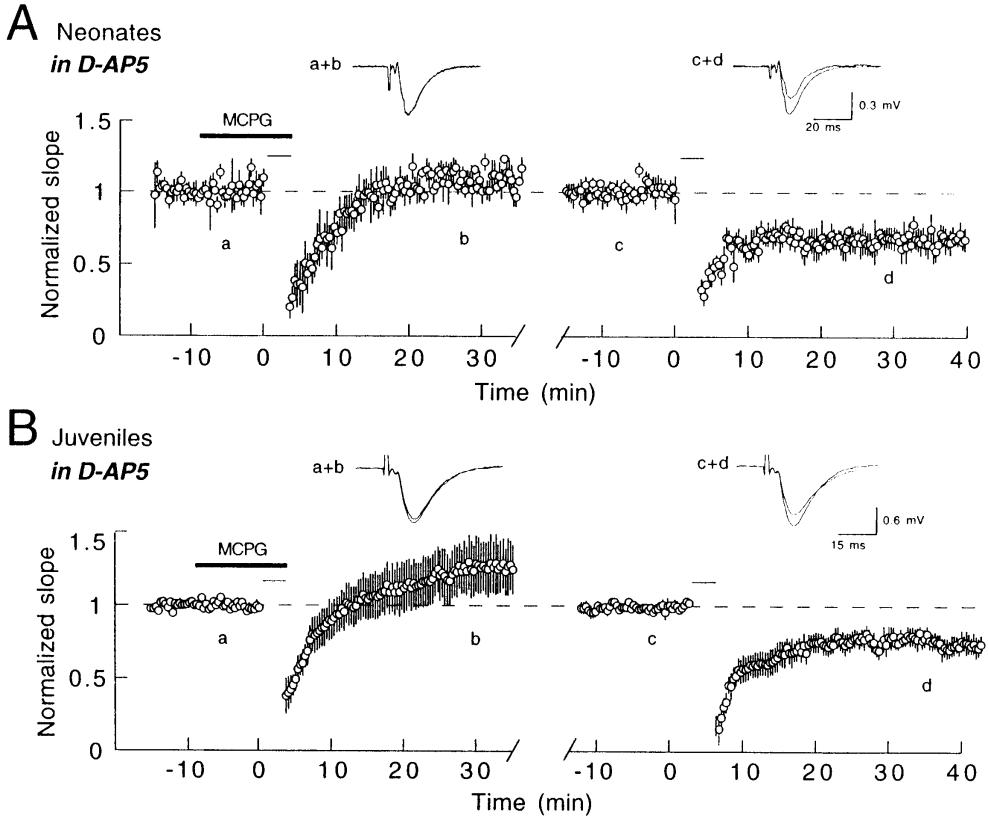

Figure 1. An mGluR-Dependent LTD Can Be Induced in Both Neonatal and Juvenile Rats

(A) In neonatal slices, a 5 Hz/3 min train of stimuli (thin line) did not induce LTD of field EPSPs when given in the presence of 100 μM D-AP5 and 0.5 mM MCPG. In the same slices (n = 5), this induction protocol elicited LTD once MCPG was washed out. The insets are averaged sample records of ten sweeps taken at the times indicated from a typical experiment.

(B) In juvenile slices, a 5 Hz/3 min train of stimuli (thin line) did not induce LTD of field EPSPs when given in the presence of 100 μM D-AP5 and 1.5 mM MCPG. In the same slices (n = 5), this induction protocol elicited LTD once MCPG was washed out.

experiment that was carried out in the neonates. In these slices, a small amount of LTD was observed in the presence of 500 μM MCPG (not shown). However, in the presence of 1.5 mM MCPG, a 5 Hz, 3 min stimulation actually evoked a small potentiation (Figure 1B). Repeating the same stimulus in the same slices in the absence of MCPG (but still in the presence of D-AP5) evoked a long lasting depression (−22.7% ± 7.5%, n = 5). These results suggest that an mGluR-dependent, NMDAR-independent form of LTD exists in slices taken from both neonatal and juvenile rats. Since no LTD was evoked in the presence of both D-AP5 and MCPG in the conditions used in the present experiments, for convenience we will refer to the LTD evoked in the presence of D-AP5 as mGluR-LTD and that evoked in the presence of MCPG as NMDAR-LTD.

Conditions Required to Induce mGluR-LTD

The observation of mGluR-LTD in neonatal slices appears to disagree with the results of Selig, et al. (1995), and the ability to generate LTD in the presence of D-AP5 in slices from juvenile rats appears to differ from the results of a number of laboratories (Dudek and Bear, 1992; Mulkey and Malenka, 1992; Oliet et al., 1996). We have, therefore, examined a number of parameters to determine what conditions were important for observing mGluR-LTD. In the study by Selig et al. (1995), the Ca^{2+}/Mg^{2+} ratio was 2.5 mM/1.3 mM, whereas in the present study the Ca^{2+}/Mg^{2+} ratio was 4 mM/4 mM. We therefore compared, in the presence of D-AP5, the ability to generate LTD in these two different Ca^{2+}/Mg^{2+} ratios. In a Ca^{2+}/Mg^{2+} ratio of 2.5 mM/1.3 mM, a 5 Hz tetanus failed to elicit LTD (4.6% ± 7.1%, n = 5), whereas, in the same slices, LTD could be elicited in the presence of a Ca^{2+}/Mg^{2+} ratio of 4 mM/4 mM (−39% ± 7%, n = 5; Figure 2A). The latter condition reduced basal synaptic transmission to 62% ± 4% (n = 5) of its original value. This was likely due, in part, to a decrease in fiber excitability, as it was associated with a decrease in the size of the presynaptic fiber volley. Since the Ca^{2+}/Mg^{2+} ratio appears to play an important role in the induction of mGluR-LTD, it is of interest to consider what the normal Ca^{2+}/Mg^{2+} ratio is in the brain. Typical values for CSF are 1.3 mM/1.3 mM (Ames et al., 1964; Heinemann et al., 1977), which, in terms of a Ca^{2+}/Mg^{2+} ratio, is similar to 4 mM/4 mM. Indeed, we were able to induce mGluR-LTD (−27% ± 10%, n = 4) in a solution with a ratio of 1.3 mM/1.3 mM, a condition which considerably reduced transmitter release (data not shown).

The level of synaptic inhibition also dictated which form of LTD could be generated. Whereas the NMDAR-LTD could be evoked in the presence (e.g., Figures 4B

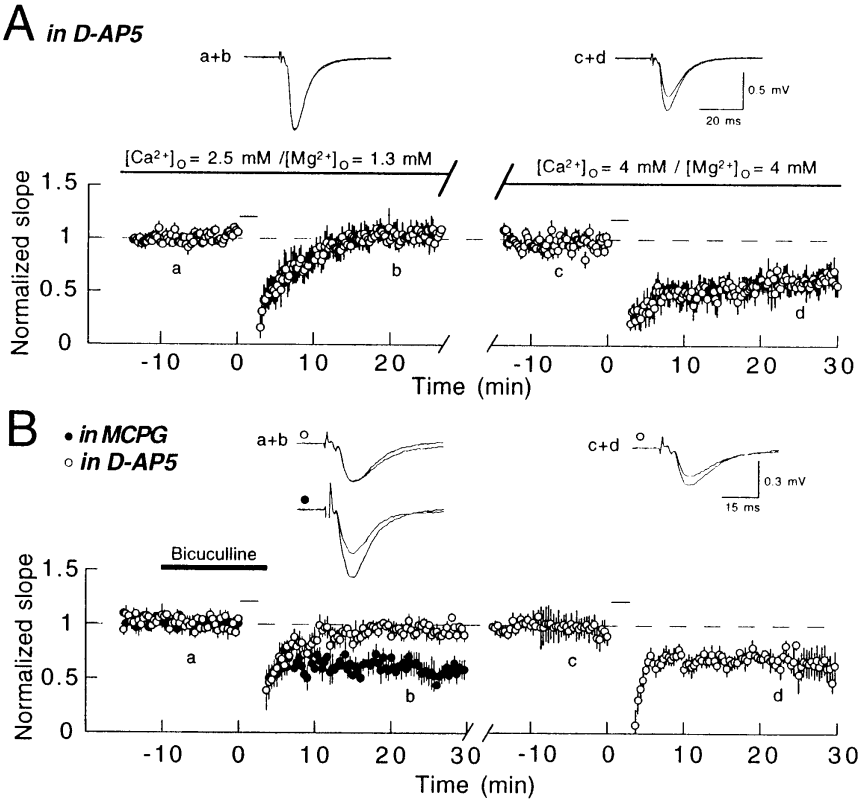

Figure 2. Induction of mGluR-LTD Depends on External Divalent Concentration and the Level of Synaptic Inhibition

(A) In the presence of 100 μM D-AP5, 2.5 mM $CaCl_2$, and 1.3 mM $MgCl_2$, mGluR-LTD of field EPSPs could not be induced. However, in the same slices (n = 5), mGluR-LTD was induced when the concentrations of $CaCl_2$ and $MgCl_2$ were raised to 4 mM.

(B) In the presence of 100 μM D-AP5 and 20 μM bicuculline, mGluR-LTD of field EPSPs was not induced (open circles). In the same slices (n = 5), mGluR-LTD was elicited once bicuculline was washed out. Bicuculline did not block NMDAR-LTD (closed circles; n = 4).

and 4E) or the absence (Figure 2B, closed circles) of $GABA_A$-mediated inhibition, mGluR-LTD was not evoked during blockade of inhibition by bicuculline but could be evoked after the washout of this $GABA_A$ antagonist (Figure 2B, open circles). Finally, mGluR-LTD (in 4 mM/4 mM) was much easier to elicit with small inputs (a slope of 0.05–0.1 mV/ms) compared to large inputs (a slope of 0.15–0.2 mV/ms). Thus, for large inputs, no LTD was observed (6% ± 0.9%), whereas, when the stimulus was reduced, LTD could be observed in these same slices (−49% ± 1.2%, n = 4; data not shown). This finding could explain why decreasing the Ca^{2+}/Mg^{2+} ratio, which decreases synaptic transmission, favors the induction of mGluR-LTD. Indeed, it was possible to induce mGluR-LTD in 2.5 mM/1.3 mM when the stimulus strength was decreased, albeit in only four of nine slices. These results indicate that mGluR-LTD is most easily evoked in slices with inhibition intact, in a bathing solution containing 4 mM Ca^{2+} and 4 mM Mg^{2+}, and with small synaptic inputs; these are therefore the conditions used for all further experiments.

Properties of mGluR-LTD

To determine if mGluR-LTD was limited to the synapses activated during the 5 Hz stimulation, we recorded synaptic responses to two overlapping but independent pathways in the stratum radiatum. Only one of the pathways was given the 5 Hz stimulation. As shown in Figure 3A, only the pathway that received the 5 Hz stimulation (open circles) exhibited LTD (−47.4% ± 7.4%, n = 11), indicating that mGluR-LTD is input specific. This form of LTD also appeared to require depolarization of the postsynaptic cell. LTD was not elicited if the 5 Hz stimulation was given when the cell was voltage clamped at −80 mV (0.6% ± 16.1%, n = 5; Figure 3B, V-clamp, −80 mV). If, however, the 5 Hz stimulation was given in the same cells at the resting membrane potential in current clamp, LTD occurred (−43.3% ± 13.2%; Figure 3B, I = 0). This finding suggests that some type of voltage-dependent Ca^{2+} entry may be required for this NMDAR-independent form of LTD. This was directly tested with dual extracellular and whole-cell recording (Figure 3C) in the presence of D-AP5. The whole-cell pipette contained the Ca^{2+} chelator BAPTA (10 mM). Between 15 and 25 min elapsed from breaking into the cell to applying the 5 Hz stimulation. While LTD was observed with the extracellular recording (−22.5% ± 7.3, n = 7), no LTD occurred in the whole-cell recording (6.6% ± 18.2%). This lack of LTD in the whole-cell recording was not due to "washout," as is typically observed with LTP, because in Figure 3B an even longer time elapsed before LTD was successfully evoked.

Role of Ca^{2+} Channels in mGluR-LTD

The prevention of mGluR-LTD by voltage clamping the cell at a hyperpolarized potential, as well as by loading

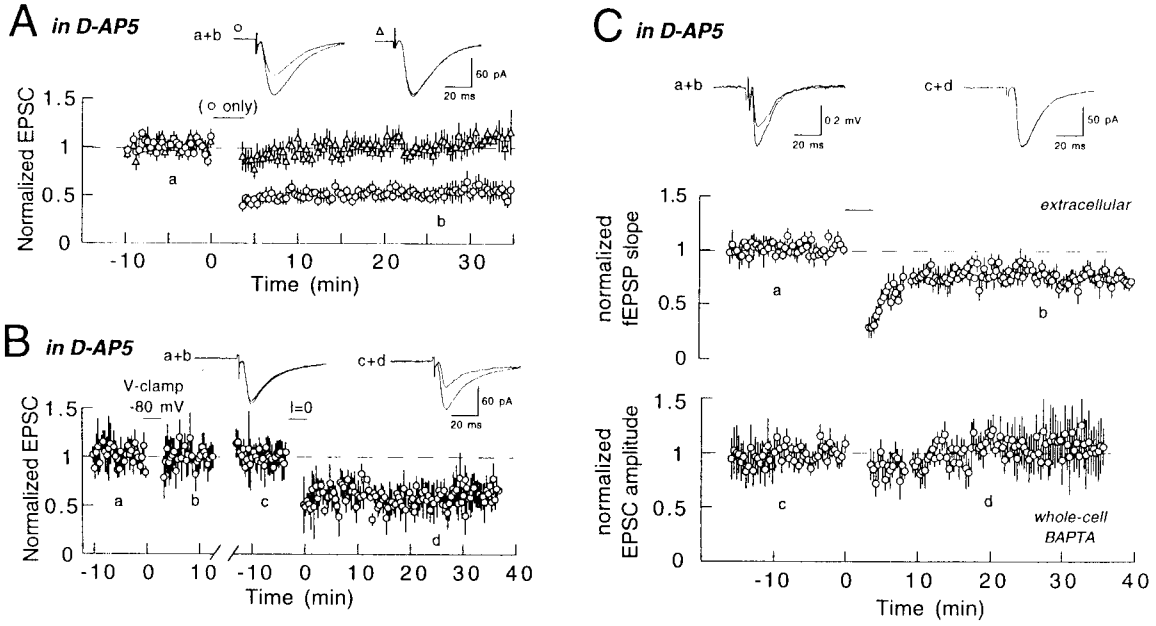

Figure 3. mGluR-LTD is Homosynaptic and Ca^{2+}-Dependent

(A) A summary graph of 11 whole-cell experiments where, in the presence of 100 μM D-AP5, a 5 Hz/3 min train of stimuli (thin line) induced LTD in the pathway receiving the stimuli but not in an independent pathway synapsing on the same cells.

(B) In the presence of 100 μM D-AP5, voltage-clamping the cells at −80 mV during the induction protocol prevented the induction of mGluR-LTD. In the same cells (n = 5), mGluR-LTD was induced when the induction protocol was given at the resting membrane potential in current clamp mode (I = 0).

(C) Inclusion of BAPTA (10 mM) in the recording electrode prevented the induction of mGluR-LTD (n = 7; lower panel). The field EPSPs simultaneously monitored during these experiments were depressed (upper panel).

the cell with a Ca^{2+} chelator, strongly implicates some type of voltage-dependent Ca^{2+} channel in this form of LTD. The L-type Ca^{2+} channel blocker nifedipine (20 μM) had no effect on either mGluR-LTD (−36.4% ± 5.9%, n = 5; Figure 4A) or NMDAR-LTD (−32.8% ± 2.2%, n = 5; Figure 4B). However, bathing slices in a concentration of Ni^{2+} (50 μM), which has been shown to be a selective antagonist of T-type Ca^{2+} channels in some neuronal cell types (Fox et al., 1987; Huguenard, 1996), blocked mGluR-LTD (−4.4% ± 9.6%, n = 5) but not NMDAR-LTD (−30.8% ± 4.5%, n = 5; Figure 4C). Following washout of the Ni^{2+}, mGluR-LTD could then be evoked (−24.2% ± 4.8%; Figure 4C). Ni^{2+} had no effect on baseline synaptic transmission, which is dependent on presynaptic N and P/Q-type Ca^{2+} channels (Luebke et al., 1993; Wheeler et al., 1994), strongly suggesting that Ni^{2+} had a selective action on T-type Ca^{2+} channels.

We also found that if cells were held in current clamp at −45 mV, a membrane potential at which the T-type Ca^{2+} channels should be entirely inactivated (Figure 4D, I-clamp, −45 mV), mGluR-LTD was not induced (−6.0% ± 7.1%, n = 5); however, if the same cells were held in current clamp at a membrane potential of −80 mV, mGluR-LTD was induced (−41% ± 8%; Figure 4D, I-clamp, −80 mV). On the other hand, if cells were held in current clamp at −80 mV (Figure 4E, I-clamp, −80 mV), a membrane potential at which the NMDAR should be largely blocked, NMDAR-LTD was not evoked (−5% ± 7%, n = 4); whereas, in the same cells, when the membrane potential was held in current clamp at −45 mV, NMDAR-LTD was induced (−42% ± 7%; Figure 4E, I-clamp, −45 mV).

Characterization of mGluR Subtypes Involved in mGluR-LTD

Metabotropic GluRs are divided into three groups; group 1 receptors are coupled to phospholipase C and, in most instances, are located postsynaptically, while group 2 and 3 receptors are negatively coupled to adenylyl cyclase and are typically localized presynaptically (Pin and Duvoisin, 1995). Thus, identification of the group of mGluR involved in mGluR-LTD would provide insight not only into the molecular mechanism of this form of LTD, but also into the location of the responsible mGluR. AIDA ([CRS]-1-aminoindan-1,5-dicarboxylic acid; 500 μM), a selective group 1 antagonist (Pellicciari et al., 1995), reversibly blocked mGluR-LTD (19% ± 6%, n = 5; Figure 5A) but had no effect on NMDAR-LTD (−23% ± 10%, n = 4; Figure 5B). The group 1 mGluRs consist of mGluR1 and mGluR5, and anatomical studies show that CA1 pyramidal cells express mGluR5, but not mGluR1 (Luján et al., 1996). The mGluR1 selective antagonist 4CPG (300 μM) had no effect on mGluR-LTD (−33% ± 6%, n = 3), providing further support for the involvement of the mGluR5 subtype of receptor.

The involvement of group 1 mGluRs, which are coupled to phospholipase C, raises the possibility that this form of LTD might require the activation of protein kinase C (PKC). To test for this possibility, we loaded cells with a PKC inhibitory peptide, PKC_{19-36} (House and Kemp, 1987), while simultaneously monitoring synaptic responses with an extracellular recording electrode (Figure 5C). No change in synaptic transmission was observed as the inhibitory peptide diffused into the cell.

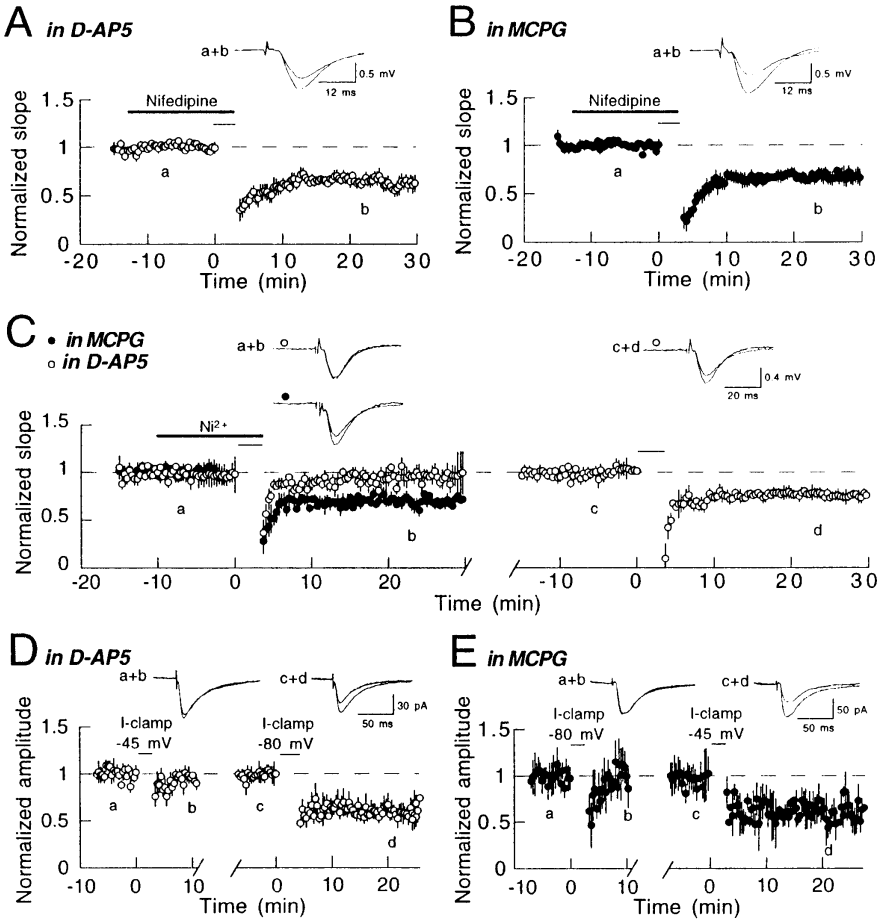

Figure 4. Activation of T-type Calcium Channels Is Required for the Induction of mGluR-LTD but Not for the Induction of NMDAR-LTD

(A-B) Both mGluR-LTD (A) and NMDAR-LTD (B) of field EPSPs were induced in the presence of 20 μM nifedipine (n = 5).

(C) In the presence of 100 μM D-AP5 and 50 μM Ni^{2+}, mGluR-LTD of field EPSPs was not induced (open circles). In the same slices (n = 6), mGluR-LTD was elicited once Ni^{2+} was washed out. Ni^{2+} did not block NMDAR-LTD (closed circles; n = 4).

(D) Induction of mGluR-LTD was prevented when the cells were held at −45 mV in current clamp. In the same cells (n = 5), mGluR-LTD was elicited when the neurons were held at −80 mV in current-clamp during the induction protocol.

(E) Induction of NMDAR-LTD was prevented by holding the cells at −80 mV in current clamp during the 5 Hz protocol. In the same cells (n = 4), NMDAR-LTD was elicited when the neurons were held at −45mV in current-clamp during the induction protocol.

However, LTD was not observed in the whole-cell recording (15% ± 12%, n = 5), while it was observed in the simultaneously recorded field EPSPs (−25% ± 9%). The PKC inhibitory peptide did not block NMDAR-LTD (Figure 5D), which also serves as a control for the positive effects observed with mGluR-LTD. These results further support the pharmacological evidence suggesting that the mGluR involved in LTD is located postsynaptically.

Role of Protein Phosphatases

It has been suggested that NMDAR-LTD (Mulkey et al., 1993; Kirkwood and Bear, 1994) and depotentiation (O'Dell and Kandel, 1994) involve a protein phosphatase cascade. It was therefore of interest to determine if mGluR-LTD converged onto this same molecular pathway. We first examined the effects of loading cells with the protein phosphatase inhibitor Microcystin LR on NMDAR-LTD. In accord with previous results, this form of LTD was blocked (8% ± 9%, n = 4; Figure 6A). However, repeating these same experiments in the presence of D-AP5 indicated that Microcystin LR had no effect on mGluR-LTD (−39 % ± 10%, n = 5; Figure 6B).

Figure 6C summarizes all of the experiments that we have performed to compare the induction properties of mGluR-LTD and NMDR-LTD. Blockade of $GABA_A$ synaptic inhibition with bicuculline blocked mGluR-LRD but not NMDAR-LTD. Blockade of L-type Ca^{2+} channels with nifedipine had no effect on either type of LTD, but blockade of T-type Ca^{2+} channels with Ni^{2+} selectively blocked mGluR-LTD. The group 1 selective mGluR antagonist AIDA, as well as the PKC inhibitory peptide ($PKC_{19–36}$), blocked mGluR-LTD but not NMDAR-LTD. The membrane potential at which the cells were held during the 5 Hz stimulation also influenced which form of LTD was elicited. When cells were held at −45 mV in current clamp, NMDAR-LTD was evoked but not mGluR-LTD, while holding cells at −80 mV in current clamp resulted in the generation of mGluR-LTD but not

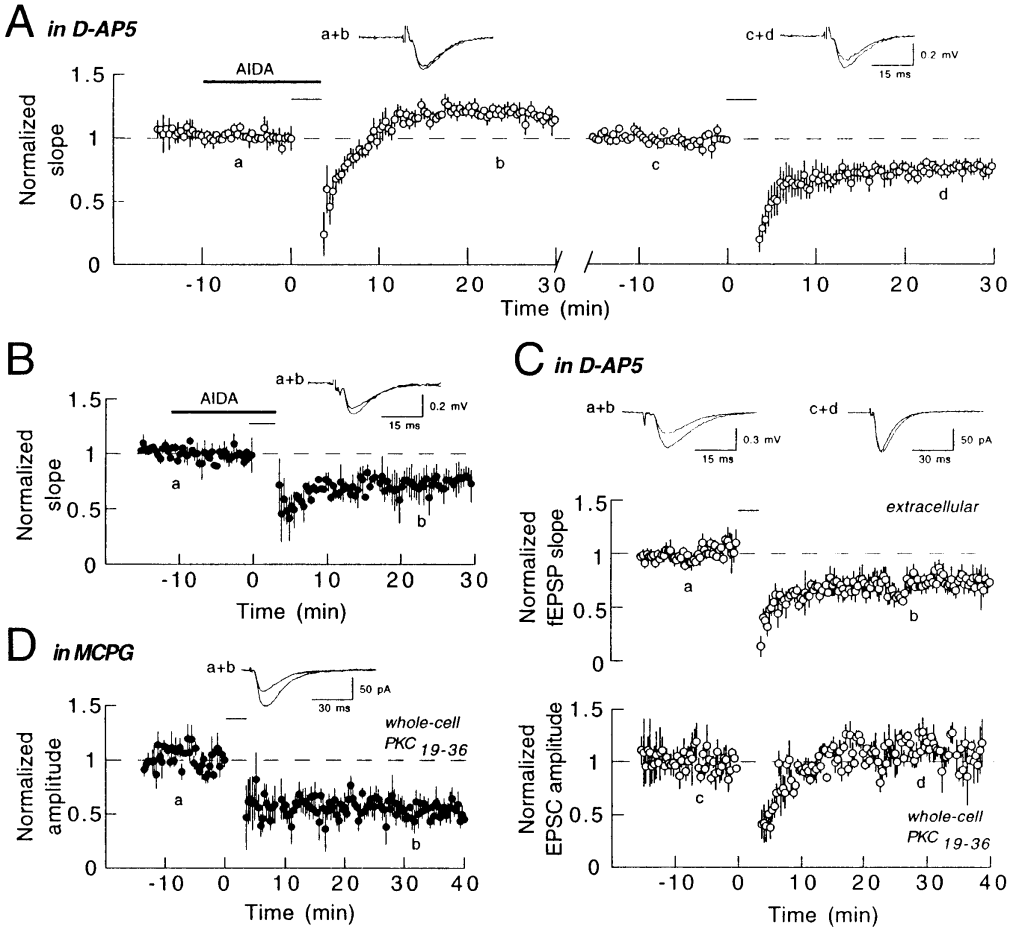

Figure 5. Activation of Group 1 mGluRs and PKC Is Required for the Induction of mGluR-LTD but Not for the Induction of NMDAR-LTD

(A) In the presence of 500 μM AIDA, mGluR-LTD of field EPSPs was prevented. In the same slices (n = 6), mGluR-LTD was induced once AIDA was washed out.

(B) AIDA did not block NMDAR-LTD (n = 3).

(C) Inclusion of PKC_{19-36} (10 μM) in the recording electrode blocked the induction of mGluR-LTD (n = 5; lower panel). Field EPSPs simultaneously monitored during these experiments were depressed (upper panel).

(D) Inclusion of PKC_{19-36} (10 μM) in the recording electrode did not prevent the induction of NMDAR-LTD (n = 4).

NMDAR-LTD. Finally, blockade of phosphatases by loading cells with Microcystin LR blocked NMDAR-LTD but not mGluR-LTD.

Effects of NMDAR-LTD and mGluR-LTD on Quantal Size and Frequency

Although the results mentioned thus far are consistent with the hypothesis that mGluR-LTD and NMDA-LTD are entirely distinct processes, it is possible that they converge onto a common target. Comparison of the expression mechanisms of these forms of LTD should help distinguish between these two possibilities. In the first series of experiments, we examined the effects of LTD on the size and frequency of quantal events by taking advantage of the finding that replacing Ca^{2+} with Sr^{2+} suppresses synchronized transmitter release but greatly enhances asynchronous release (Miledi, 1966; Goda and Stevens, 1994). Since the asynchronous release only occurs at the subset of synapses that are stimulated, it permits a detailed analysis of quantal events originating from these synapses (Oliet et al., 1996). Two independent pathways were monitored with whole-cell recording, and LTD was induced in one of the pathways with the other serving as a control. After establishing that the LTD was stable, Ca^{2+} was replaced with Sr^{2+}, and bouts of 2 Hz stimulation to the two pathways were alternated. This permitted a comparison of the quantal events that were collected from each pathway. In previous work (Oliet et al., 1996), we found that the size and frequency of quantal events generated by two independent pathways of similar size synapsing on the same cell are the same, thus validating this cross-pathway comparison.

We initially examined NMDAR-LTD and excluded any contamination from mGluR-LTD by conducting these experiments in the presence of the mGluR antagonist MCPG (1.5 mM). An example of a single experiment is shown in Figures 7A and 7B, and a summary of all 5 experiments is shown in Figures 7C–7E. In confirmation of previous results (Oliet et al., 1996), we found a decrease in the average size of quanta during NMDAR-LTD (−20.8% ± 7.1%, n = 5; $p < 0.05$), which appears

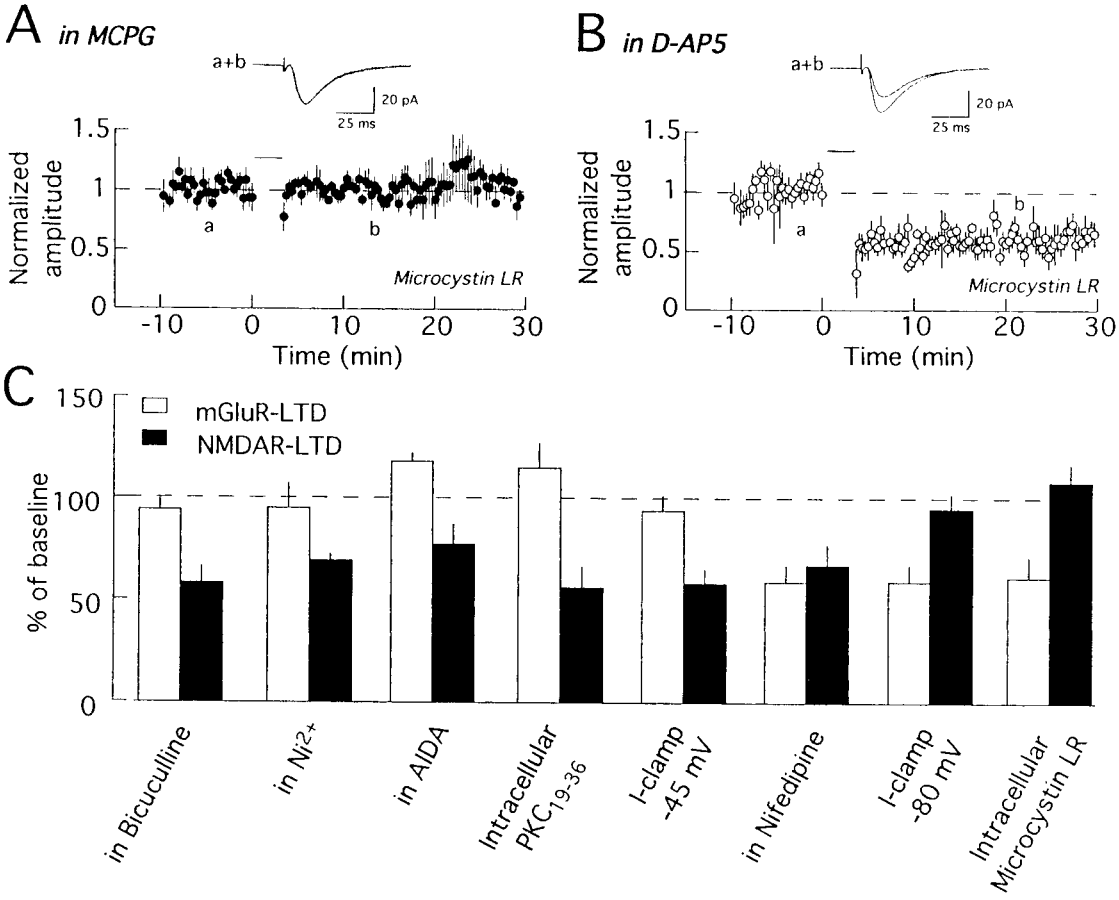

Figure 6. Phosphatase Inhibition Prevents the Induction of NMDAR-LTD but Not the Induction of mGluR-LTD

(A) Inclusion of microcystin LR (10 μM) in the recording electrode blocked the induction of NMDAR-LTD (n = 4).

(B) Microcystin LR had no effect on the induction of mGluR-LTD (n = 5).

(C) A summary of the different manipulations on the percent depression associated with NMDAR- and mGluR-LTD.

as a shift to the left of the cumulative probability plot (Figure 7D). While there was also a decrease in the frequency of events, this was not significant (−12.7% ± 10%, n = 5; $p > 0.6$; Figure 7E).

These experiments were repeated for the mGluR-LTD, which was studied in isolation by applying D-AP5 (100 μM) to prevent NMDAR-LTD. A typical experiment is shown in Figures 8A and 8B, and a summary of all 5 experiments is shown in Figure 8C–8E. In contrast to NMDAR-LTD, no decrease in quantal size was detected ($p > 0.3$; Figure 8D), but there was a substantial decrease in the frequency of events (−40.4% ± 2.9%, n = 6; $p < 0.02$; Figure 8E).

mGluR-LTD Does Not Occlude NMDAR-LTD

If, as suggested by the examination of quantal events, the two forms of LTD have independent expression mechanisms, they should not exhibit occlusion. To test this prediction, we monitored two pathways; in one, mGluR-LTD was saturated by giving repeated bouts (3–6 times) of 5 Hz stimulation in the presence of D-AP5 until no further depression occurred. D-AP5 was washed from the slice, and MCPG was applied. We then compared the magnitude of LTD induced on the pathway expressing mGluR-LTD to that induced on the naive pathway (Figure 9). As is evident in the graph, NMDAR-LTD could still be induced on a pathway expressing saturated mGluR-LTD (−27.1% ± 6.2%, n = 5), and the magnitude of the LTD was the same as in a naive pathway (−23.3% ± 5%). These results provide further evidence indicating that the two forms of LTD are distinct.

NMDAR-LTD, but Not mGluR-LTD, Reverses LTP

Considerable evidence suggests that NMDAR-LTD and NMDAR-LTP are reversible modifications of a common process (Mulkey and Malenka, 1992; Dudek and Bear, 1993; Mulkey et al., 1993). Thus, following saturation of LTP, low frequency stimulation can unsaturate LTP, a phenomenon often referred to as depotentiation. That this is a true reversal of LTP is indicated by the fact that, following the depotentiation induced by the low frequency stimulation, it is possible to restore the LTP to its previous level with high frequency stimulation. This is shown in Figure 10A. After LTP was saturated with 3–4 series of tetanic stimulation (the last two are shown in the figure), MCPG was applied, and 5 Hz stimulation was given. This caused a stable depression in synaptic

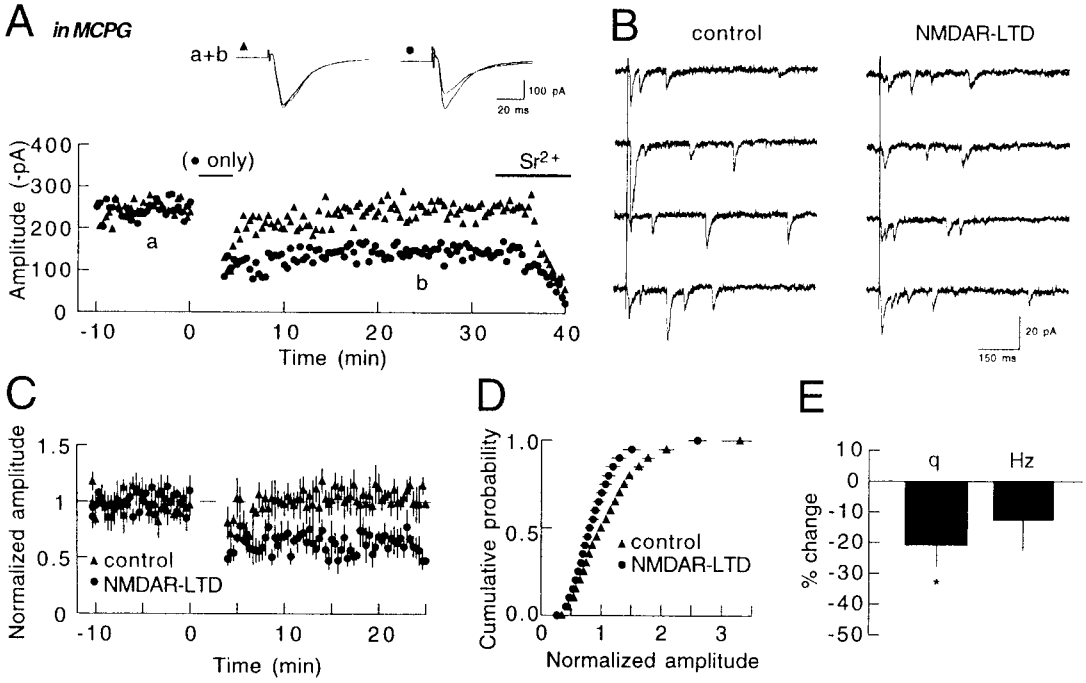

Figure 7. Quantal Changes Associated with NMDAR-LTD

(A) An example of a whole-cell recording where LTD was induced in one pathway (circles) in the presence of 1.5 mM MCPG. Once LTD was stable, Sr^{2+} was washed in. The sample records are superimposed traces (averages of 10 sweeps) taken at the indicated times in both control and test pathways.

(B) Representative sample traces of evoked EPSCs originating from the control and the depressed pathway in the presence of Sr^{2+}.

(C) A summary graph of five experiments.

(D) The corresponding cumulative amplitude distributions of the events associated with Sr^{2+}-induced asynchronous release, obtained from the depressed (circles) and control (triangles) pathways.

(E) A plot of the average changes in the frequency (Hz) and size (q) of the Sr^{2+}-induced asynchronous events associated with NMDAR-LTD ($n = 5$).

transmission ($-21\% \pm 6\%$, $n = 5$). When another tetanus was applied, the saturated level of LTP was reestablished. The same experimental protocol was given in another set of slices, except that the 5 Hz stimulation was given in the presence of D-AP5 (Figure 10B). This stimulation again caused a stable depression in synaptic transmission ($-21\% \pm 5\%$), but, unlike in the previous experiment, no LTP could be evoked following the 5 Hz stimulation. Thus, unlike NMDAR-LTD, mGluR-LTD does not involve a reversal of the mechanisms mediating NMDAR-LTP.

Discussion

LTD has been observed in a variety of regions in the CNS and has been variously described as being dependent on NMDARs or on mGluRs. Even within the CA1 region of the hippocampus, considerable controversy exists as to whether NMDARs (Dudek and Bear, 1992; Mulkey and Malenka, 1992; Izumi and Zorumski, 1993; Debanne et al., 1994; Stevens et al., 1994; Xiao et al., 1994; Kerr and Abraham, 1995; Wagner and Alger, 1995; Selig et al., 1995; Hrabetova and Sacktor, 1996; Heynen et al., 1996) or mGluRs (Stanton et al., 1991; Bolshakov and Siegelbaum, 1994; Yang et al., 1994; Otani and Connor, 1996) are required for the induction of LTD. The finding of an mGluR-dependent form of LTD in neonates (Bolshakov and Siegelbaum, 1994), which contrasts with most previous studies in older animals, reporting an NMDAR-dependence, suggested that the type of LTD might be developmentally regulated. However, we provide evidence here that in the same CA1 pyramidal cell, depending on the recording conditions, either a NMDAR-LTD or a mGluR-LTD can be induced. We have defined some of the conditions that govern which type of LTD is induced, characterized some of the cellular mechanisms involved in mGluR-LTD, and compared their mechanism of expression. Surprisingly, both induction and expression mechanisms of mGluR-LTD appear to be completely different from those responsible for NMDAR-LTD.

Induction Requirements

A number of conditions were found that influenced the ability to generate mGluR-LTD. First, the Ca^{2+}/Mg^{2+} ratio was an important variable; mGluR-LTD was difficult to induce in a ratio of 2.5 mM/1.3 mM, but could easily be induced in a ratio of 4 mM/4 mM, which decreased the size of the synaptic response. Second, the level of $GABA_A$-mediated synaptic inhibition determined if mGluR-LTD could be induced. In the absence of inhibition, mGluR-LTD was not induced. While these results can explain why Selig et al. (1995), who used a ratio of 2.5 mM/1.3 mM and picrotoxin, only observed NMDAR-LTD, they

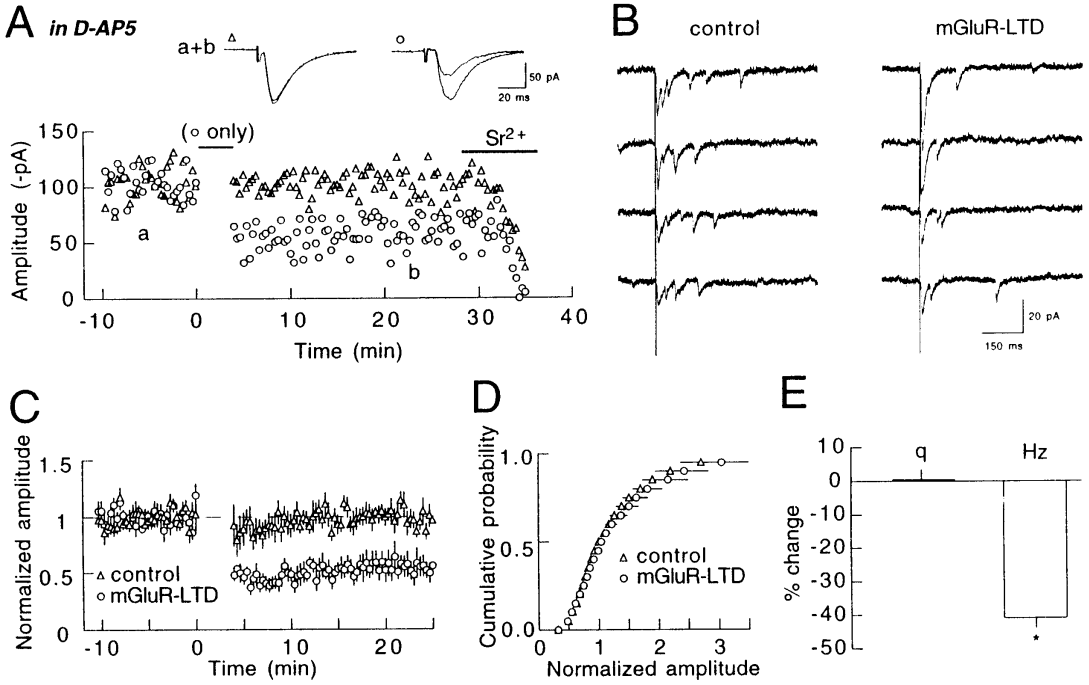

Figure 8. Quantal Changes Associated with mGluR-LTD

(A) An example of a recording where LTD is induced in one pathway (circles) in the presence of 100 μM D-AP5. Once LTD was stable, Sr^{2+} was washed in. The sample records are superimposed traces (averages of 10 sweeps) taken at the indicated times in both control and test pathways.

(B) Representative sample traces of evoked EPSCs originating from the control and the depressed pathway in the presence of Sr^{2+}.

(C) A summary graph of six experiments.

(D) The corresponding cumulative amplitude distributions of the events associated with Sr^{2+}-induced asynchronous release, obtained from the depressed (circles) and control (triangles) pathways.

(E) A plot of the average changes in the frequency (Hz) and size (q) of the Sr^{2+}-induced asynchronous events associated with mGluR-LTD (n = 6).

differ from the results of Bolshakov and Siegelbaum (1994), who studied mGluR-LTD in a ratio of 2.5 mM/ 1.3 mM. The finding that low frequency stimulation in the presence of GABA can induce an mGluR dependent form of LTD (Yang et al., 1994) is also consistent with our observations. Third, mGluR-LTD was easier to evoke with small inputs compared with large inputs. Fourth, the membrane potential at which the induction protocol was given influenced the type of LTD evoked, such that at hyperpolarized potentials, mGluR-LTD is favored over

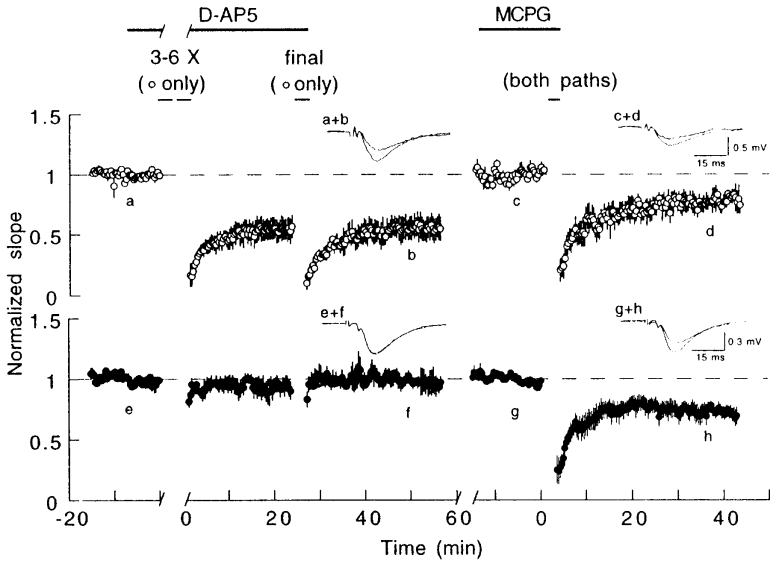

Figure 9. NMDAR-LTD and mGluR-LTD Do Not Occlude

A summary of experiments (n = 5), in which several trains of 5 Hz/3 min stimulations were applied to one pathway (open circles), in the presence of 100 μM D-AP5, to saturate mGluR-LTD. These trains had no effect on the control pathway (closed symbols). MCPG (1.5 mM) was then washed onto the slices, and another train of 5 Hz stimulation was given for 3 min to both pathways to induce NMDAR-LTD. The insets are sample traces taken at the indicated times.

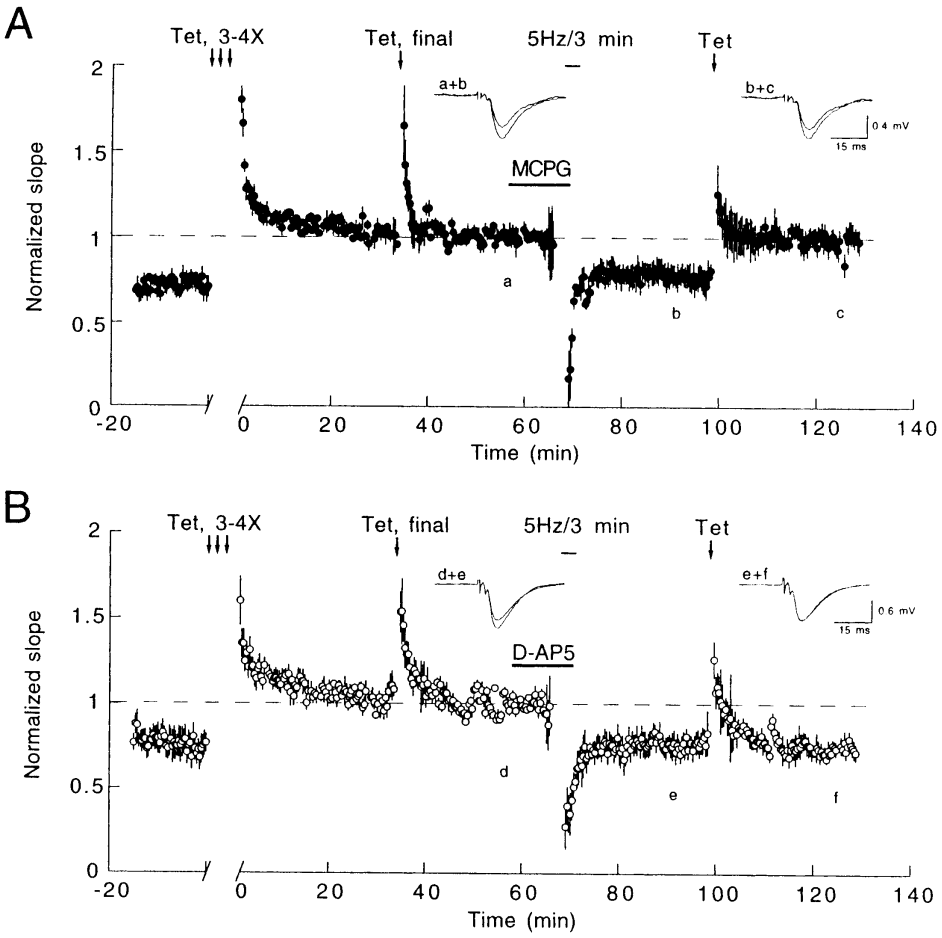

Figure 10. mGluR-LTD Is Not the Reversal of LTP

(A) Following saturation of LTP by several tetani, NMDAR-LTD (solid symbols; n = 5) was induced by 5 Hz stimulation for 3 min in MCPG (1.5 mM). Subsequent high frequency tetani increased the EPSP to its original level.

(B) When depotentiation was induced in the presence of D-AP5 (100 μM), a subsequent tetanus failed to repotentiate the EPSP.

NMDAR-LTD, whereas at depolarized potentials, NMDAR-LTD is favored over mGluR-LTD. Except for the membrane potential at which LTD was induced, none of the variables discussed above appeared to effect appreciably the induction of NMDAR-LTD, although it has been reported that the level of GABAergic inhibition can influence the induction of NMDAR-LTD (Wagner and Alger, 1995). All of the conditions that favor the induction of mGluR-LTD would result in a small depolarization during the 5 Hz stimulation.

While mGluR-LTD and NMDAR-LTD are superficially similar in that they are of similar magnitude, are pathway specific, have a voltage dependence to their induction, and require a rise in postsynaptic Ca^{2+}, the mechanisms responsible for these induction properties are very different. The pathway specificity for the two forms of LTD presumably comes from the local activation of either the NMDAR or the mGluR on the activated synapses, while the Ca^{2+} dependence for NMDAR-LTD comes from Ca^{2+} permeability of the NMDAR, and the Ca^{2+} dependence of mGluR-LTD comes from the gating of T-type Ca^{2+} channels. The presence of T-type Ca^{2+} channels in CA1 neurons (Takahashi et al., 1991) and their presence and activation in the dendrites by subthreshold EPSPs (Magee and Johnston, 1995) are consistent with this proposal. Although the ability to define pharmacologically T-type Ca^{2+} channels in the hippocampus is problematic (Takahashi and Akaike, 1991), the inability to induce mGluR-LTD at a holding potential of −45 mV is consistent with the role of T-type, but not L-type, Ca^{2+} channels in the induction of this form of LTD. The lack of effect of the L-type Ca^{2+} channel blocker nifedipine also favors the involvement of T-type over L-type Ca^{2+} channels, as has recently been suggested for LTD in the dentate gyrus (Wang et al., 1997). The involvement of T-type Ca^{2+} channels may explain the requirement for synaptic inhibition in mGluR-LTD, since inhibition would help to deinactivate these channels. Interestingly, in neonatal slices the L-type Ca^{2+} channel is required for mGluR-LTD (Bolshakov and Siegelbaum, 1994), suggesting a developmental switch in the type of Ca^{2+} channel that may be involved.

Signal Transduction

We found that AIDA, an antagonist of group 1 mGluRs (consisting of mGluR1 and mGluR5), blocks mGluR-LTD. We favor the involvement of mGluR5, and not mGluR1,

because the mGluR1 selective antagonist 4CPG was ineffective, and anatomical studies have shown that CA1 pyramidal cells express mGluR5 but not mGluR1 (Luján et al., 1996). The action of MCPG is also consistent with the involvement of mGluR5 (Saugstad et al., 1995; but see Joly et al., 1995).

Group I mGluRs are coupled to phospholipase C, suggesting that this signaling pathway may be involved in mGluR-LTD. The finding that a PKC inhibitory peptide ($PKC_{19–36}$) injected into the postsynaptic cell blocked mGluR-LTD, but not NMDAR-LTD, supports a postsynaptic localization of the mGluR and also the activation of the PKC limb of the PI turnover pathway. On the other hand, we could find no evidence for a role of PKC in NMDAR-LTD. While the role of PKC in mGluR-LTD could be to increase T-type Ca^{2+} channel current, PKC activation appears to inhibit T-type Ca^{2+} channels (see Huguenard, 1996). Thus, we favor a model in which PKC and Ca^{2+} act cooperatively on some downstream process.

We confirmed that postsynaptic application of the phosphatase inhibitor Microcystin LR blocks NMDAR-LTD (Mulkey et al., 1993), yet has no effect on mGluR-LTD. Recently, it has been proposed that NMDAR-LTD in CA1 pyramidal cells is due to a decrease in PKC activity, based, in part, on the finding that PKC inhibitors depress synaptic responses and that this occluded with LTD (Hrabetova and Sacktor, 1996). The present results are difficult to reconcile with this conclusion. Loading cells with the PKC inhibitory peptide did not block NMDAR-LTD, nor did it affect baseline synaptic transmission; the latter result indicates that the inability to evoke mGluR-LTD in the presence of this peptide is due to a block of LTD rather than to an occlusion. Thus, we conclude that mGluR-LTD requires the activation of PKC. It has been proposed that PKC activation is also involved in the generation of LTP (Bliss and Collingridge, 1993; Wang and Kelly, 1995), suggesting that additional factors are required for either one or both of these forms of plasticity.

Expression Mechanisms

An important conclusion of our study is that the expression mechanisms of mGluR-LTD and NMDAR-LTD are distinct. mGluR-LTD is associated with a marked decrease in the frequency of quantal events but no change in their size, while NMDAR-LTD is associated with a decrease in quantal size. The change in frequency is consistent with a presynaptic expression mechanism (Bolshakov and Siegelbaum, 1994) and the requirement for a retrograde messenger or, alternatively, with the all-or-none down regulation of clusters of AMPA receptors, a mechanism analogous to that proposed for LTP (Isaac et al., 1995; Liao et al., 1995). The diffusible messenger NO does not appear to be the retrograde messenger, since mGluR-LTD could still be evoked after blockade of NO synthase by L-N^G-nitroarginine (100 μM; n = 2). Another candidate retrograde messenger, for which some evidence has recently been proposed, is arachidonic acid (Bolshakov and Siegelbaum, 1995), but we did not explore its possible role. The decrease in quantal size associated with NMDAR-LTD (Oliet et al., 1996) is consistent with a postsynaptic expression mechanism.

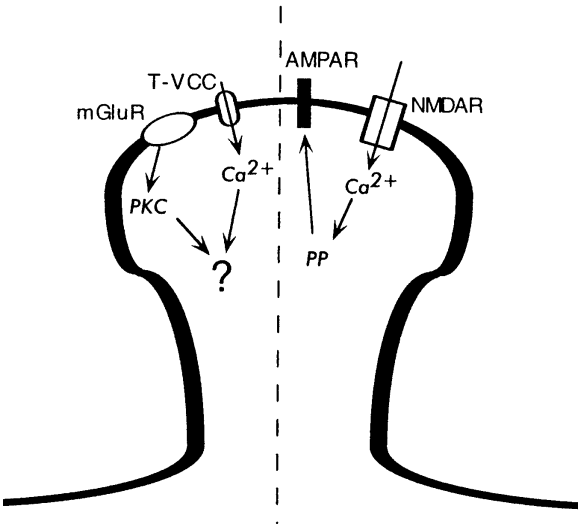

Figure 11. Schematic Diagram Illustrating the Different Steps Involved in the Induction of NMDAR-LTD and mGluR-LTD (PP Stands for Protein Phosphatase and T-VCC Stands for T-Type Voltage-Dependent Ca^{2+} Channel)

Perhaps the most important distinction between NMDAR-LTD and mGluR-LTD is that the former reverses NMDAR-LTP while the latter does not. This observation, together with the finding that NMDAR-LTD, but not mGluR-LTD, is associated with a decrease in quantal size, while NMDAR-LTP is associated with an increase in quantal size, suggests that the NMDAR-LTD and NMDAR-LTP are reversible modifications of a common process, while mGluR-LTD uses a distinct expression mechanism. These findings also raise the question of whether there is a form of LTP that is the converse of mGluR-LTD. It has been suggested that LTP elicited by activation of voltage-dependent Ca^{2+} channels may utilize signal transduction cascades different from those involved in NMDAR-LTP (Huber et al., 1995; Cavus and Teyler, 1996). However, it remains unclear whether the final expression mechanisms of these forms of LTP are different. It has also been suggested that NMDAR-LTP may involve different mechanisms depending on the induction protocols (Haley et al., 1993). Again, this possibility has not been explored thoroughly at a mechanistic level. Finally, evidence has been presented that mGluRs may be involved in LTP (Bortolotto and Collingridge, 1993; Bashir et al., 1993; Bortolotto et al., 1994; but see Selig et al., 1995), but the expression mechanism is proposed to be the same as that of NMDR-LTP. Thus, at the present time, there is no clear evidence demonstrating a form of LTP that is the converse of mGluR-LTD.

Conclusions

We have presented evidence that mGluR-LTD and NMDAR-LTD in CA1 pyramidal cells are independent forms of synaptic plasticity, in that their induction requirements, expression mechanisms, and signal transduction pathways all differ. We favor the conclusion, as diagrammed in Figure 11, that these two forms of LTD coexist in single dendritic spines. This would require that the Ca^{2+} entry through T-type Ca^{2+} channels is

unable to activate the mechanisms mediating NMDAR-LTD. In contrast, repetitive activation of L-type Ca^{2+} channels (Christofi et al., 1993; Cummings et al., 1996) or photo release of caged Ca^{2+} (Neveu and Zucker, 1996) can cause a long-lasting depression of synaptic strength that partially occludes with NMDAR-LTD. These conclusions require a rather remarkable compartmentalization in the spine, which limits the activation of specific signal transduction pathways to the appropriate trigger. Indeed, it has been shown that glutamate receptor subtypes are differentially localized at the postsynaptic spine, with the mGluRs localized perisynaptically and the ionotropic glutamate receptors localized at the postsynaptic density (Luján et al., 1996). Compartmentalization could also be accomplished by tethering the signaling transduction molecules to the immediate vicinity of the triggering receptor and/or channel (Rosenmund et al., 1994; Faux and Scott, 1996; Brenman et al., 1996).

An alternative explanation for our results is that the two forms of LTD are anatomically segregated to separate spines on a given cell. While formally a possibility, we think this is much less likely, since there is no evidence for segregation of mGluRs and NMDARs to different spines, and this scenario would require a remarkable, highly specific targeting of specific molecules to specific spines. Given that the expression mechanisms of the two forms of LTD are clearly different, and only NMDAR-LTD reverses established LTP, it would appear extremely unlikely that the two forms of LTD are not distinct but in fact eventually converge on some common process.

A final important question is: how might these forms of LTD be engaged in the behaving animal? While we have not carried out an exhaustive search for the rules that determine which form of LTD is induced, we have identified three important physiological variables: the membrane potential of the cell, the level of synaptic inhibition, and the size of the synaptic input. All of these variables will affect the level of membrane depolarization that occurs during the 5 Hz stimulation, with larger depolarizations favoring the induction of NMDAR-LTD. The behavioral state of the animal would be expected to greatly influence these three variables. For instance, in the behaving animal, the level of inhibition, synchronous firing, and burst firing can change dramatically (Wilson and McNaughton, 1993; Chrobak and Buzsaki, 1996), all of which would alter the relative contribution of NMDAR-LTD and mGluR-LTD. Further studies will be needed to determine what particular computational advantage is provided by the existence of two independent forms of LTD in hippocampal neurons.

Experimental Procedures

The hippocampal slices (500 μm) were prepared from 6–35 day-old Sprague-Dawley rats as described previously (Castillo et al., 1994). The slices were allowed to recover for at least 1 hr and then were transferred to a recording chamber, where they were submerged in an artificial CSF saturated with 95% O_2 and 5% CO_2. The composition of the artificial CSF (pH 7.4; 295–305 mOsm kg^{-1}) was 123 mM NaCl, 2.5 mM KCl, 1 mM NaH_2PO_4, 26.2 mM $NaHCO_3$, 4 mM $CaCl_2$, 4 mM $MgCl_2$, and 10 mM glucose. When bicuculline was present in the perfusing solution, a cut was made between CA3 and CA1 to reduce epileptiform activity. The experiments were carried out at room temperature.

Standard whole-cell patch-clamp and extracellular field recording techniques were used. One or two independent pathways were stimulated (0.05 Hz), using bipolar stainless steel stimulating electrodes placed in the stratum radiatum equidistant from the pyramidal layer on each side of the recording site. The field recording electrodes were filled with 3 M NaCl. The patch electrodes (2–5 MΩ) were filled with an internal solution (pH 7.1; 295–305 mOsm kg^{-1}) containing 123 mM K-gluconate, 8 mM NaCl, 10 mM HEPES, 0.2 mM EGTA, 10 mM glucose, 2 mM Mg^{2+}-adenosine triphosphate, and 0.3 mM Na_3-guanosine triphosphate. In some experiments, either 10 mM BAPTA (substituted for an equimolar amount of K-gluconate), 10 μM microcystin LR, or 10 μM PKC_{19-36} were included in the internal solution. Series and input resistance were monitored throughout each experiment. Cells were excluded from data analysis if more than a 20% change in the series resistance or the input resistance occurred during the course of the experiment. For the experiments with BAPTA, microcystin LR, and PKC_{19-36}, 15–20 min elapsed after break-in before the LTD induction protocol was applied.

The data were collected with an axopatch-1D (Axon Instruments, Foster City, CA) filtered at 1 kHz, sampled at 2 kHz, and analyzed on-line as previously described (Mulkey and Malenka, 1992) using Neurophysiologica, software developed by D. Selig. Unless otherwise stated, all averages are listed as mean ± SEM. The initial slopes of the field EPSPs were measured from approximately 10%–40% of the rising phase, using a least-squares regression. The amplitudes of the EPSCs were measured by taking the average of a 2–3 ms window around the peak of the EPSC relative to the baseline. The data were normalized to the averaged value obtained during the 10–15 min period prior to applying the LTD inducing stimulus, which in all cases was a 5 Hz/3 min train of stimuli. Sr^{2+}-induced asynchronous events were collected and analyzed as previously described (Oliet et al., 1996). Briefly, 4 mM $CaCl_2$ was replaced by 4 mM $SrCl_2$ and 0.1 mM picrotoxin in the superfusing solution. Once the EPSCs amplitude reached a steady-state level in the presence of Sr^{2+}, each pathway was stimulated alternatively (every 30 s) at 2Hz for 10 s for 15–20 min. Asynchronous events were measured during the 400 ms period after the end of the synchronous response and were analyzed as previously described (Manabe et al., 1992; Wyllie and Nicoll, 1994). Entries in the cumulative amplitude histograms ranged from 182–848 events. The data were compared statistically with either the nonparametric Kolmogorov-Smirnov test or with the paired Student's t test. Averaged cumulative histograms were obtained by normalizing each distribution to the corresponding median value obtained in the control pathway.

Tetanus-induced long-term potentiation was obtained by using a 100 Hz stimulus for 1 s, repeated four times at 20 s intervals. For the experiments of saturation of LTP, this procedure was repeated four to five times to reach saturation. The field EPSP slopes were normalized to the value obtained at saturation. For the experiments where LTD was saturated, four to seven trains of 3 min at 5 Hz were given to the test pathway. The data were renormalized after saturation.

The drugs used were D(−)-2-amino-5-phosphonopentanoic acid (D-AP5), (+)α-methyl-4-carboxyphenylglycine (MCPG), AIDA, (S)-4-carboxyphenylglycine (4CPG; Tocris Cookson), Nifedipine, Ni^{2+}, Bicuculline methiodide, 1,2-bis(2-aminophenoxy)ethane-N,N,N^1,N^1-tetraacetic acid (BAPTA; Sigma), Microcystin LR, and PKC_{19-36} (Research Biochemicals International).

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