Evidence for Postsynaptic Induction and Expression of NMDA Receptor Independent LTP

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Grover, Lawrence M. Evidence for postsynaptic induction and expression of NMDA receptor independent LTP. J. Neurophysiol. 79: 1167–1182, 1998. Whole cell/patch-clamp and extracellular field potential recordings were used to study the induction and expression of N-methyl-D-aspartate (NMDA) receptor independent long-term potentiation (LTP) in area CA1 of the in vitro rat hippocampus. Induction of NMDA receptor independent LTP was prevented by manipulations that inhibited postsynaptic depolarization during tetanic stimulation: direct hyperpolarization of postsynaptic neurons and bath application of an α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and kainate receptor antagonist. NMDA receptor independent LTP also was blocked by intracellular application of the lidocaine derivative, N-(2,6-dimethylphenylcarbamoylmethyl)triethylammonium bromide (QX-314), to CA1 pyramidal neurons. These results complement the previous findings that NMDA receptor independent LTP was inhibited by postsynaptic injections of the calcium chelator 1,2-bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid (BAPTA) (Grover and Teyler 1990) and by extracellular application of the dihydropyridine calcium channel antagonist, nifedipine (Cavus and Teyler 1996; Grover and Teyler 1990). Additional experiments (Little et al. 1995) suggested a requirement for metabotropic glutamate receptors in the induction of this LTP. Maintenance of NMDA receptor independent LTP was not affected by the broad spectrum serine/threonine kinase inhibitor H-7, (Cavus and Teyler 1996; Grover and Teyler 1995), but maintenance was prevented by the tyrosine kinase inhibitors genistein and lavendustin A (Cavus and Teyler 1996). These data suggest that a phosphorylational change is needed for expression of NMDA receptor independent LTP, but the location (pre- or postsynaptic) of this change is not known.

In this paper, several issues related to the induction of NMDA receptor independent LTP in the CA1 area are investigated. First, this paper tests the hypothesis that the induction site for NMDA receptor independent LTP is in postsynaptic neurons through a series of experiments designed to limit postsynaptic changes in membrane potential during LTP induction. Second, this paper examines the site of change underlying maintenance of NMDA receptor independent LTP by testing for LTP of pharmacologically isolated α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)- and NMDA-receptor–mediated excitatory postsynaptic potentials (EPSPs) and by testing for changes in paired pulse facilitation (PPF), an index of presynaptic function (Zucker 1989).

METHODS

Slice preparation

Male and female Sprague-Dawley rats (6- to 16-wk old) were sedated by inhalation of a CO2/air mixture and decapitated. The skull was opened, and the brain was removed and placed into chilled, modified artificial cerebrospinal fluid (modified ACSF) with composition (in mM) of 124.0 NaCl, 26.0 NaHCO3, 3.0 KCl, 0.5 CaCl2, 5.0 MgSO4, and 10.0 d-glucose, bubbled with 95% O2-5% CO2 (pH 7.35). The brain was trimmed down to a block containing the hippocampus, which was glued to the stage of a vibrotome (Campden Vibroslice), and immersed in chilled modified modified ACSF. Coronal sections 400-μm thick were cut. Sections containing the hippocampus in a transverse orientation were collected. Individual hippocampal slices were cut free from surrounding structures using two 25-gauge needles. In experiments where mag-
nesium-free medium was used (described later), the CA3 region was cut away from the slices.

Hippocampal slices were transferred to a holding chamber where they were stored at room temperature. The holding chamber was filled with a standard ACSF composed of (in mM) 124.0 NaCl, 26.0 NaHCO₃, 3.4 KCl, 2.0 CaCl₂, 2.0 MgSO₄, 1.2 NaH₂PO₄, and 10.0-glucose, pH 7.35 gassed with 95% O₂-5% CO₂. Slices were incubated in the holding chamber for a minimum of 1 h before use in experiments.

For electrophysiology, slices were transferred to a small volume (200 µL) interface recording chamber heated to 35°C. The recording chamber was perfused at a rate of 1.2–1.5 ml/min with standard ACSF. Upper surfaces of the slices were exposed to a warmed, humidified 95% O₂-5% CO₂ atmosphere. Slices were allowed a minimum 30-min recovery period after being transferred into the recording chamber before beginning an experiment.

In some experiments, slices were perfused with a magnesium-free ACSF, which differed from the standard ACSF solely in the omission of MgSO₄. Drugs were applied by addition to the ACSF perfusing the slices.

**Electrophysiology**

Whole cell recordings were obtained from the somata of CA1 pyramidal neurons by the method of Blanton et al. (1989). Patch electrodes (4–6 MΩ) were filled with a solution of 140 mM cesium gluconate, 10 mM sodium N-[2-hydroxyethyl]piperazine-N′-[2-ethanesulfonic acid], 3 mM MgCl₂, 3 mM sodium ATP, and 0.2 mM sodium GTP. In some experiments the pipette solution also contained 5 mM of the lidocaine derivative, N-[2,6-dimethylphenylcarbamoylmethyl] triethylammonium bromide (QX-314), to block action potential generation (Connors and Prince 1982; Courtney 1975; Nunez and Buno 1992). Positive pressure was applied to the back of the patch electrodes as they were lowered into the somatic layer of area CA1, and the electrode resistance was monitored continuously. When electrode resistance increased, positive pressure was released, and gentle negative pressure was applied to form a high-resistance seal (>1 GΩ, typically 2–5 GΩ) with the cell membrane. The membrane patch then was ruptured to obtain the whole cell recording configuration.

Membrane potentials were measured with an Axoclamp 2B (Axon Instruments) operating in continuous current clamp mode. Access resistance was measured and compensated using the Axoclamp bridge balance circuitry. Cell input resistance was monitored throughout experiments by passing small hyperpolarizing and depolarizing currents into the cell (up to ±200 pA). Cells were discarded if either access or input resistances showed large, abrupt, irreversible changes. In all but one experiment, resting membrane potentials were maintained at a constant level near the normal CA1 pyramidal cell resting potential (−65 to −70 mV) by injecting current through the recording electrode. In one experiment, neurons were hyperpolarized to between −100 and −120 mV during tetanic stimulation but otherwise were held at a constant potential between −65 and −70 mV.

Field potentials were recorded from a broken patch electrode, filled with ACSF and placed into the middle of the apical dendritic region in stratum radiatum.

Stimulating electrodes were placed into the midstratum radiatum to activate Schaffer collateral/commissural fibers. Two stimulating electrodes, one on each side of the recording site, were used in all whole cell and some field potential experiments. In the remaining field potential experiments, a single stimulating electrode was used. Constant current test stimuli were delivered every 30 s, when a single stimulating electrode was used, and were delivered every 15 s (alternating between the 2 electrodes) when two stimulating electrodes were used.

Test stimulus intensities were set by first determining the intensity to consistently evoke an orthodromic action potential (in whole cell experiments) or to evoke a clearly discernible population spike (in field potential experiments). Test stimuli were delivered at one-half the intensity needed to evoke firing. Using this method, test stimuli averaged 76 ± 5 µA (mean ± SE; range 20–250 µA) with a stimulus duration of 0.1 ms. Evoked synaptic potentials were low-pass filtered (at 1–2 kHz for whole cell responses, 2–3 kHz for field responses), amplified (gain of 10–100 for whole cell, 100–1000 for field), digitized (10–40 kHz), and stored on a 80486-based personal computer. Individual synaptic potentials were measured by fitting a straight line to the initial rising (whole cell) or falling (field) phase of the EPSP using least squares linear regression or, in some cases, as noted later, by measuring amplitude at a fixed latency after stimulus delivery.

In some slices, test stimuli were presented as pairs, separated by a 50-ms interval. Using this stimulus protocol, the EPSP evoked by the second stimulus is facilitated relative to the first response (PPF). In these experiments, PPF was quantified as a ratio

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slope EPSP_1 / slope EPSP_2
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PPF provides a relative index of the probability of neurotransmitter release from the presynaptic terminal (Douglas and Haas 1985; Hess et al. 1987; Otmakhov et al. 1993): increased probability of release is associated with a decrease in PPF, while decreased probability of release is accompanied by an increase in PPF.

LTP was induced by four 200-Hz, 0.5-s-long stimulus trains, delivered at intervals of 5 s. Stimulus intensity during tetanization was set to twice the test stimulus intensity. Tetanic stimuli always were delivered in the presence of the competitive NMDA receptor antagonist D,L-2-amino-5-phosphonopentanoic acid (APV, 50–100 µM), to block NMDA receptor dependent LTP (Grover and Teyler 1990, 1992, 1994). As described later, in some slices, the NMDA receptor glycine site antagonist 5,7-dichlorokynurenic acid (DCK, 100 µM) was applied with APV during tetanization, whereas in other slices, the noncompetitive NMDA receptor antagonist dizocilpine (MK-801, 20 µM) was applied with APV. Tetanic stimulation was delivered only after a stable baseline recording period (minimum of 5 min in whole cell experiments and 10 min in field potential experiments).

**Data analysis**

For each cell or slice, the slopes of the evoked EPSPs were normalized by comparison with the mean EPSP slope value obtained during the baseline recording period. These values are reported as percentage change from the mean of the baseline. Grouped data are given as means ± SE. Statistical significance was determined by use of the Student’s t-test (for independent or dependent samples, as appropriate).

**Results**

**NMDA receptor independent LTP during whole cell recording**

Whole cell patch clamp recording perfuses the interior of the cell (Pusch and Neher 1988), which can remove important cytosolic components from the cell. For this reason, the pretetanus baseline period during whole cell recording was kept short (<16 min in all cells). Because the pretetanus recording period was short, APV was applied to slices to block NMDA receptors as soon as a high resistance (GΩ) seal was formed. After breakthrough to the whole cell con-
configuration, a few minutes usually were required for the evoked EPSPs to stabilize. In all cells, a minimum 5-min period of stable baseline recording was required before tetanization. To control for nonspecific changes in synaptic responses, in all whole cell experiments, two afferent pathways were tested.

Tetanic stimulation, at 200 Hz, was delivered to one of the two pathways at the end of the baseline period. EPSPs evoked by test stimulation of the tetanized pathway showed an immediate, large increase (see Fig. 1). This enhancement, resembling posttetanic potentiation (PTP), decayed within 2–3 min and was followed by a more slowly developing, and sustained increase in the EPSP, which typically required from 10–15 min to reach a maximum, sustained amplitude. The magnitude and time course of the persistent EPSP potentiation closely resemble those of the NMDA receptor independent LTP reported previously in studies where field potential and conventional intracellular recording techniques were used (Cavus and Teyler 1996; Grover and Teyler 1990, 1992, 1994, 1995; Little et al. 1995). In contrast to the changes observed in the tetanized pathway, EPSPs in the control (nontetanized) pathway showed no persistent changes (see Fig. 1), although a short-term, heterosynaptic depression (lasting 2–5 min) sometimes was observed in the control pathway (Grover and Teyler 1992, 1993a,b).

A total of 10 cells (in 10 slices) were studied. LTP was obtained in 7 of these 10 cells. The magnitude of LTP was determined by averaging the change in EPSPs in the tetanized pathway during a period from 20–30 min posttetanus. LTP (for all 10 cells) was 38 ± 10%, whereas the control pathway changed by −5 ± 7% (see Fig. 6). This difference was significant (P < 0.001).

**Postsynaptic hyperpolarization during tetanic stimulation**

Previous studies (Cavus and Teyler 1996; Grover and Teyler 1990) have shown that NMDA receptor independent LTP in area CA1 is prevented by nifedipine, a dihydropyridine compound that antagonizes some voltage-dependent calcium channels (L-type calcium channels). In addition, loading of postsynaptic neurons with BAPTA, a calcium chelator, also prevented NMDA receptor independent LTP (Grover and Teyler 1990). Other observations have indicated a predominantly postsynaptic localization of dihydropyridine sensitive (L-type) voltage-dependent calcium channels (Jones and Heinemann 1987; Westenbroek et al. 1990). These data suggest that calcium influx through L-type channels into CA1 pyramidal neurons during tetanization is a trigger for the induction of NMDA receptor independent LTP (Teyler et al. 1994). If this hypothesis is correct, then hyperpolarization of postsynaptic neurons during tetanic stimulation should prevent induction of NMDA receptor independent LTP by preventing the activation of postsynaptic, voltage-dependent calcium channels.

To test this hypothesis, CA1 pyramidal cells were held between −100 and −120 mV during tetanic stimulation by injecting current through the whole cell recording pipette. Consistent with expectations, hyperpolarization did prevent induction of NMDA receptor independent LTP (see Figs. 2 and 6). For cells hyperpolarized during tetanization, the

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**FIG. 1.** Sample whole cell recording illustrating N-methyl-D-aspartate (NMDA) receptor independent long-term potentiation (LTP). Two stimulating electrodes were placed into stratum radiatum to activate 2 independent sets of afferent fibers. Stimuli were delivered at 15-s intervals, alternating between the 2 pathways. NMDA receptor antagonist, D,L-2-amino-5-phosphonopentanoic acid (APV, 100 μM), was added to the artificial cerebrospinal fluid (ACSF) immediately after seal formation and before breakthrough into the whole cell configuration to ensure sufficient time for APV to reach the slice (wash-in time for APV was ~5 min, see Fig. 7B). At 0 min, one of the pathways was tetanized (●). Tetanized pathway showed a posttetanic increase in excitatory postsynaptic potential (EPSP), which was present within 15 s of the tetanic stimulation but lasted for only 2–3 min; a slowly emerging, NMDA receptor independent LTP followed. EPSPs evoked by stimulation of the control pathway were not potentiated at any time after tetanization. Sample EPSPs shown (top) are averages of 5 successive responses recorded before (1 and 3) and at 30 min after (2 and 4) tetanization.
FIG. 2. Hyperpolarizing cells during tetanic stimulation prevented NMDA receptor independent LTP. Two stimulating electrodes were placed into stratum radiatum; stimuli were delivered at 15-s intervals alternating between the 2 pathways. NMDA receptor antagonist, APV (100 μM), was added to the ACSF immediately after seal formation. At 0 min, the cell was hyperpolarized to between −100 and −120 mV, and 1 of the pathways (●) was tetanized. In contrast to results obtained under normal conditions (see Fig. 1), the posttetanic increase in EPSP was reduced greatly, and no LTP was evident. EPSPs evoked by stimulation of the control pathway were stable after tetanization. Sample EPSPs shown (top) are averages of 5 successive responses recorded before (1 and 3) and at 30 min after (2 and 4), tetanization.

The difference between tetanized (−16 ± 17%) and control (−34 ± 13%) pathways was not significant (P > 0.20). On average, EPSPs in both the tetanized and control pathways were reduced below baseline by 20–30 min posttetanization (Fig. 6). This nonspecific reduction might be a consequence of the whole cell recording, which could have caused both control and tetanized EPSPs to run down in the cells being recorded from. Alternatively, tetanization during hyperpolarization might have caused a slow onset, nonspecific depression of EPSPs. Either of these possibilities would be consistent with the gradual decline in EPSPs shown in Fig. 2. Regardless of these considerations, LTP induction clearly failed when cells were hyperpolarized during tetanization. This result is consistent with an induction mechanism requiring postsynaptic depolarization during tetanic stimulation to activate postsynaptic voltage-dependent calcium influx.

Surprisingly, hyperpolarization also attenuated the PTP-like effect seen in the tetanized pathways (compare Figs. 1 and 2). Under control conditions, an immediate posttetanic enhancement (measured 15 s after the final 200-Hz stimulus train) was observed in all 10 cells and averaged 97 ± 16%. When cells were hyperpolarized during tetanic stimulation, a posttetanic increase in EPSPs was seen in only one-half of the cells (3/6), and the mean posttetanic change in EPSP slope was significantly reduced (P < 0.05) to 37 ± 22%.

AMPA receptor blockade

If postsynaptic depolarization is required during tetanic stimulation, then blocking AMPA receptors, which provide the depolarization (Grover and Teyler 1990) should prevent induction of NMDA receptor independent LTP. To test this possibility, field potential recordings were made from stratum radiatum in area CA1. Both APV and the AMPA receptor antagonist 6,7-dintroquinoxaline-2,3-dione (DNQX, 3 μM) were applied to the slices at the end of the baseline recording period. A relatively low concentration of DNQX was used in this experiment to facilitate washout after tetanization. In addition, because washout of DNQX is slow and generally incomplete, two stimulating electrodes were placed into stratum radiatum so that two afferent pathways could be tested. Immediately after tetanic stimulation of one pathway, washout of DNQX and APV was begun. Any NMDA receptor independent LTP induced by the tetanic stimulation would be apparent as an increase in the tetanized pathway relative to the control (nontetanized) pathway during the washout period.

As Fig. 3 shows, the combination of DNQX and APV reduced EPSPs to ~20% of their baseline levels. The inability of DNQX + APV to completely suppress EPSPs can be attributed to the low concentration of DNQX used. To ensure that the intended effect (reduced postsynaptic depolarization during tetanic stimulation) was achieved, whole cell recordings of the postsynaptic membrane potential during tetanization were obtained from another group of slices. These cells first received repeated series of 200-Hz stimulus trains, in the presence of APV, until stable responses were obtained. Then DNQX (3 μM) was applied along with APV, and the cells were again tetanized at 200 Hz. In all cells examined, the addition of DNQX to the perfusate considerably reduced depolarization during tetanization (see Fig. 3, inset), although some residual depolarization, especially during the early portion of the 200-Hz trains, remained. Nonetheless, the application of DNQX + APV considerably decreased the depolarization of postsynaptic neurons during tetanization.

Consistent with the data obtained by hyperpolarizing postsynaptic neurons (Figs. 2 and 6), application of DNQX prevented the induction of NMDA receptor independent LTP (Fig. 3). This can be seen by comparing the tetanized and control pathways; during the 55- to 60-min posttetanesis period, tetanized responses were −23 ± 21% of baseline, and control responses were −43 ± 6% of baseline. This difference was not significant (P > 0.15).
FIG. 3. Partial blockade of γ-aminobutyric acid-A (GABA_A) receptors with 6,7-dinuoroquinoxaline-2,3-dione (DQX, 3 μM) prevented the induction of NMDA receptor independent LTP. An extracellular electrode in stratum radiatum was used to monitor field EPSPs. DNQX and APV were added to the ACSF, starting 15 min before tetanization. Because EPSP recovery during washout of DNQX is slow, 2 afferent pathways were tested (only 1 pathway was tetanized). Although the tetanized pathway might not show an increase relative to the baseline, if LTP was induced, the EPSPs would show at least a relative increase, compared with the control responses, during the washout period. Although EPSPs were inhibited by only ~80% with the concentration of DNQX used in this experiment, NMDA receptor independent LTP was prevented. The error bars show ± SE. Whole cell recordings during tetanic stimulation were used to determine if 3 μM DNQX inhibited postsynaptic depolarization during tetanization. Inset: application of 3 μM DNQX greatly reduced, but did not abolish, the depolarization obtained during tetanization. Inset, left: 4 superimposed responses recorded in APV. Inset, right: final 4 responses recorded after washing in 3 μM DNQX.

Blockade of postsynaptic action potentials

Action potential firing in hippocampal pyramidal neurons is especially effective for stimulating voltage-gated calcium influx into the dendrites (Magee and Johnston 1995; Miyakawa et al. 1992; Spruston et al. 1995). This observation, coupled with the apparent involvement of postsynaptic, voltage-dependent calcium channels in the induction of NMDA receptor independent LTP, suggests that blocking generation of postsynaptic action potentials might prevent NMDA receptor independent LTP. This possibility is consistent with the results of the first two experiments reported here because hyperpolarization and AMPA receptor blockade reduced action potential firing in the CA1 pyramidal neurons during tetanization. To directly test the possible role of postsynaptic action potential firing, CA1 pyramidal cells were loaded with the local anesthetic derivative, QX-314. Intracellular QX-314 completely suppressed action potential firing in response to 200-Hz tetanic stimulation (see Fig. 4).

As with postsynaptic hyperpolarization and AMPA receptor blockade, loading cells with QX-314 prevented NMDA receptor independent LTP (see Figs. 5 and 6). Changes in EPSP slope measured between 20 and 30 min posttetanus did not differ significantly between tetanized (~3 ± 14%) and control (9 ± 9%) pathways (P > 0.30). In addition, cells loaded with QX-314 showed a pronounced postsynaptic depression in the tetanized pathway with a mean change in EPSP slope of ~90 ± 2%. This change was significantly different from the PTP-like increase in EPSP slope seen in the tetanized pathways of control cells (P < 0.001). The posttetanic depression observed in QX-314 loaded cells was specific to the tetanized pathway because EPSPs evoked by stimulation of the control pathway in these same cells showed a minimal change (11 ± 12%).

Selective changes in AMPA-receptor-mediated EPSPs

Under physiological conditions, EPSPs at Schaffer collateral/CA1 synapses are mediated primarily by non-NMDA (AMPA) glutamate receptors (Andreasen et al. 1989; Collingridge et al. 1983; Koerner and Cotman 1982). To reveal a NMDA-receptor-mediated EPSP, slices were incubated in magnesium-free ACSF. These slices also were treated with the γ-aminobutyric acid-A (GABA_A) receptor antagonist, bicuculline (10 μM), to further uncover the NMDA-receptor-mediated EPSP. Under these conditions, slices are prone to both evoked and spontaneous bursting. To minimize this bursting, slices were treated with low a concentration of the AMPA receptor antagonist DNQX (1–2 μM), and the CA3 region was cut away from the slices. Under these conditions, stimulation of afferent fibers in stratum radiatum evoked an EPSP with a prolonged time course (Fig. 7A1, trace 1). The AMPA-receptor-mediated portion of the EPSP (AMPA-EPSP) could be isolated by application of APV (Fig. 7A1, trace 2), and the NMDA-receptor-mediated portion of the EPSP (NMDA-EPSP) then could be obtained by subtraction (Fig. 7A2, trace 1-2). As Fig. 7A1 (trace 3) shows, the original response recovered after washing out APV. This procedure was used to isolate and measure AMPA- and NMDA-EPSPs: first, before tetanic stimulation and then again during the maintenance phase of NMDA receptor independent LTP (between 30 and 40 min posttetan-
FIG. 4. Inclusion of 5 mM QX-314 in the whole cell pipette solution completely prevented firing of action potentials during tetanic stimulation. Recordings shown here are from 2 different cells. When the standard pipette solution (control, top) was used, all cells depolarized strongly in response to 200-Hz tetanization, and action potentials were fired in an accommodating pattern. In contrast, when cells were perfused internally with a pipette solution containing 5 mM QX-314, despite the strong depolarization, action potentials were not fired. In QX-314-loaded cells, neither 200-Hz tetanization (as shown bottom) nor direct current injection (not shown) could evoke action potentials.

nus). If NMDA receptor independent LTP is maintained by a persistent increase in glutamate release, then both NMDA- and AMPA-EPSPs should show comparable posttetanic changes (provided that neither receptor type is saturated by pretetanus levels of glutamate release, see DISCUSSION).

In this LTP experiment, two pathways were tested. One pathway was tetanized and the second pathway served as a control, to detect any nonspecific changes in either component of the EPSP. An example from this experiment, which was repeated in a total of six slices, is shown in Fig. 7B. This example shows the time course of the changes in amplitude of early and late portions of the EPSP. The time point for measuring the early EPSP was selected by finding the peak of the isolated AMPA-EPSP (Fig. 7A1, left arrow). The late EPSP was measured at a time point that followed the decay of the isolated AMPA-EPSP (Fig. 7A1, right arrow). As can be seen in Fig. 7, A1 and B1, the early EPSP contained both AMPA-receptor- and NMDA-receptor-mediated components because this portion of the response was reduced partially by APV. The late EPSP, on the other hand, was measured at a time point where there was minimal contribution from AMPA receptors because this portion of the response was blocked almost completely by APV.

Pharmacologically isolated AMPA- and NMDA-EPSPs from both the tetanized and control pathways are shown in Fig. 7B2. The AMPA-EPSPs were obtained during the first, pretetanus, APV application (2) and during the second, posttetanus, application (4). The AMPA-EPSPs were increased in the tetanized (top) but not control (bottom) pathway. Isolated NMDA-EPSPs were obtained by subtraction (as in Fig. 7A2). NMDA-EPSPs in both tetanized and control pathways were not changed by tetanization.

Results for pharmacologically isolated AMPA- and NMDA-EPSPs were averaged across all six slices (Fig. 7C). AMPA-EPSPs were measured at their peak amplitude. NMDA-EPSPs were measured at the point of maximum difference between the isolated AMPA-EPSP and the composite EPSP. The AMPA-EPSPs were potentiated selectively,
and this potentiation was seen only in the tetanized pathway. In the control pathway, neither AMPA- nor NMDA-EPSPs were altered by tetanic stimulation. Although the late component of the EPSP (measured at a time point after the AMPA-EPSP had decayed) in the tetanized pathway frequently was increased (Fig. 7B1) and this increase could be discerned as soon as APV began to wash out, there was no long-lasting change in this component of the postsynaptic response. Thus although the NMDA-receptor-mediated component of the EPSP may have increased for a short time period after tetanization, persistent changes were apparent only for AMPA-EPSPs. These data suggest that maintenance of NMDA receptor independent LTP is limited to a postsynaptic change in AMPA receptor function. The short-term change in the late EPSP might, however, have resulted from a short-term increase in glutamate release, which returned to the baseline level within ~30 min. To test this possibility, a second method, measurement of PPF, was used.

Changes in PPF

As described earlier (METHODS), PPF is altered in consistent ways by changes in the probability of neurotransmitter release: increased release of glutamate leads to decreases in PPF, whereas decreased release of glutamate leads to increases in PPF. PPF therefore was measured and used as a test for possible changes in presynaptic function during maintenance of NMDA receptor independent LTP. Because this experiment was performed under “standard” conditions (2.0 mM magnesium in the ACSF, no GABA or AMPA receptor antagonists) and it was previously shown that NMDA receptor independent LTP is expressed selectively only in a tetanized pathway under these conditions (Fig. 1) (Grover and Teyler 1992), a control (nontetanized) pathway was not tested in this experiment. Instead, stimulus pairs (50-ms interstimulus interval) were delivered during both pretetanus baseline and posttetanus periods. The procedure used for LTP induction, however, was identical to that of the previous experiments.

Figure 8A1 shows mean changes in EPSP slope for the first response of the pair, plotted during a 10-min baseline period and a 30-min posttetanus period. The PPF ratio for the corresponding time points is shown in Fig. 8A2. As in whole cell experiments (Fig. 1), tetanic stimulation in the presence of APV caused an immediate posttetanic increase in EPSP slope which lasted for 2–3 min. NMDA receptor independent LTP emerged slowly during the next 10–15 min before stabilizing at a potentiated level relative to the pretetan baseline.

Initially, the PPF ratio showed a large, posttetanic decrease. This was followed by a steady recovery toward the baseline PPF ratio. This recovery appeared to occur in two phases: an initial rapid recovery during the first 1–2 min posttetanus followed by a slower recovery during the next 30 min (Fig. 8A2). While the PPF ratio was, on average, decreased at 30 min posttetanus, the magnitude of this effect was very small (average change of only ~5 ± 2%). Nonetheless, the PPF results suggest that for a period of time, lasting up to ~30 min posttetanus, there is an increase in the probability of glutamate release.

We previously showed that application of the metabotropic glutamate receptor (mGluR) antagonist (R,S)-α-methyl-4-carboxyphenylglycine (MCPG, 500 μM) prevented NMDA receptor independent LTP (Little et al. 1995). MCPG therefore might be expected to also prevent the tetanus associated changes in PPF ratio. This possibility was examined by repeating the PPF experiment in the presence of both NMDA and mGluR receptor antagonists.

Consistent with our prior report, MCPG inhibited NMDA receptor independent LTP (Fig. 8C1). EPSPs (measured 25–30 min posttetanus) were increased by a mean of 31 ± 6% after tetanization in APV but were increased by only 7 ± 4% after tetanization in APV + MCPG (P < 0.01). In addition, MCPG altered the immediate posttetanic change in EPSP slope, which increased by 41 ± 8% after tetanization in APV but decreased by 35 ± 5% after tetanization in APV + MCPG (P < 0.002). MCPG also altered the immediate posttetanic decrease in PPF ratio (~35 ± 5% in APV; ~26 ± 9% in APV + MCPG), but this effect was not significant (P > 0.30). Slices tetanized in APV + MCPG showed a small, persistent decrease in PPF ratio, which averaged ~2 ± 3% (measured 25–30 min posttetanic) This change in PPF ratio was not significantly different from that seen in slices tetanized in APV (P > 0.40). Overall, MCPG significantly decreased both PTP and LTP but failed to alter the changes in PPF at these two times.

Because the posttetanus changes in PPF were steady, but not completely, reversing during the first 30 min posttetanus, PPF was measured at later time points in a subset (7) of the 12 slices shown in Fig. 8. When the observation period was extended (Fig. 9), PPF continued to recover toward the baseline level. Results from one of these seven slices are given in Fig. 9 (A and B). Although the LTP was maintained at a constant level, the PPF ratio eventually recovered to its pretetan baseline. This finding was mirrored in the other slices, which showed equivalent degrees of LTP at both
Although the results were not altered if measurements of AMPA-EPSP slope and NMDA-EPSP area were used instead.

Evident only in the AMPA-EPSPs of the tetanized pathways. Data shown here are from measurements of EPSP amplitudes, after tetanization. NMDA-receptor–mediated component was determined by subtraction at the same 2 time points. LTP was of 6 slices was examined using this procedure. AMPA-EPSPs were isolated and measured in APV before and at 35 ± 40 min required to washout APV, it was not possible to determine if the NMDA-receptor–mediated component showed an immediate posttetanic enhancement but some degree of potentiation of the late component usually was seen during the washout period.

Delivered at 0 min, resulting in normal posttetanic and long-term potentiation of the early EPSP in the tetanized pathway. APV (100 μM) was added to the ACSF perfusing the slice. Isolated AMPA-receptor–mediated EPSPs were measured in APV before (2) and 35–40 min after (4) tetanization. Averaged AMPA-EPSPs from these 2 time periods are shown in B2. NMDA-receptor–mediated EPSPs (NMDA-EPSPs) were determined by subtracting the AMPA-EPSPs recorded in APV (2 and 4) from the composite EPSPs recorded before the APV application (1 and 3). Pretetanus (1 and 2) and posttetanus (3 and 4) NMDA-EPSPs determined by this method are shown in B2. Tetanic stimulation was delivered at 0 min, resulting in normal posttetanic and long-term potentiation of the early EPSP in the tetanized pathway. Washout of APV began after tetanization to allow posttetanus measurement of the NMDA-EPSP. Because of the time required to washout APV, it was not possible to determine if the NMDA-receptor–mediated component showed an immediate posttetanic enhancement but some degree of potentiation of the late component usually was seen during the washout period.

APV was reapplied to the slice, beginning 30 min after tetanization, to allow measurement of the isolated AMPA-receptor–mediated component of the EPSP and for determination of the NMDA-receptor component by subtraction. C: a total of 6 slices was examined using this procedure. AMPA-EPSPs were isolated and measured in APV before and at 35–40 min after tetanization. NMDA-receptor–mediated component was determined by subtraction at the same 2 time points. LTP was evident only in the AMPA-EPSPs of the tetanized pathways. Data shown here are from measurements of EPSP amplitudes, although the results were not altered if measurements of AMPA-EPSP slope and NMDA-EPSP area were used instead.

25–30 min and 35–40 min posttetanus times (Fig. 9C); however, PPF was reduced at the earlier, but not the later, of these two time periods (Fig. 9D). These observations demonstrate that LTP maintenance at posttetanus times >30 min occurred without detectable changes in PPF.

A final series of experiments was performed to ensure that data obtained in the previous experiments was not contaminated by NMDA receptor dependent synaptic plasticity. In these experiments, LTP was studied in the presence of: APV plus a competitive antagonist of the glycine site on the NMDA receptor (DCK) or APV plus a noncompetitive NMDA receptor antagonist, MK-801. Tetanizing slices in the presence of pharmacologically distinct types of NMDA receptor antagonists should safeguard against residual (APV resistant) activation of NMDA receptors by glutamate during tetanic stimulation. However, before conducting these final LTP experiment, it was necessary to determine an effective concentration range for DCK and the number of stimuli needed in the presence of MK-801 because the block by MK-801 is use dependent (Huettner and Bean 1988).

Isolated NMDA-receptor–mediated EPSPs were studied by perfusing slices with magnesium-free ACSF and applying bicuculline (10 μM; to block GABA_A receptors) and DNQX (15 μM; to block AMPA receptors). DCK then was added.
NMDA RECEPTOR INDEPENDENT LTP

FIG. 8. NMDA receptor independent LTP was associated with a temporary decrease in paired-pulse facilitation (PPF). Posttetanic potentiation and LTP, but not the corresponding changes in PPF, were blocked by (R,S)-a-methyl-4-carboxyphe-nylglycine (MCPG). A1: changes in field EPSP slope (first EPSP of the pair) in response to 200-Hz tetanization in the presence of APV. There was large posttetanic increase in EPSP slope, which decayed during the initial 2 min. This was followed by a slowly developing potentiation that was maintained without decrement from 10 to 30 min posttetanus. A2: PPF ratio (EPSP2/EPSP1) showed a large decrease immediately after tetanization. However, the PPF ratio, after a rapid but partial initial recovery, steadily returned toward the baseline level. This pattern of posttetanus changes contrasts with the changes in EPSP slope, which persisted without decrement during the latter two-thirds of the posttetanus period. B: sample EPSPs from 1 slice tetanized in APV. Responses were evoked immediately before (1) and 15 s (2) and 30 min (3) after tetanic stimulation. At the 15-s posttetanus time, the slope of the 1st EPSP was increased, whereas there was no change in the slope of the 2nd EPSP, leading to a large decrease in the PPF ratio (in this slice, from 1.83 to 1.28). Over time, the 2nd EPSP increased so that the PPF ratio by 30 min posttetanus (1.90) was no longer reduced. C1: unlike slices tetanized in APV alone, slices tetanized in APV and MCPG did not show the immediate posttetanic increase in EPSP slope nor did these slices show LTP. The only consistent change observed was a transient depression that recovered within ~5 min. C2: despite the lack of potentiation, slices tetanized in APV and MCPG did show a large posttetanic decrease in PPF. D: sample EPSPs from 1 slice tetanized in APV and MCPG. Responses were evoked immediately before (1) and 15 s (2) and 30 min (3) after tetanic stimulation. At the 15-s posttetanus time, the slope of both EPSPs was decreased, but there was a proportionally larger decrease in the 2nd EPSP of the pair (50% decrease) compared with the 1st (21% decrease), causing the PPF ratio to decrease from 1.95 to 1.24. At 30 min posttetanus, EPSP slopes and PPF ratio had returned to pretetanus levels.

Concentrations of DCK up to 10 μM did not reduce the NMDA-receptor-mediated EPSP. Concentrations of DCK were applied to slices at a clearly effective concentration. If any portion of the NMDA-receptor-mediated EPSP was blocked. Concentrations greater than 100 μM failed to completely inhibit the response. Concentrations greater than 100 μM were not tested because a nonspecific depression of AMPA-receptor-mediated responses became apparent at the 100 μM concentration (see Fig. 10C). For comparison, addition of 50 μM APV to the ACSF (previously determined to be a maximally effective concentration for antagonizing NMDA-receptor-mediated EPSPs) (Grover and Teyler 1990, 1994) completely suppressed the NMDA-receptor-mediated EPSPs (Fig. 10A and B).

Although DCK could not be used at a concentration producing 100% block of NMDA receptors, it was applied to slices at a clearly effective concentration. If any portion of the potentiation obtained in the previous experiments was contaminated by residual NMDA receptor activation, occurring despite the presence of APV, then the LTP should be reduced by application of both APV and DCK. In this case, less LTP would be observed in slices tetanized in APV + DCK compared with APV alone. Results from this experiment are summarized in Figs. 10C and 11C. There was no difference in magnitude of LTP (measured at 25–30 min posttetanus) induced by tetanization in APV compared with tetanization in APV + DCK. However, there was an apparent difference in LTP time course between these two groups, with the slices tetanized in APV + DCK requiring a longer posttetanus time to reach the maximum level of potentiation. This difference in time course may reflect a nonspecific depressant effect of DCK on EPSPs. This depressant effect was observed during the baseline recording period, where...
DCK application reduced baseline EPSP slopes by 12 ± 8%. Recovery from the DCK-induced depression presumably occurred during the posttetanus period as DCK washed off the slices. Because the washout of the DCK coincided with the time when NMDA receptor independent LTP was developing and because EPSPs were normalized relative to the predrug baseline period, it is not possible to determine whether the difference in LTP time course is real or is instead an artifact resulting from a slow recovery from the depression of DCK. Regardless, the final level of LTP was identical whether slices were tetanized in APV alone or APV + DCK, confirming that NMDA receptors do not contribute to the final level of potentiation obtained.

The noncompetitive NMDA antagonist MK-801 also was tested for any additional inhibitory effect against the LTP obtained by tetanizing slices in APV. Because the block by MK-801 is use dependent, it was anticipated that a number of pretetanus stimuli would be required, in the presence of pretetanus stimuli would be required, in the presence of

FIG. 9. Changes in PPF during NMDA receptor independent LTP eventually reverse. Time course of EPSP and PPF changes was followed for ≥40 min after tetanization in 7 slices. Data from 1 of these slices is shown in A (EPSP slope) and B (PPF ratio). Posttetanic decrease in PPF eventually recovered, although the potentiation of EPSP was maintained without decrement. Notice that in this slice, despite the large posttetanic decrease in PPF, there was no corresponding posttetanic potentiation of the EPSP. C: mean changes in EPSP slope for all 7 slices. On average, these slices showed a posttetanic increase in EPSP slope (15 s posttetanic measurement). Magnitude of NMDA receptor independent LTP observed at the 25–30 min posttetanus and the 35–40 min posttetanus times was identical. D: all of the 7 slices showed a large decrease in PPF ratio at the 15-s posttetanus time point. Posttetanic decrease in PPF had largely, but not completely, recovered by the 25–30 min posttetanus measurement. By 35–40 min posttetanus, the PPF ratio was not distinguishable from the pretetanus measurement.

FIG. 10. NMDA receptor mediated synaptic potentials were inhibited by the glycine site antagonist 5,7-dichlorokynurenic acid (DCK), but slices tetanized in both DCK and APV showed the same magnitude of LTP as slices tetanized in APV alone. A: slices were perfused with a Mg²⁺-free medium containing 10 μM bicuculline and 15 μM DNQX. Under these conditions, single stimuli evoked large NMDA receptor mediated postsynaptic potentials. Responses from 1 slice are shown here. When applied at low concentrations (≤10 μM), DCK had no effect on the NMDA receptor mediated EPSPs, whereas higher concentrations produced a partial block. At the highest concentration examined, 100 μM, the inhibition by DCK was substantial but was not still complete, as shown by the subsequent application of 50 μM APV. B: DCK was applied to a total of 4 slices. ■, percentage inhibition obtained with 1–100 μM DCK; □, inhibition obtained with 50 μM APV (mean ±1 SE). C: final level of LTP was similar regardless of whether slices were tetanized in APV (●; same data shown in Fig. 8A1) or APV + DCK (▲). The combination of APV and DCK depressed EPSPs during the baseline period, suggesting that the high concentration of DCK used may have partially antagonized AMPA receptors because no depression was seen when APV alone was applied.
of two per minute. Results are shown in Fig. 11

NMDA-EPSPs were isolated as described earlier. Then MK-

APV

MK-801 was added to the ACSF with stimuli delivered at a rate of two per minute. Results are shown in Fig. 11A. Between 5 and 10 min (10–20 stimuli) was required before the NMDA-

EPSPs began to decline. This lag may indicate a slow penetration of the drug into the tissue because this time is longer than the 2–3 min required for APV to begin blocking the response (see Fig. 7B1). NMDA-EPSPs were reduced by ~50% within 15 min after addition of the drug to the ACSF (30 stimuli). The drug effect was asymptotic, although not complete, after 30 min (60 stimuli).

A total of 10 slices were tested for LTP in the presence of APV (100 μM) + MK-801 (20 μM). In five of these slices, a second (nontetanized) pathway also was tested as a control for any nonspecific changes. The results are shown in Fig. 11B. LTP (average increase of 53 ± 16% in EPSP slope) was obtained in the slices tetanized in APV + MK-801. There was no difference in LTP magnitude between the five slices where a second, control pathway was tested (55 ± 15%) and the other five slices where a second pathway was not tested (51 ± 19%). Although the LTP obtained in the presence of APV + MK-801 was on average greater than the LTP obtained in APV alone, this difference was not significant (P > 0.15). The slices tetanized in APV + MK-801 were given 94 ± 40 (range 21–454) stimuli in MK-801 before tetanization. There was no correlation between the number of pretetan- 

stimuli given in MK-801 and the magnitude of LTP obtained (r = 0.16, P > 0.55).

As was the case for slices tetanized in APV alone (Fig. 8A), slices tetanized in APV + MK-801 showed both posttetan- 

tic (1st response posttetanus, P < 0.05) and long-term decreases in PPF (25–30 min post, P < 0.01). The changes in EPSP slope and PPF observed after tetanization in APV + MK-801 were restricted to the tetanized pathway (Fig. 11B). No changes were observed in control pathway EPSPs, where the mean change, measured 25–30 min post, was −1 ± 8% (P < 0.05 compared with the tetanized pathway). Likewise, there were no posttetan- 

cus changes in PPF in the control pathway (P > 0.15 for the 1st posttetanus trial, and P > 0.15 for the 25- to 30-min posttetanus period).

Figure 11C summarized the effects on LTP of the NMDA

FIG. 11. MK-801 inhibited NMDA receptor me-
diated EPSPs but did not inhibit LTP obtained by tetanizing slices in APV. A: NMDA-EPSPs were ob-
tained by perfusing slices with nominally Mg2+-free ACSF to which bicuculline (10 μM) and DNQX (15 μM) were added. Stimuli were delivered every 30 s. Fifteen minutes after the addition of MK-801 to the ACSF (30 stimuli), NMDA-EPSPs were reduced by ~50%. MK-801 block reached a maximum after ~30 min (60 stimuli). B: a total of 10 slices were tetanized in APV + MK-801. In 5 of these slices, a 2nd, control (nontetanized) pathway was tested. LTP was obtained after 200-Hz tetanization in APV + MK-801 (top). No change in EPSP slope was seen in the control pathways ( ). Tetanized pathways showed a posttetan- 

cus decrease in PPF (middle) that was similar to that seen in slices tetanized in APV alone (see Fig. 8A2). No change in PPF was seen in the control pathways (bottom). C: summary of LTP results for slices tetanized in APV (data taken from Fig. 8A), APV + DCK (data from Fig. 10C), APV + MK-

C801 (data from this figure, B), and APV + MC 

singularly affected LTP, whereas MCPG did.

FIG. 12. An increase in axon excitability does not account for the increase in EPSP slope observed during NMDA receptor independent LTP. A: averaged pretetan- 

tic response and a single response recorded 15 s after 200-Hz tetanization. Tetanization increased the latency to the peak of the fiber volley and slightly decreased the amplitude of the fiber volley. B: fiber volley recovered quickly after tetanization. C: at 30 min posttetan-

us, the fiber volley remained at the pretetan- 

cus amplitude, whereas the EPSP was potentiated. After tetrodotoxin (TTX) was added to the ACSF, both the presynaptic fiber volley and the EPSP were blocked, leaving only the stimulus artifact (which has been partially blanked). D: fiber volley data from a subset of the slices tetanized in APV, APV + DCK, and APV + MK-801. In many slices, the fiber volley was either too small to measure or could not be distinguished from the stimulus artifact; these slices were excluded from this analysis. Fiber volley amplitude was normalized (relative to the mean of the pretetan- 

cus baseline) and plotted over time. There were no differences among the slices tetanized in APV alone, APV + DCK, and APV + MK-801, so the data were combined. Tetanization was at 0 min. Fiber volley amplitude was reduced slightly during the first few minutes posttetan- 

us but returned to the pretetan- 

cus level for the remainder of the recording period.
and metabotropic glutamate receptor antagonists. Neither DCK ($P > 0.85$) nor MK-801 ($P > 0.15$) significantly affected the magnitude of LTP, whereas MCPG ($P < 0.05$) significantly reduced the LTP.

In addition to measuring EPSP slope, the presynaptic fiber volley was measured in a subset of the slices shown in Figs. 8, 10, and 11. This subset consisted of slices where a fiber volley could be clearly discerned: 9 of the 12 slices tetanized in APV, 3 of the 5 slices tetanized in APV + DCK, and 2 of the 10 slices tetanized in APV + MK-801. As shown in Fig. 12, fiber volley amplitude was not affected by tetanic stimulation with the exception of a transient and small decrease seen in some of the slices. When present, this depression of the fiber volley reversed within the first 30–60 s posttetanus. In addition, in most of the slices examined, there was a posttetanic increase in the latency to the peak of the fiber volley (Fig. 12A). This apparent slowing of axonal conduction recovered rapidly (Fig. 12B). The lack of long-term changes in the fiber volley rules out the possibility that a persistent increase in presynaptic axon excitability underlies maintenance of NMDA receptor independent LTP.

**Discussion**

**Postsynaptic induction of NMDA receptor independent LTP**

Evidence presented here favors a postsynaptic site for the induction of NMDA receptor independent LTP: LTP was prevented by direct hyperpolarization of postsynaptic neurons, LTP was blocked when postsynaptic depolarization was reduced by the AMPA receptor antagonist DNQX, and loading postsynaptic neurons with QX-314 also abolished the LTP. These observations are consistent with previous experiments, which demonstrated a requirement for dihydropyridine-sensitive, voltage-dependent calcium channels (Cavus and Teyler 1996; Grover and Teyler 1990), which have a postsynaptic distribution in area CA1 (Jones and Heinemann 1987; Westenbroek et al. 1990). In addition, NMDA receptor independent LTP was blocked by loading postsynaptic neurons with BAPTA (Grover and Teyler 1990). Taken as a whole, these observations indicate that induction of NMDA receptor independent LTP requires voltage dependent influx of calcium into CA1 pyramidal neurons through dihydropyridine-sensitive calcium channels. Moreover, the gating of these calcium channels appears to require the firing of action potentials in postsynaptic neurons because both postsynaptic action potential firing and NMDA receptor independent LTP were blocked by QX-314. This suggested role for postsynaptic action potential firing is consistent with reports demonstrating that action potential waveforms are effective stimuli for the gating of high-threshold, voltage-dependent calcium channels (McCobb and Bean 1991; Scroggs and Fox 1992) and with recent data demonstrating a direct role for postsynaptic action potential firing in synaptic potentiation (Magee and Johnston 1997; Markham et al. 1997).

The potential involvement of postsynaptic action potentials in the induction of NMDA receptor independent LTP will require confirmation using other methods, because it is possible that QX-314 had other effects on the cells studied, in addition to the block of voltage-gated sodium channels. For instance, QX-314 inhibits some transmitter gated (Alreja and Aghajanian 1994; Andrade 1991; Nathan et al. 1990; Otis et al. 1993) and voltage gated (Oda et al. 1992; Perkins and Wong 1995) potassium channels. However, most of these channels are blocked by intracellular cesium (Gahwiler and Brown 1985; Otis et al. 1993). Because cesium was the major cation in the intracellular solution for all whole cell experiments and NMDA receptor independent LTP could easily be induced in cesium loaded cells, the inhibition of potassium channels by QX-314 is unlikely to explain the loss of LTP in QX-314 loaded cells. There are still other potential effects of QX-314, including inhibition of voltage gated calcium channels (Talbot and Sayer 1996) [although calcium spikes can still be evoked in QX-314 loaded hippocampal pyramidal neurons (Connors and Prince 1982; Nunez and Buno 1992; unpublished observations)] and inhibition of ryanodine receptors (Martin et al. 1993; Shoshan-Barmatz and Zschut 1993), which cannot at present be excluded. Regardless of the cause, the fact that postsynaptic QX-314 blocked NMDA receptor independent LTP supports the conclusion that induction of this form of LTP occurs in the postsynaptic neuron.

**Postsynaptic induction of PTP?**

Observations reported here suggest that the short duration, PTP-like effect, is dependent on tetanus-induced postsynaptic depolarization and action potential firing. These observations seem at odds with many previous studies indicating that PTP is a presynaptic phenomenon (Zucker 1989). It is possible, although, that the PTP observed in this study is not a ‘‘true’’ PTP. The PTP-like, short-term enhancement shown here may be a transient form of postsynaptic enhancement, which, coincidentally, has a time course similar to the presynaptic PTP that has been observed at many types of synapses. An additional possibility that should be considered is that the PTP-like enhancement observed here, while induced in the postsynaptic neurons, is expressed by enhanced release of glutamate from the presynaptic terminals. This possibility, which is considered in more detail later, would require the generation of a retrograde messenger within the postsynaptic neurons which would then alter the release of glutamate from the presynaptic terminals. Several compounds have been suggested as retrograde messengers, which can link postsynaptic NMDA receptor function to changes in presynaptic function (Bohme et al. 1991; O’Dell et al. 1991; Schumann and Madison 1992; Williams et al. 1989; Zhuo et al. 1993). Because generation of these retrograde messengers requires elevation of postsynaptic calcium concentration, one or more of these messengers could have been generated in the cells studied here by the influx of calcium through postsynaptic voltage-gated calcium channels.

**Evidence for postsynaptic expression of NMDA receptor independent LTP**

Evidence reported here favors a postsynaptic locus for the long term maintenance of NMDA receptor independent LTP. Only the AMPA receptors of the postsynaptic neurons participated in LTP maintenance; there was no evidence for persis-
tent enhancement of NMDA-receptor–mediated synaptic potentials. In addition, while there were posttetanic changes in PPF, these changes lasted for only ~30 min, an insufficient time to explain the persistent potentiation of the EPSPs. Moreover, the changes in PPF that did occur could not be related specifically to the LTP because similar changes in PPF were observed in the presence of MCPG, which blocked the LTP.

It is not surprising that some changes in PPF occurred during these experiments. For instance, the repeated, high-frequency stimulation that was used to induce NMDA receptor independent LTP might have altered any of several aspects of presynaptic function (Augustine et al. 1994; Green-gard et al. 1993; Verhage et al. 1994), for instance, by depleting the pool of readily releasable vesicles or altering the processes of vesicle mobilization or docking. Presynaptic changes like these could have contributed to the changes in PPF that were observed. One potential presynaptic change, an increase in axon excitability, can be ruled out, because there was no persistent change in the amplitude or time course of the presynaptic fiber volley. *Most importantly*, the changes in presynaptic function suggested by the changes in PPF did not last long enough to contribute to the persistent maintenance of NMDA receptor independent LTP.

Although the evidence presented here favors postsynaptic maintenance of NMDA receptor independent LTP, no such claim can be made for the NMDA receptor independent PTP-like enhancement. While PTP induction was sensitive to postsynaptic manipulations (hyperpolarization, QX-314, antagonism of AMPA receptors), the site of expression of the PTP is not clear. The time course of the PTP-like enhancement closely matched the rapid phase of recovery of PPF seen during the first 1–2 min posttetanus, but this result is not decisive because slices which did not show PTP— including slices tetanized in APV + MCPG, and some of the slices tetanized in APV alone—still could show large posttetanic decreases in PPF. It would be interesting to know how NMDA-receptor–mediated EPSPs also showed a PTP-like enhancement, but the NMDA receptor antagonist used (APV) required 15–20 min to wash out, a much longer period than the duration of the PTP. If the PTP-like enhancement was mediated by a presynaptic change, then some sort of retrograde signal would be required because the PTP was sensitive to the postsynaptic manipulations employed in this paper.

In some slices (for example, Fig. 7B1), an intermediate duration (lasting up to ~30 min) enhancement of the late EPSP, which was largely mediated by NMDA receptors, was observed. At present, there is no reason to favor either a pre- or postsynaptic explanation for this effect. Because enhancement of the late EPSP overlapped with the time course for the slow phase of PPF recovery, it is tempting to speculate that the enhancement was caused by a temporary increase in release of glutamate from the presynaptic terminals that was sensed by both NMDA and AMPA receptors. But postsynaptic alternatives for the temporary increase in the late EPSP can be offered. One possibility is a postsynaptic modulation of NMDA receptor function (Ben-Ari et al. 1992) by a mGluR-mediated signaling pathway that culmi-nates in phosphorylation and enhanced function of NMDA receptors. This possibility could be tested by repeating the experiments of Fig. 7 in the presence of an mGluR antagonist, such as MCPG.

Methodological issues and site of persistent change in NMDA receptor independent LTP

When NMDA- and AMPA-receptor–mediated EPSPs were isolated, it was found that AMPA receptor, but not NMDA receptor, mediated EPSPs showed LTP. This can be taken as evidence against certain presynaptic mechanisms (such as increased glutamate release) only if it is assumed that NMDA receptors were not saturated by the pretetanus level of transmitter release and that NMDA-receptor–mediated EPSPs therefore could be increased if glutamate release was enhanced. Likewise, changes in PPF may indicate a presynaptic alteration, but it is possible for postsynaptic alterations to change PPF (Wang and Kelly 1996). These limitations were overcome, to at least some extent, by the use of two dissimilar methods to assess the locus of change.

For example, the observed pattern of changes—a long-lasting increase in AMPA-receptor–mediated EPSPs without a corresponding enhancement of NMDA mediated EPSPs—could be explained by a presynaptic change if NMDA receptors were saturated before tetanization, but AMPA receptors weren’t. In this case, even if there was a persistent increase in probability of glutamate release, AMPA-receptor–mediated EPSPs still would appear to be selectively increased. However, this type of presynaptic change—an increase in the probability of release—should be accompanied by a decrease in PPF (Dunwiddie and Haas 1985; Hess et al. 1987; Otmakhow et al. 1993). While such a change in PPF might indicate a postsynaptic alteration (Wang and Kelly 1996) in addition to or instead of a presynaptic alteration, the finding that PPF eventually returned to the baseline level argues against both the postsynaptic change described by Wang and Kelly (1996) and against an increased probability of glutamate release as mechanisms for maintaining NMDA receptor independent LTP.

There is a type of presynaptic change that could occur without any change in PPF: the formation of new sites for transmitter release that have the same probability of release as the original sites. This type of presynaptic alteration could increase the total quantity of glutamate released in response to a single presynaptic action potential but would not be accompanied by a change in PPF. For this mechanism to reproduce the observed pattern of results, postsynaptic NMDA receptors, but not AMPA receptors, would have to be saturated by the amount of glutamate released before tetanization.

Two candidate mechanisms, one postsynaptic (enhanced AMPA receptor function) and one presynaptic (formation of new release sites), are diagrammed schematically in Fig. 13. At present, the postsynaptic explanation seems preferable. The presynaptic mechanism depends on the assumption that new release sites would have the same probability of release as previously existing sites, otherwise there should have been persistent changes in PPF. In addition, the post-synaptic mechanism has the virtue of greater simplicity because a retrograde signal would be required to couple the postsynaptic induction of NMDA receptor independent LTP to any presynaptic mechanism for expression. A more certain
FIG. 13. Two models that can explain the selective increase in AMPA-receptor–mediated EPSPs during NMDA receptor independent LTP. A: NMDA receptor independent LTP is caused by an increase in the number of functional AMPA receptors in the postsynaptic membrane. These could be new receptors or preexisting receptors the function of which has been enhanced. There is no change in the number of functional NMDA receptors. B: NMDA receptor independent LTP occurs through an increase in the number of functional release sites without any change in the postsynaptic membrane. New release sites have the same probability of vesicle release as the original sites, and there is no interaction between the original and the new sites, allowing the degree of vesicle release to remain constant. AMPA receptors were not saturated by the level of glutamate release before LTP but NMDA receptors were.

choice between these two possibilities could be made if the relative degrees of AMPA and NMDA receptor saturation were known. Unfortunately, this issue is both complicated and unresolved (Frerking and Wilson 1996).

**Potential roles for mGluRs in PTP and LTP**

Data presented here (Fig. 8) and in a previous experiment (Little et al. 1995) indicate that mGluRs are required for the induction of both NMDA receptor independent LTP and the PTP-like enhancement. However, activation of mGluRs alone cannot be sufficient to trigger either the LTP or the PTP-like enhancement. First, mGluR activation should have been unaffected during experiments in which cells were hyperpolarized or loaded with QX-314 or in which AMPA receptors were blocked, yet LTP did not occur and the PTP-like effect was either reduced or absent. Second, previous experiments have shown that postsynaptic, voltage-dependent calcium channels are required for the LTP. Thus although activation of mGluRs is necessary, this activation is not sufficient for induction of either NMDA receptor independent LTP or the PTP-like effect.

Although mGluRs appear to be needed, many questions regarding the specific function of the mGluRs remain. First, the required mGluRs could be located in either presynaptic terminals or in the postsynaptic membranes because MCPG-sensitive mGluRs (Watkins and Collingridge 1994) are expressed by both CA3 and CA1 pyramidal neurons (Fotuhi et al. 1994; Shigemoto et al. 1992). Second, postsynaptic mGluRs, by generating a second messenger with limited ability to diffuse within the cell, might confer the input specificity of the NMDA receptor independent LTP (Figs. 1, 6, and 7) (Grover and Teyler 1992). If this second messenger is calcium (Nakanishi 1992; Schoepf 1994), then mGluR activation also would contribute to the generation of the postsynaptic calcium signal that triggers induction of NMDA receptor independent LTP. On the other hand, presynaptic mGluR activation during tetanization might enhance the release of glutamate and, in this way, indirectly increase the level of postsynaptic depolarization so that LTP is induced. Although presynaptic mGluR activation typically inhibits glutamate release (Schoepf 1994; Watkins and Collingridge 1994), a mechanism through which mGluR activation facilitates glutamate release has been described (Herrero et al. 1992; Vásquez et al. 1994). An mGluR-mediated facilitation of glutamate release could explain the MCPG sensitivity of the PTP-like effect. Of course, none of these suggested pre- and postsynaptic mGluR functions are exclusive and all may contribute to induction of NMDA receptor independent LTP.

**Conclusions**

The evidence for postsynaptic induction of NMDA receptor independent LTP at the Schaffer collateral/CA1 synapse is strong. The maintenance of NMDA receptor independent LTP in area CA1 is also likely to be postsynaptic, although this interpretation could be disputed because at least one presynaptic mechanism could explain the results reported here. The features of NMDA receptor independent LTP in the CA1 area described in this paper contrast with features of the NMDA receptor independent LTP found at mossy fiber/CA3 synapses, which is induced presynaptically ([Zalutsky and Nicoll 1990] but may also contain a postsynaptically induced component (Jaffe and Johnston 1990; Urban and Barrionuevo 1996) and is accompanied by a sustained decrease in PPF (Staubli et al. 1990; Zalutsky and Nicoll 1990).

Identification of the signaling pathway that links induction to maintenance may help to resolve this issue if specific components of this pathway can be localized to either presynaptic or postsynaptic elements. Likewise, more certain evidence for or against postsynaptic expression of the LTP would guide investigations into the signaling pathway, for
instance, by indicating whether or not a retrograde messenger is required.

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