Distinct LTP Induction Mechanisms: Contribution of NMDA Receptors and Voltage-Dependent Calcium Channels

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SUMMARY AND CONCLUSIONS

1. Our results indicate that there are two distinct components of long-term potentiation (LTP) induced by the K+ channel blocker tetraethylammonium chloride (TEA) at synapses of hippocampal CA1 pyramidal neurons. Preincubation of hippocampal slices in the N-methyl-D-aspartate (NMDA) receptor antagonist D,L-2-amino-5-phosphononovalerate (D,L-APV, 50 μM), reduced the magnitude of TEA LTP. In addition, the L-type voltage-dependent Ca2+ channel (VDCC) antagonist nifedipine (10 μM) attenuated TEA LTP. Only the combined application of D,L-APV plus nifedipine blocked the induction of TEA LTP.

2. Occlusion experiments demonstrated that saturation of VDCC-dependent TEA LTP did not reduce or occlude NMDA-receptor-dependent TEA LTP. These results indicate that the mechanisms underlying VDCC and NMDA receptor components of TEA LTP are different and do not share a common saturable mechanism.

3. TEA LTP was strictly dependent on NMDA receptor activity in slices with CA3-CA1 connections severed (isolated CA1 slices). In contrast to results obtained in slices with intact CA3-CA1 connections, the NMDA receptor antagonists APV (50 μM) or MK-801 dizocilpine (10 μM) completely blocked TEA LTP in isolated CA1. Consistent with this observation, the properties of TEA LTP in isolated CA1 were very similar to other types of NMDA-receptor-dependent plasticity such as tetanus-induced LTP; TEA LTP required presynaptic stimulation, displayed pathway specificity, and was occluded by tetanus-induced LTP.

4. A variety of conditions were tested to facilitate the induction of VDCC-dependent TEA LTP in isolated CA1 slices. High-frequency stimulation (80-ms pulses at 25 Hz) to Schaffer collaterals or CA1 axons (i.e., antidromic stimulation) in conjunction with TEA application induced LTP in the presence of APV (100–200 μM). This potentiation was completely blocked by the combined application of APV (100–200 μM) and nifedipine (50 μM), indicating that induction of VDCC-dependent TEA LTP is frequency dependent, similar to other types of VDCC-dependent plasticity.

5. Using a 25-Hz stimulation protocol in two pathway experiments, we observed that induction of VDCC-dependent TEA LTP was not pathway specific, which contrasts with NMDA-receptor-dependent TEA LTP and suggests that the route of Ca2+ entry during LTP induction can determine synapse specificity.

6. These results demonstrate that different Ca2+-dependent processes may be activated by VDCCs and NMDA receptors that induce long-lasting potentiation with different properties.

INTRODUCTION

Long-term potentiation (LTP) of synapses on hippocampal CA1 neurons is induced by a high frequency tetanus (100 Hz) and requires increases in postsynaptic Ca2+ (Lynch et al. 1983; Malenka et al. 1988) mediated by the activation of N-methyl-D-aspartate (NMDA) receptors (Collingridge et al. 1983, 1988). Other forms of long-lasting potentiation in CA1 have been demonstrated that are also Ca2+ dependent but do not appear to require NMDA receptor activity. NMDA receptor-independent LTP can be induced with 200-Hz stimulation, which requires voltage-dependent Ca2+ channel (VDCC) activation (Grover and Teyler 1990). The K+ channel blocker tetraethylammonium chloride (TEA) induces long-lasting potentiation (TEA LTP) that is believed to be independent of NMDA receptor activation (Aniksztejn and Ben-Ari 1991). TEA LTP is blocked by L-type VDCC antagonists or the postsynaptic injection of the Ca2+ chelator bis-(o-aminophenoxy)-N,N,N',N'-tetraacetic acid (Aniksztejn and Ben-Ari 1991; Huang and Malenka 1993). In contrast, VDCCs do not appear to contribute to tetanus-induced LTP (Huang and Malenka 1993; Kullmann et al. 1992; Taube and Schwartzkroin 1986). Together, these results indicate that there are two routes of Ca2+ influx that can induce LTP, one via NMDA receptors and the other through VDCCs. These observations generate many questions. For example, are the cellular mechanisms activated by these different Ca2+ routes distinct? What conditions facilitate the induction of LTP via NMDA receptors versus VDCCs? Dendritic spines have been hypothesized to localize NMDA-receptor-mediated Ca2+ increases and therefore to be responsible for the synapse specificity of tetanus-induced LTP (Zador et al. 1990). In contrast, VDCCs have been shown to be localized on the soma and proximal dendrites of hippocampal neurons and mediate Ca2+ increases in the dendritic shaft (Müller and Connor 1991; Westenbroek et al. 1990). Therefore potentiation that relies on Ca2+ influx through VDCCs may not display synapse specificity. To test this hypothesis, we set out to determine whether LTP induced by Ca2+ influx through VDCCs displayed synapse or pathway specificity. These experiments could determine whether the route of Ca2+ entry during LTP induction is responsible for synapse specificity or whether other mechanisms exist that can detect coincident pre- and postsynaptic activity. In addition, analysis of NMDA- and VDCC-dependent LTP should provide important information about the compartmentation of postsynaptic Ca2+ and the convergence or divergence of Ca2+-activated processes important for LTP induction.

We have utilized TEA LTP to investigate potentiation mechanisms that rely primarily on the activation of VDCCs versus NMDA receptors. Using this model, we performed occlusion experiments to examine the similarities between...
LTP mechanisms activated by Ca\(^{2+}\) influx through VDCCs and/or NMDA receptors. We investigated induction conditions that favor VDCC-dependent or NMDA-receptor-dependent potentiation. Finally, we examined the properties of each form of potentiation to determine their requirements for presynaptic stimulation and if they displayed pathway or synapse specificity.

**METHODS**

We prepared hippocampal slices (400 \(\mu\)m thick) as described (Cormier et al. 1993) from pentobarbital-sodium-anesthetized (50 mg/kg) adult (10–14 wk, 250–300 g) Harlan Sprague-Dawley rats. Hippocampi were dissected in ice-cold medium containing 10 mM MgCl\(_2\) and no added CaCl\(_2\), and 2 mM CaCl\(_2\) was added to the slice incubation buffer to 2 mM and the temperature of the medium was gradually warmed to 30°C over 30 min. Slices were then transferred to standard medium and incubated for \(\approx\)30–45 min before being transferred to a submersion recording chamber (31°C) and constantly perfused at 2 ml/min. Standard medium for electrophysiological recordings consisted of (in mM) 124 NaCl, 3 KCl, 1 MgCl\(_2\), 2 CaCl\(_2\), 2 NaI\(_4\)PO\(_4\), 26 NaHCO\(_3\), 10 dextrose, and 10 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid. pH 7.35, media were continuously gassed with 95% O\(_2\)-5% CO\(_2\). Changes in the standard medium are noted in the text and figure legends. All chemicals were purchased from Sigma. MK-801 dizocilpine was a generous gift from J. Aronowski (University of Texas Medical School, Houston, TX). A nifedipine stock (10 mM in dimethylsulfoxide) was made fresh daily, protected from light, and diluted 1:1000 immediately before use.

We obtained field potentials from stratum radiatum in area CA1 of hippocampal slices using 1- to 3-M\(\Omega\) recording electrodes filled with standard medium. Schaffer collaterals were stimulated at a rate of 0.05–0.1 Hz with tungsten monopolar (20–50 \(\mu\)m) stimulating electrodes (Frederick Haer, Brunswick, ME). Data were digitized on a Nicolet 410 oscilloscope and analyzed on a computer with custom software that computed excitatory postsynaptic potential (EPSP) slopes and amplitudes. Values of potentiation, reported in the text as means \(\pm\) SE, and figures were computed from average values between 55 and 60 min after TEA washout. Our criterion for TEA LTP was a \(\geq\)20% increase in EPSP slope (relative to baseline) that lasted 60 min after the washout of TEA. The data presented in all figures, except Figs. 3C and 5A, represent averaged results from all slices studied under that experimental condition. Independent t-tests were conducted on data from isolated CA1 slices. Paired and one sample t tests were used for two-way and occlusion experiments, respectively, utilizing a critical \(P\) value of 0.05. A one-way analysis of variance (ANOVA) and a subsequent Tukey multiple comparison test (critical \(P\) value of 0.05) were performed on data from intact slices.

**RESULTS**

Both VDCC and NMDA receptor activities contribute to TEA LTP in intact slices

Similar to previous reports (Aniksztejn and Ben Ari 1991; Huang and Malenka 1993), we observed that application of 25 mM TEA for 10 min to hippocampal slices induced a robust and sustained increase in synaptic transmission (72 \(\pm\) 8%, mean \(\pm\) SE; \(n=15\) of 16; Fig. 1A and D) as measured by the initial slope of EPSPs (Fig. 1A). Aniksztejn and Ben-Ari (1991) demonstrated that TEA application has only a transient effect (<30 min) on cell excitability and therefore the resulting potentiation is not due to residual TEA. We observed in our experiments that TEA rapidly washed out of slices (16 \(\pm\) 2 min, \(n=9\)), as measured by its effect on the amplitude and duration of the presynaptic fiber volley. The number of slices that exhibited TEA LTP under each experimental condition (\(n\)) is expressed as a ratio of the total number of slices tested for that condition. For example, in intact slices the number of slices that exhibited TEA LTP was 15 of 16 slices (i.e., \(n=15\) of 16; see above). We observed that TEA LTP was only partially attenuated by 10 \(\mu\)M nifedipine, an L-type VDCC antagonist (Tsien et al. 1988; 44 \(\pm\) 6%; \(n=11\) of 11; Fig. 1, B and D). Likewise, the NMDA receptor antagonist D,L-2-amino-5-phosphonovalerate (D,L-APV) (50 \(\mu\)M) reduced but did not block TEA LTP in intact slices (39 \(\pm\) 9%; \(n=6\) of 9; Fig. 1, B and D). An APV-resistant component of TEA LTP was still observed at higher concentrations of D,L-APV (200 \(\mu\)M; 27 \(\pm\) 7%; \(n=3\) of 4; data not shown). However, with the combined application of nifedipine and APV, only one of six slices exhibited significant potentiation (14 \(\pm\) 4%, Fig. 1C). A one-way ANOVA of the percent potentiation 1 h after TEA application revealed a significant difference among all conditions [\(F(3,38)=45.08, P<0.001\)]. A subsequent Tukey test revealed that APV, nifedipine alone, or APV plus nifedipine all significantly attenuated TEA LTP compared with control TEA LTP (\(P<0.001\)); moreover, APV plus nifedipine significantly reduced TEA LTP compared with the APV or nifedipine alone conditions (\(P<0.005\)). These results indicate that two routes of Ca\(^{2+}\) entry contribute to the induction of TEA LTP in hippocampal slices, one through VDCCs and a second via NMDA receptors, making TEA LTP an attractive model to test the convergence and/or independence of NMDA- and VDCC-dependent mechanisms.

**NMDA-receptor- and VDCC-dependent components of TEA LTP utilize distinct mechanisms**

Because the VDCC and NMDA receptor components of TEA LTP appear to be additive and display different time courses (Fig. 1, B and D), it is possible that different Ca\(^{2+}\)-dependent mechanisms are utilized by the two components. Occlusion experiments are frequently used to determine whether different forms of synaptic plasticity share common, saturable mechanisms (Cormier et al. 1993; Kauer et al. 1988; Kullmann et al. 1992; Muller et al. 1990). We performed occlusion experiments by first inducing the VDCC-dependent component of TEA LTP in the presence of D,L-APV (37 \(\pm\) 5%; \(n=13\) of 13; Fig. 2A). APV was then replaced with nifedipine (10 \(\mu\)M) in the perfusate and slices were then subjected to a second TEA application to induce the NMDA receptor component of TEA LTP. The second TEA application induced additional potentiation (51 \(\pm\) 14%; \(n=13\) of 13; \(P<0.002, 1\)-sample t-test), giving a total of 88 \(\pm\) 17% potentiation relative to initial EPSP slope values (Fig. 2A). This result indicates that the VDCC component of TEA LTP does not occlude the NMDA receptor component and that mechanisms underlying these components do not share a common, saturable process.
To ensure that the VDCC and NMDA receptor components of TEA LTP were saturated in occlusion experiments, we performed two controls. VDCC-dependent TEA LTP was induced in D,L-APV (38 ± 6%; n = 5 of 5; Fig. 2B). A second TEA application (60 min later) in APV induced no further potentiation (1 ± 5%; n = 0 of 5; P > 0.5), indicating that VDCC-dependent TEA LTP was saturated. The NMDA component of TEA LTP was also saturated with one TEA application in the presence of nifedipine (10 μM; 49 ± 1%; n = 4 of 4), as demonstrated by the fact that a second TEA application in nifedipine induced no additional potentiation (5 ± 2%; n = 0 of 4; P > 0.1; data not shown). These results demonstrated that the VDCC- and NMDA-receptor-dependent components of TEA LTP were saturated by a single induction protocol. Moreover, these occlusion experiments suggested that NMDA receptors and VDCCs can activate distinct cellular pathways that lead to potentiation and prompted additional experiments to characterize the properties of each and examine their similarities with tetanus-induced LTP.

**TEA LTP in isolated CA1 requires NMDA receptor activity**

The original studies of TEA LTP were performed in isolated CA1 slices (accomplished with a knife cut between CA3 and CA1 regions; Aniksztejn and Ben-Ari 1991), where TEA LTP was found to be largely dependent on VDCC activity. Therefore, in contrast to TEA LTP in intact slices, which displayed both NMDA and VDCC components, TEA LTP in isolated CA1 would appear ideal for elucidating mechanisms underlying VDCC-dependent TEA LTP. Although TEA induced a robust and sustained increase in synaptic transmission in isolated CA1 preparations (41 ± 8%; n = 7 of 9), it was attenuated but not blocked by nifedipine (20 ± 8%; n = 4 of 8; P < 0.05, independent t-test; Fig. 3A). These results indicated that although VDCC activity contributed to TEA LTP, a nifedipine-resistant component remained.

To examine the contribution of NMDA receptor activity in the induction of TEA LTP, we carried out experiments in isolated CA1 in the presence of the NMDA receptor antagonist D,L-APV (50 μM). Stable TEA LTP was obtained in only 8 of 34 slices incubated in D,L-APV, with an average potentiation of 9 ± 3% (data not shown). To avoid any nonspecific affects of the L-isomer of APV (Coleman and Miller 1988; Massey and Miller 1990), we conducted additional experiments in D-APV (25 μM, Fig. 3B). Before TEA application, 3 of 8 slices received tetanic stimulation (100 Hz for 1 s) to verify D-APV’s ability...
NMDA- AND VDCC-DEPENDENT TEA LTP

2.2. D,L-APV and nifedipine

![Graph](image)

**FIG. 2.** VDCC and NMDA receptor components of TEA LTP utilize distinct cellular mechanisms. A: before the 1st TEA application, tetanic stimulation to intact slices (+) was given to test the efficacy of APV applications. Induction of the VDCC component of TEA LTP (application of TEA in the presence of 50 μM D,L-APV) does not occlude NMDA-receptor-dependent TEA LTP (TEA application in the presence of 10 μM nifedipine, n = 13). B: VDCC component of TEA LTP is saturated under these induction conditions because a 2nd application of TEA in APV induced no additional potentiation (n = 4). Representative EPSPs (1-min average) are shown at the times indicated. Scale bars: 5 ms and 0.5 mV.

D-APV not only prevented TEA LTP in 7 of 8 slices (Fig. 3B), but synaptic transmission was slightly depressed (~18 ± 8%) after TEA applications in D-APV. Stimulation intensity was then increased to obtain EPSP slopes similar to baseline values (Fig. 3B, ▲). Robust TEA LTP (44 ± 8%, n = 8 of 8) was induced in all slices after the second TEA application in the absence of D-APV. These results show that D-APV reliably blocked the induction of TEA LTP, and that the small depression of EPSP slopes observed after TEA applications in D-APV was not due to poor slice viability. As a second test of the dependence of TEA LTP on NMDA receptor activity, the effects of the noncompetitive NMDA receptor antagonist MK-801 (Coan et al. 1987) were examined. Preincubation in MK-801 (10 μM for 1–5 h) reliably blocked TEA LTP (8 ± 6%, n = 2 of 9), whereas control slices placed in the recording chamber with MK-801-pretreated slices exhibited stable TEA LTP (36 ± 11%; n = 4 of 5; data not shown). These results indicate that NMDA receptor activity is absolutely necessary for the induction of TEA LTP in isolated CA1, and suggest that VDCCs may simply act to facilitate NMDA receptor activity, perhaps by increasing postsynaptic depolarization.

**TEA activates NMDA-receptor-mediated EPSPs**

In support of our results, which demonstrated that TEA induced NMDA-receptor-dependent TEA LTP in isolated CA1, APV-sensitive components of EPSPs were evident during TEA applications (Fig. 3C). EPSPs were analyzed ~2 min (Fig. 3B, asterisk) after TEA applications in D-APV (25 μM) and compared with EPSPs recorded during a second TEA application in the absence of APV. A large APV-sensitive component was observed and quantitated by integrating the difference between the EPSPs obtained with and without APV (Fig. 3C). Consistent with the reported time course of NMDA-receptor-mediated synaptic poten-
facilitation paradigm across pathways (i.e., stimulating S1). Evidence of synaptic pathways was verified by a paired-pulse protocol. EPSPs were measured 10-45 ms after evoked stimulation (Fig. 3C, shaded area). The magnitude of this APV-sensitive component during TEA applications was 274 ± 44 mV·ms (n = 8). These results support our contention that TEA induced an NMDA-receptor-dependent TEA LTP, and indicate that NMDA receptors are functional in the presence of TEA (see also Wright et al. 1991).

NMDA-receptor-dependent TEA LTP is pathway specific

The properties of the NMDA receptor are believed to underlie the synapse-specific and associative properties of tetanus-induced LTP (Gustafsson et al. 1987; Kelso et al. 1986; Wigström and Gustafsson 1988). Therefore NMDA-receptor-dependent TEA LTP should display similar synapse- or pathway-specific properties. To test the pathway specificity of TEA LTP we utilized two pathway experiments. Using this design, one pathway (S1) was stimulated while evoked stimulation to a second pathway (S2) was turned off during TEA applications (Fig. 4A). The independence of synaptic pathways was verified by a paired-pulse facilitation paradigm across pathways (i.e., stimulating S1 100 ms before S2 and stimulating S2 100 ms before S1; Huang and Malenka 1993). Only slices that displayed no detectable paired-pulse facilitation between the two pathways were used.

TEA LTP was strictly pathway specific in isolated CA1 (Fig. 4A). TEA LTP was observed only in the pathway (S1) or synapses receiving evoked stimulation during TEA applications (32 ± 5%; n = 8 of 8; Fig. 4A). Although transient potentiation (32 ± 4%) was observed in S2 pathways (stimulation off) when stimulation was resumed, EPSP slopes returned to baseline in <1 h after TEA washout (6 ± 2%, n = 0 of 8). In some experiments, TEA was applied a second time with stimulation on in both pathways; in four of five slices in which the unstimulated pathway (S2) failed to display LTP after the first TEA application, the second TEA application induced potentiation (32 ± 5%). Interestingly, VDCC activity did not appear to compromise the pathway specificity of TEA LTP. Similar results were obtained in the presence of nifedipine, in which TEA LTP was obtained only in the pathway receiving evoked stimulation (60 ± 13%, n = 5 of 6) and not in the stimulation off pathway (11 ± 3%; n = 2 of 6; data not shown). These results indicate that like tetanus-induced LTP, NMDA-receptor-dependent TEA LTP is pathway specific. Because the pathway specificity was observed in the presence or absence of nifedipine, VDCC activity may function to enhance NMDA receptor activation in isolated CA1.

Tetanus-induced LTP occludes NMDA-receptor-dependent TEA LTP in isolated CA1

We performed occlusion experiments (described above) to determine similarities between NMDA-receptor-dependent TEA LTP and tetanus-induced LTP. Occlusion experiments utilized simultaneous recordings from two slices. In one slice, high-frequency stimulation (2 × 100 Hz for 1 s; 20-s interval) was given to Schaffer collaterals three times, each separated by 5 min, to saturate LTP (Fig. 4B). Tetanus LTP was then applied 5 min after a new baseline was established. Tetanus-induced LTP not only blocked TEA LTP, but a depression of synaptic transmission was evident after TEA washout (46 ± 4%, n = 10 of 10). Robust TEA LTP (41 ± 10%, n = 5 of 5) was reliably induced in control slices (slice 2) receiving only low-frequency (1/15 s) stimulation. These results suggest that tetanus-induced LTP and NMDA-receptor-dependent TEA LTP rely on similar cellular mechanisms.
Increased postsynaptic depolarization and Ca\(^{2+}\) influx did not induce TEA LTP in APV

It is possible that the conditions we used to induce TEA LTP in d-APV were subthreshold for sufficient activation of VDCCs. Therefore we explored conditions that could facilitate the induction of TEA LTP via VDCC activation. In addition, this strategy could be useful in determining experimental conditions that could account for the differences between our results and previous reports (Aniksztejn and Ben-Ari 1991; Huang and Malenka 1993). The TEA induction protocol was modified to increase the likelihood of activating VDCCs by increasing postsynaptic depolarization and action potentials. Doubling stimulation intensity to elicit population spikes in field recordings was insufficient to induce TEA LTP in 25 μM d-APV (−8 ± 8%; n = 1 of 7; data not shown). Conditions expected to increase Ca\(^{2+}\) influx through VDCC’s during TEA applications, such as increasing extracellular Ca\(^{2+}\) from 2 to 3 mM and decreasing Mg\(^{2+}\) from 1 to 0.5 mM, were unsuccessful in inducing TEA LTP in d-APV (−5 ± 5%; n = 0 of 8; data not shown). Increasing extracellular K\(^{+}\) from 3.5 to 5 mM, in conjunction with high Ca\(^{2+}\) (3 mM) and low Mg\(^{2+}\) (0.5 mM), also failed to induce TEA LTP in d-APV (−9 ± 5%; n = 0 of 4; data not shown). Finally, the addition of picrotoxin (50 μM) to block γ-aminobutyric acid-A-mediated inhibition did not produce TEA LTP in the presence of d-APV (n = 0 of 2; data not shown).

VDCC-dependent TEA LTP is facilitated by high-frequency stimulation

NMDA-receptor-independent TEA LTP was readily obtained in slices with intact CA3-CA1 connections (Fig. 1, B and D). Therefore we hypothesize that the bursting activity of CA3 neurons that is known to occur during TEA applications (Fueita and Avoli 1993; Rutecki et al. 1990) may enhance and/or induce bursts of Ca\(^{2+}\) spikes in CA1 neurons and induce VDCC-dependent TEA LTP. Such bursting activity of CA3 neurons was evident in extracellular EPSPs recorded during and after TEA applications in slices with intact CA3-CA1 connections (Fig. 5A). The multiple spikes were strongly attenuated by nifedipine and were absent in EPSPs recorded in isolated CA1 slices (Fig. 5A). The appearance of these multiple nifedipine-sensitive spikes in intact slices supports our hypothesis that intact CA3-CA1 connections are necessary to facilitate Ca\(^{2+}\) spikes in CA1 neurons during TEA applications and induce VDCC-dependent TEA LTP. Robust TEA LTP (35 ± 9%; n = 8 of 10; data not shown) was observed in intact slices in APV when evoked stimulation was turned off during TEA applications. This result indicates that spontaneous activity of CA3 neurons during TEA was sufficient to induce the VDCC component of TEA LTP.

To test the hypothesis that high-frequency stimulation of CA1 neurons facilitates the induction of VDCC-dependent TEA LTP, we mimicked the bursting activity of CA3 neurons (observed during TEA) by stimulating Schaffer collaterals in isolated CA1 slices. We were successful in obtaining significant potentiation (26 ± 4%; n = 8 of 12) in the presence of dL-APV (100–200 μM) by delivering 80-ms pulses of 25 Hz stimulation every 5 s to Schaffer collaterals during TEA application (Fig. 5B). This potentiation was blocked by the combined application of APV (100–200 μM) plus nifedipine (10–50 μM; 5 ± 3%; n = 0 of 6; Fig. 5B, inset). These results indicate that short bursts of high-frequency stimulation are required for the activation of VDCCs sufficient to induce TEA LTP in APV.

VDCC-dependent TEA LTP is not pathway specific and does not require evoked stimulation

Our results in isolated CA1 indicated that TEA LTP is stimulation dependent and pathway specific because of its dependence on NMDA receptor activation. If NMDA receptor activation determines the synapse specificity of LTP, then the component of TEA LTP that relies on Ca\(^{2+}\) influx...
through VDCCs may not require evoked stimulation nor display synapse specificity. Using two independent pathway experiments in isolated CA1, we induced VDCC-dependent TEA LTP in one pathway (26 ± 4%, n = 8 of 12) by delivering bursts of 25-Hz stimulation to Schaffer collaterals during TEA applications in APV (described above; see Fig. 5B). Comparable potentiation (27 ± 3%, n = 10 of 12) was observed in the second pathway (Stim off) when evoked stimulation was turned off 5 min before and until 30 min after TEA washout. These results indicated that the induction of VDCC-dependent TEA LTP was independent of evoked stimulation and was not pathway specific. In contrast, we demonstrated that NMDA-receptor-dependent TEA LTP was pathway specific and required evoked stimulation (Fig. 4A). These major differences suggest that the route of Ca\(^{2+}\) entry during the induction of TEA LTP can determine the synapse and/or pathway specificity of potentiation.

**Antidromic stimulation of CA1 neurons during TEA induces VDCC-dependent TEA LTP**

To extend our results using 25 Hz synaptic stimulation, we tested the induction of VDCC-dependent TEA LTP in isolated CA1 without Schaffer collateral stimulation. We demonstrated that VDCC-dependent TEA LTP was not pathway specific (Fig. 5B); however, excess glutamate released during 25-Hz stimulation to one pathway during TEA application may stimulate adjacent synapses and induce potentiation in the stimulation off pathway. To prevent excess glutamate release, we ceased Schaffer collateral stimulation during and 30 min after TEA application and antidromically stimulated CA1 neurons with alvear stimulation (Fig. 6, inset). Antidromic spikes were monitored for the duration of each experiment with an extracellular recording electrode placed in stratum pyramidale. To ensure that these responses were antidromic, as opposed to synaptic, we applied Ca\(^{2+}\)-free media to some slices. The magnitude of antidromic responses was unaffected by the application of Ca\(^{2+}\)-free media (n = 3, data not shown).

We performed antidromic experiments in isolated CA1 slices in the presence of D,L-APV (100-200 μM). A high-frequency tetanus was given to each slice to test the efficacy of APV applications. Schaffer collateral stimulation was turned off 5 min before and until 30 min after TEA application. During TEA applications, 80-ms pulses of 25-Hz stimulation were delivered to CA1 axons once every 5 s. The duration of the antidromic spike increased during TEA application (Fig. 6, antidromic spikes). This stimulation protocol resulted in potentiation of EPSPs when stimulation was resumed (34 ± 8%), which decayed to 24 ± 4% (n = 8 of 10) 1 h after TEA washout (Fig. 6, •). Low frequency antidromic stimulation (0.07 Hz) during TEA application did not result in EPSP potentiation when Schaffer collateral stimulation was resumed (data not shown). The magnitude of TEA LTP was significantly reduced when experiments were performed in APV and nifedipine (50 μM, P < 0.02); EPSP slopes were only 9 ± 3% (n = 0 of 7) above baseline 1 h after TEA washout (Fig. 6, □). This result indicates that postsynaptic L-type VDCCs play a role in the induction of TEA LTP by antidromic stimulation. The decremental potentiation observed in APV plus nifedipine in antidromic experiments (Fig. 6) and in intact slices (Fig. 1, C and D) may indicate that L-type VDCCs were not completely blocked by nifedipine, or that other types of VDCCs may be activated with antidromic stimulation during TEA application. Increased nifedipine concentrations (>50 μM) were used to test the latter hypothesis, but effects on basal synaptic transmission were observed (O'Regan et al. 1991). In addition, there was no difference in the magnitude of TEA LTP using either 100 or 200 μM APV. Nonetheless, these results are consistent with experiments using 25-Hz synaptic stimulation and support the hypothesis that the induction of VDCC-dependent TEA LTP is not synapse specific and does not require evoked synaptic stimulation.

**Discussion**

In intact hippocampal slices, TEA LTP is composed of two distinct components, one dependent on NMDA receptor activity and another on VDCC activation. We observed robust TEA LTP in D,L-APV; moreover, TEA LTP was obtained in the presence of nifedipine. Each antagonist alone decreased the degree of potentiation to approximately half of that obtained in control slices, and TEA LTP was significantly attenuated with APV plus nifedipine. These results indicate that the contribution of Ca\(^{2+}\) influx through NMDA receptors and VDCCs is additive and/or synergistic during the induction of TEA LTP. The demonstration that high-frequency-stimulation (200 Hz)-induced LTP appears to
have both NMDA receptor and VDCC components (Grover and Teyler 1990) is consistent with our findings on TEA LTP. If the magnitude of potentiation is related to the total increase in postsynaptic Ca\(^{2+}\), Ca\(^{2+}\) influx through NMDA receptors and VDCCs could be additive, with maximal potentiation resulting when both are activated. Postsynaptic Ca\(^{2+}\) may be acting at the same downstream effector targets independent of its site of entry. However, our results showing that the VDCC component of TEA LTP does not occlude or reduce the magnitude of the NMDA receptor component argue against this hypothesis and suggest that potentiation due to Ca\(^{2+}\) influx via VDCCs versus NMDA receptors relies on distinct intracellular mechanisms. Additionally, the peak magnitude and temporal characteristics of TEA LTP are different in nifedipine versus APV (Fig. 1B), suggesting that Ca\(^{2+}\) influx through VDCCs or NMDA receptors activates potentiation mechanisms with different kinetics. Thus two different routes of Ca\(^{2+}\) entry can produce long-lasting potentiation through apparently distinct pathways and TEA LTP can be used to better understand these Ca\(^{2+}\)-dependent pathways. A recent report (Hanse and Gustafsson 1994) that indicates that TEA LTP may induce two distinct potentiations of the EPSP, one via the NMDA receptor and another through the activation of VDCCs, is in agreement with our findings.

To examine in detail the properties of the NMDA component of TEA LTP, we utilized isolated CA1 slices. Contrary to previous findings in isolated CA1 (Aniksztejn and Ben-Ari 1991; Huang and Malenka 1993), our results indicate that TEA LTP is strictly dependent on NMDA receptor activation. In contrast to intact slices, NMDA receptor antagonists completely blocked TEA LTP in isolated CA1. The fact that we observed a large APV-sensitive component and the absence of multiple spikes in EPSPs during TEA applications supports our contention that the induction of TEA LTP in isolated CA1 relies on the activation of NMDA receptors. Nifedipine attenuated but did not block TEA LTP in isolated CA1, indicating VDCC activity may contribute to TEA LTP but is not sufficient to induce long-lasting potentiation with low-frequency test stimulation. VDCCs may contribute to the activation of NMDA receptors primarily by increasing postsynaptic depolarization; however, such a contribution clearly does not alter the pathway-specific characteristics of TEA LTP in isolated CA1 (Fig. 4A).

Additional studies in isolated CA1 demonstrated that NMDA-dependent TEA LTP and tetanus-induced LTP are very similar. Our results show that NMDA-receptor-dependent TEA LTP requires evoked presynaptic stimulation and displays pathway specificity. These findings are consistent with the hypothesis that the NMDA receptor acts as a detector of pre- and postsynaptic activity and is responsible for the input specificity of TEA LTP, similar to tetanus-induced LTP (Gustafsson et al. 1987; Kelso et al. 1986). Occlusion experiments indicate that the NMDA receptor component of TEA LTP utilizes similar cellular mechanisms as LTP induced by tetanic stimulation. Preliminary experiments indicate that, like tetanus-induced LTP, the NMDA receptor component of TEA LTP is also dependent on protein kinase activity (Huber et al. 1993). We conclude that NMDA-receptor-dependent TEA LTP and tetanus-induced LTP utilize common cellular mechanisms. This implies that similar potentiation mechanisms are engaged by different protocols that activate NMDA receptors. Therefore studies of TEA LTP may be instrumental in investigating the cellular mechanisms of tetanus-induced LTP.

Different stimulation conditions were required for the induction of VDCC versus NMDA-receptor-dependent TEA LTP. We demonstrated that high-frequency bursts (80 ms, 25 Hz) of stimulation to CA1 neurons during TEA applications facilitated the induction of VDCC-dependent TEA LTP. These bursts can be delivered by Schaffer collateral or alvear stimulation of CA1 neurons in isolated CA1, or by TEA-induced bursting of CA3 neurons in intact slices. Thus high-frequency stimulation conditions may be required to increase postsynaptic depolarization to sufficiently activate VDCCs and engage potentiation mechanisms. In addition, stimulation frequency may also be important in reducing VDCC inactivation (Cavalié et al. 1986; Eckert and Chad 1984). In support of this conclusion, Kullmann et al. (1992) demonstrated that repeated depolarizing pulses to CA1 neurons were much more efficient in producing VDCC-dependent potentiation compared with a sustained depolarization. In addition, Grover and Teyler (1990) demonstrated that potentiation induced by high-frequency stimulation (200 Hz) is NMDA independent and blocked by nifedipine, and low-frequency stimulation (25 Hz) was insufficient to produce potentiation in D,L-APV. These results, together with ours, suggest that NMDA receptors are the major or more utilized route for Ca\(^{2+}\) influx during the induction of TEA LTP and tetanus-induced LTP, because extremely high stimulation frequencies are required to induce potentiation via VDCCs. These results also suggest that the compartmentation of Ca\(^{2+}\) increases is important for the induction of LTP. Ca\(^{2+}\) influx through NMDA receptors located on spine heads (Müller and Connor 1991; Wigström and Gustafsson 1988) may have greater access to potentiation mechanisms than through VDCCs located on dendritic shafts (Nicolli et al. 1988; Regehr et al. 1989; Westenbroek et al. 1990). Therefore VDCC-dependent potentiation mechanisms may only be engaged under high-frequency stimulation conditions (e.g., 200 Hz) or during bursting/seizurelike activity. In this context, such high frequencies may occur during certain physiological or behavioral conditions (Buzsáki et al. 1992).

We have found differences in the routes of Ca\(^{2+}\) that are utilized for TEA LTP induction in intact versus isolated CA1 hippocampal slices. It is noteworthy that the slice preparations studied herein behave differently than those used by Ben-Ari (Aniksztejn and Ben-Ari 1991) or Huang and Malenka (1993), who observed TEA LTP that was predominately NMDA receptor independent. However, as mentioned above, a recent report has concluded that TEA induces potentiation via the NMDA receptor and is not totally blocked by the combined VDCC antagonists flunarizine and nifedipine in guinea pig hippocampal slices (Hanse and Gustafsson 1994). Zhang and Morrisett (1993) also observed that D-APV can reduce the magnitude of TEA LTP, which is consistent with our findings. In this respect, we observed a prominent NMDA-receptor-dependent component in EPSPs during TEA applications in isolated CA1 (Fig. 3C). Similar to our findings, the LTP model described by Grover and Tyler...
(1990), induced with 200-Hz stimulation, clearly contains both NMDA receptor and VDCC components. The relative contribution of VDCCs or NMDA receptors to TEA LTP induction may be related to the general biophysical state of hippocampal slices, such as their susceptibility to seizurelike activity and/or Ca\(^{2+}\) spiking (see above). Even though previous studies have utilized isolated CA1 regions to reduce TEA-induced seizures, we have prepared slices in 10 mM Mg\(^{2+}\) with no added Ca\(^{2+}\) to prevent cell damage due to excitotoxicity (Feig and Lipton 1990). In addition, we stimulated Schaffer collaterals/commissural axons with small-diameter (20–50 μm) monopolar tungsten electrodes to elicit EPSPs, whereas stainless steel electrodes are more likely to induce seizurelike activity (Campbell et al. 1984; Gall and Lauterhorn 1992). Thus we adjusted our conditions to reduce excitotoxicity during slice preparation and seizure activity during TEA treatments. The only condition in which we were successful in inducing TEA LTP in isolated CA1 was using evoked stimulation at higher frequencies (25 Hz). In contrast, reliable VDCC-dependent TEA LTP was induced in APV using intact slices and low frequency (0.07 Hz), where it is known that TEA induces bursting activity in CA3 neurons (Fueta and Avoli 1993; Rutecki et al. 1990). Regardless of the precise technical reasons for the differences between our results and previous work, we emphasize that our goal in studying TEA LTP was to determine the similarities, differences, and underlying mechanisms of NMDA-receptor- and VDCC-dependent potentiation. We have extensively characterized the properties of these forms of potentiation under the conditions used herein and believe that the information provided from our experiments increases our understanding of other forms of NMDA- and VDCC-dependent LTP.

We observed that different routes of Ca\(^{2+}\) influx induce potentiation with different properties. VDCC-dependent potentiation does not require evoked stimulation and is not pathway specific. These properties contrast sharply with those of NMDA-receptor-dependent TEA LTP and indicate that the route of Ca\(^{2+}\) entry during LTP induction can determine synapse specificity and therefore modulate neuronal information processing. Our results demonstrating the lack of pathway specificity of VDCC-dependent TEA LTP differ from those of Kullmann et al. (1992), who observed that synaptic stimulation in conjunction with postsynaptic depolarization (in the presence of APV) was required to induce long-lasting potentiation. However, this same group (Wyllie et al. 1994) found that depolarization in the presence of calciuculin A, a protein phosphatase inhibitor, resulted in a long-lasting potentiation without synaptic stimulation. The TEA LTP induction protocol may result in a different magnitude or localization of Ca\(^{2+}\) influx that sufficiently activates protein kinases to potentiate synapses regardless of synaptic stimulation. Our results support the hypothesis that the localization of NMDA receptors on spines, and/or the efficient Ca\(^{2+}\) buffering of spine heads/necks, is responsible for Ca\(^{2+}\) localization and therefore synapse specificity during LTP induction (Müller and Cumur 1991; Nicoll et al. 1988; Zador et al. 1990). Ca\(^{2+}\) entering through VDCCs located on dendritic shafts may potentiate nonspine synapses or overcome the Ca\(^{2+}\) buffering mechanisms in spine necks to potentiate synapses regardless of their activity. If the route of Ca\(^{2+}\) entry during LTP induction controls synapse specificity, as suggested by our experiments, then synaptic plasticity that relies on VDCC activation is nonassociative and cell-wide. In this context, L-type VDCCs have been localized to the soma and proximal dendrites of hippocampal neurons and have been proposed to regulate cellular events involved in synaptic plasticity, such as gene expression or protein synthesis (Westenbroek et al. 1990).

There are several potential sites during LTP induction in which a divergence of potentiation mechanisms could exist. As mentioned above, the location of Ca\(^{2+}\) influx and its subcellular compartmentation (e.g., spine head vs. dendritic shaft) during the induction of TEA LTP may determine which potentiation mechanisms are activated. The observation that NMDA-receptor- and VDCC-dependent TEA LTP mechanisms appear distinct suggests that potentiation mechanisms may exist in the dendritic shaft that are activated by VDCCs. This result also implies that Ca\(^{2+}\) that enters through VDCCs does not have access to NMDA-receptor-regulated mechanisms in the spine head and may only potentiate synapses on the dendritic shaft. Other possible scenarios to explain the divergence of NMDA receptor and VDCC-dependent mechanisms include the activation of Ca\(^{2+}\)-dependent enzymes by VDCCs that are then transported/translocated to synapses on spine heads. In addition, VDCCs could stimulate the production of a membrane-permeable retrograde messenger that acts on presynaptic terminals of adjacent synapses (Schuman and Madison 1991; Williams et al. 1989). Recent work (Schuman and Madison 1994) indicates that nitric oxide may potentiate synapses of neighboring neurons regardless of their activity. Information regarding Ca\(^{2+}\)-dependent pathways regulated by VDCCs could be instrumental in determining whether NMDA receptors and VDCCs activate similar enzyme cascades that are separately compartmentalized or whether distinct enzyme cascades (Lerea and McNamara 1993) are involved in each form of potentiation. If VDCC-dependent TEA LTP is also dependent on protein kinase activity and/or retrograde messengers, this will provide new information about the biochemical cascades activated by Ca\(^{2+}\) influx through VDCCs versus NMDA receptors during LTP induction.

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