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Examination of TEA-induced Synaptic Enhancement in Area CA1 of the Hippocampus: The Role of Voltage-dependent Ca^{2+} Channels in the Induction of LTP

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The role of voltage-dependent Ca^{2+} channels (VDCCs) in the induction of long-term potentiation (LTP) in the CA1 region of rat hippocampus was determined by examining the relationship between LTP and the long-lasting synaptic enhancement induced by extracellular application of tetraethylammonium (TEA). Consistent with previous findings (Aniksztejn and Ben-Ari, 1991), the TEA-induced synaptic enhancement did not require NMDA receptor activation. It was blocked by the L-type VDCC antagonist nifedipine or by intracellular injection of the Ca^{2+} chelator 1,2-bis(2-aminophenoxy)ethane *N,N,N',N'*-tetra-acetic acid (BAPTA) and could be mimicked by direct activation of VDCCs with repetitive depolarizing current pulses. In contrast to its effect on TEA-induced synaptic enhancement, nifedipine had no effect on the magnitude or duration of NMDA receptor–dependent LTP. Saturation of NMDA receptor–dependent LTP reduced the magnitude of the TEA-induced synaptic enhancement. Similarly, increasing synaptic strength by initial application of TEA reduced the magnitude of the subsequent tetanus-induced LTP. Like LTP, the TEA-induced synaptic enhancement did not significantly affect paired-pulse facilitation. These results suggest that dihydropyridine-sensitive VDCCs do not normally contribute to the induction of NMDA receptor–dependent LTP even though their repetitive activation can generate an increase in synaptic strength. The mutual occlusion of LTP and TEA-induced synaptic enhancement suggests that they share a common expression mechanism and perhaps are generated by activation of common Ca^{2+}-dependent intracellular processes. This would occur if a large rise in intracellular Ca^{2+} solely due to repetitive activation of VDCCs that are excluded from dendritic spines can overcome endogenous buffering mechanisms and activate biochemical processes within the spine. However, because the occlusion between LTP and TEA-induced synaptic enhancement was not complete, the possibility that there are some mechanistic differences between the two forms of synaptic enhancement cannot be ruled out.

[Key words: long-term potentiation, calcium, calcium channels, hippocampus, synaptic plasticity, NMDA receptors]

The induction of long-term potentiation (LTP) in hippocampal CA1 pyramidal cells is thought to require a rise in postsynaptic Ca^{2+} as a consequence of synaptic activation of postsynaptic NMDA receptors during concomitant postsynaptic depolarization (Nicoll et al., 1988). The experimental evidence supporting a critical role for postsynaptic Ca^{2+} increases in LTP induction is reasonably compelling. First, the NMDA receptor ionophore is permeable to calcium (MacDermott et al., 1986; Jahr and Stevens, 1987; Mayer and Westbrook, 1987; Ascher and Nowak, 1988), and in the hippocampal slice preparation NMDA receptor activation leads to significant rises in Ca^{2+} levels in the dendrites of CA1 pyramidal cells (Regehr and Tank, 1990). Second, injection of Ca^{2+} chelators into CA1 cells reduces or blocks LTP (Lynch et al., 1983; Malenka et al., 1988), as does strong postsynaptic depolarization (Malenka et al., 1988; Perkel et al., 1991), which decreases the driving force for Ca^{2+} influx. Finally, directly increasing postsynaptic Ca^{2+} by releasing Ca^{2+} from the photolabile Ca^{2+} chelator Nitr-5 can cause an enhancement of synaptic transmission (Malenka et al., 1988), although the relationship of this form of potentiation to LTP remains to be determined.

Although it is often assumed that the rise in postsynaptic Ca^{2+} required for LTP induction is due to entry of Ca^{2+} via the NMDA receptor ionophore, another source for increasing postsynaptic Ca^{2+} is voltage-dependent Ca^{2+} channels (VDCCs). The dendrites of CA1 pyramidal cells appear to contain both N-type and L-type VDCCs (Jones et al., 1989; Westenbroek et al., 1990), and like NMDA receptors, their activation can lead to significant increases in intradendritic Ca^{2+} (Regehr et al., 1989; Jaffe et al., 1992). Recently evidence has been presented that repetitive activation of L-type VDCCs can generate a long-lasting synaptic enhancement that resembles LTP yet is not blocked by the NMDA receptor antagonist D-2-amino-5-phosphonovalerate (D-APV) (Grover and Teyler, 1990; Aniksztejn and Ben-Ari, 1991).

The ability of Ca^{2+} influx through L-type VDCCs to increase synaptic efficacy raises two important questions about the role of L-type VDCCs in the induction of LTP. First, does the Ca^{2+} influx via L-type VDCCs contribute to the generation of "normal," NMDA receptor–dependent LTP? Second, does the Ca^{2+} increase due to L-type VDCCs activate the same intracellular mechanisms as those activated by Ca^{2+} influx via the NMDA receptor ionophore, or does it activate distinct processes resulting in a form of LTP mechanistically different from NMDA receptor–dependent LTP? To address these questions, we have examined the relationship between LTP and the synaptic enhancement elicited by application of the potassium channel blocker tetraethylammonium (TEA) (Aniksztejn and Ben-Ari, 1991).

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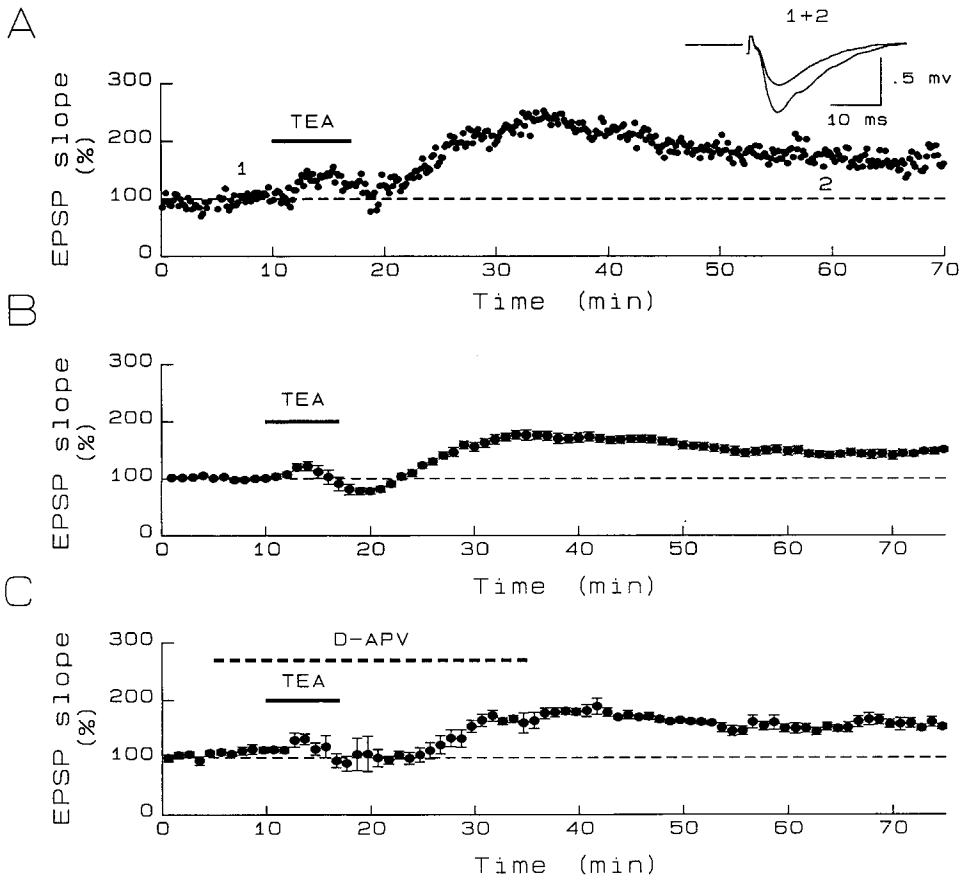

Figure 1. TEA application causes an APV-insensitive long-lasting potentiation of synaptic transmission. *A*, Example of an experiment in which 7 min of application caused synaptic potentiation. TEA was applied to the slice for the period indicated by the *bar*. The *inset* shows sample data traces taken at the time indicated by the *numbers* on the graph. *B*, Summary of experiments ($n = 10$) like that shown in *A*. *C*, Summary of experiments ($n = 7$) in which TEA was applied in the presence of the NMDA receptor antagonist D-APV (25–50 μM). All points on this and subsequent summary graph figures are mean ± SEM.

Materials and Methods

Standard methods were used to make extra- and intracellular recordings from hippocampal slices prepared from Sprague–Dawley rats (Malenka, 1991; Colino et al., 1992). The composition of the superfusing solution was (in mM) NaCl, 119; KCl, 2.5; $MgSO_4$, 1.3; NaH_2PO_4, 1.0; $NaHCO_3$, 26.2; $CaCl_2$, 2.5; and glucose, 11. The solution temperature was maintained at 29–31°C, and it was saturated with 95% O_2, 5% CO_2 before application. Extracellular field EPSPs were recorded in stratum radiatum with electrodes filled with 3 M NaCl. Intracellular recordings were made with electrodes filled with 3 M CsCl or 3 M potassium acetate using the bridge circuit of an Axoclamp 2A amplifier (Axon Instruments). During synaptic stimulation, cells were held between −70 and −80 mV under current clamp. Bipolar stainless steel electrodes were placed in stratum radiatum to activate Schaffer collateral/commissural afferents at 0.1 Hz. Before placement of the electrodes, a cut was made between the CA3 and CA1 regions to prevent any epileptiform activity in CA3 cells from affecting the recordings in CA1. When experiments required activation of two independent pathways, stimulation electrodes were placed on opposite sides of the recording electrodes. The independence of the two pathways was confirmed by the absence of any discernible paired-pulse facilitation (PPF; interstimulus interval, 50 msec) between the two inputs.

Data were collected and analyzed on line (2–10 kHz sampling rate) using a 386 IBM PC clone computer programmed with AXOBASIC (Axon Instruments). Initial slopes of EPSPs were calculated using a least-squares regression. When recording extracellular field EPSPs, LTP was elicited by applying a tetanus (100 Hz, 1 sec) three times with an intertetanus interval of 1–2 min. Averaged summary graphs were constructed as described (Malenka et al., 1989b; Huang et al., 1992). The significance of the differences between control and experimental manipulations was tested using a two-tailed *t* test. Stock solutions of nifedipine (50 mM, Sigma) were made up in dimethyl sulfoxide (DMSO), stored in the dark, and diluted to the appropriate final concentration in the perfusing medium immediately prior to application. Application of the DMSO (0.05%) alone had no effect on LTP.

Results

In agreement with a previous report (Anikszteijn and Ben-Ari, 1991), a 7 min extracellular application of TEA (25 mM) to the hippocampal slice preparation caused a long-lasting increase in the initial slope of the field EPSP (Fig. 1*A*). The average magnitude of the potentiation measured 40 min following the washout of TEA was 55 ± 6% ($n = 10$) (Fig. 1*B*). Application of the NMDA receptor antagonist D-APV, (25 μM, $n = 5$; 50 μM, $n = 2$) had no discernible effect on the duration or magnitude of the TEA-induced synaptic enhancement (59 ± 8%; Fig. 1*C*), indicating that NMDA receptors were not required nor did they contribute to the generation of the observed synaptic enhancement. Maintaining the osmolality of the bathing solution by reducing the concentration of NaCl also had no effect on the TEA-induced synaptic enhancement ($n = 3$).

Application of TEA broadens the action potential in CA1 pyramidal cells (Lancaster and Nicoll, 1987; Storm, 1987). Consistent with this finding, a transient and completely reversible increase in the duration of the fiber volley was often observed during the application and washout of TEA (not illustrated). This interfered with the measurement of the initial negative slope of the field EPSP because the slope measurement, made at a fixed latency using linear regression, now often included the latter portion of the fiber volley. Intracellular recording (see Fig. 3) confirmed that this measured reduction in field EPSP initial slope did not reflect a true decrease in the strength of synaptic transmission.

To test whether the TEA-induced synaptic enhancement was due to activation of VDCCs, as previously suggested (Anikszteijn

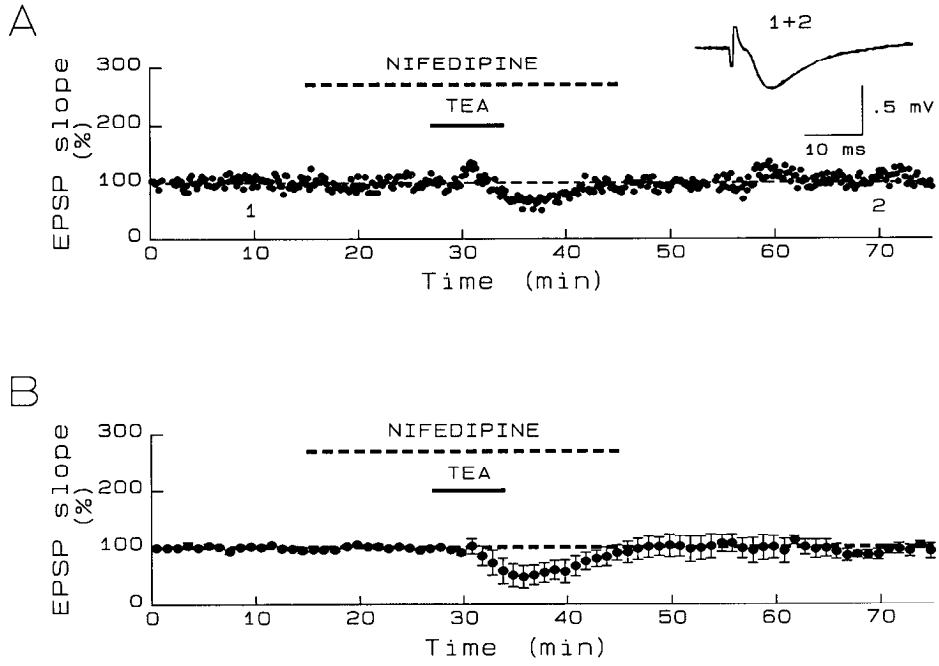

Figure 2. The TEA-induced synaptic enhancement is blocked by nifedipine. *A*, Example of experiment in which TEA was applied in the presence of nifedipine (12 μM). *Solid* and *broken bars* indicate the duration of drug applications. The *inset* shows raw data traces taken at the times indicated by the *numbers* on the graph. *B*, Summary of experiments ($n = 6$) like that shown in *A*.

and Ben-Ari, 1991), we examined the effects of the L-type VDCC blocker nifedipine (Tsien et al., 1988). Nifedipine has been reported to decrease strongly the magnitude of postsynaptic Ca^{2+} influx into CA1 pyramidal cells (Jones and Heinemann, 1987) and to decrease the NMDA receptor–independent synaptic potentiation induced by 200 Hz tetanic stimulation (Grover and Teyler, 1990). In the presence of nifedipine (10–12 μM), application of TEA resulted in no significant synaptic enhancement (Fig. 2; $n = 6$).

Because extracellular application of nifedipine will affect both pre- and postsynaptic dihydropyridine-sensitive VDCCs, it was important to determine whether preventing an increase in postsynaptic Ca^{2+} levels would reduce or block the effects of TEA on synaptic transmission. To accomplish this, cells were im-

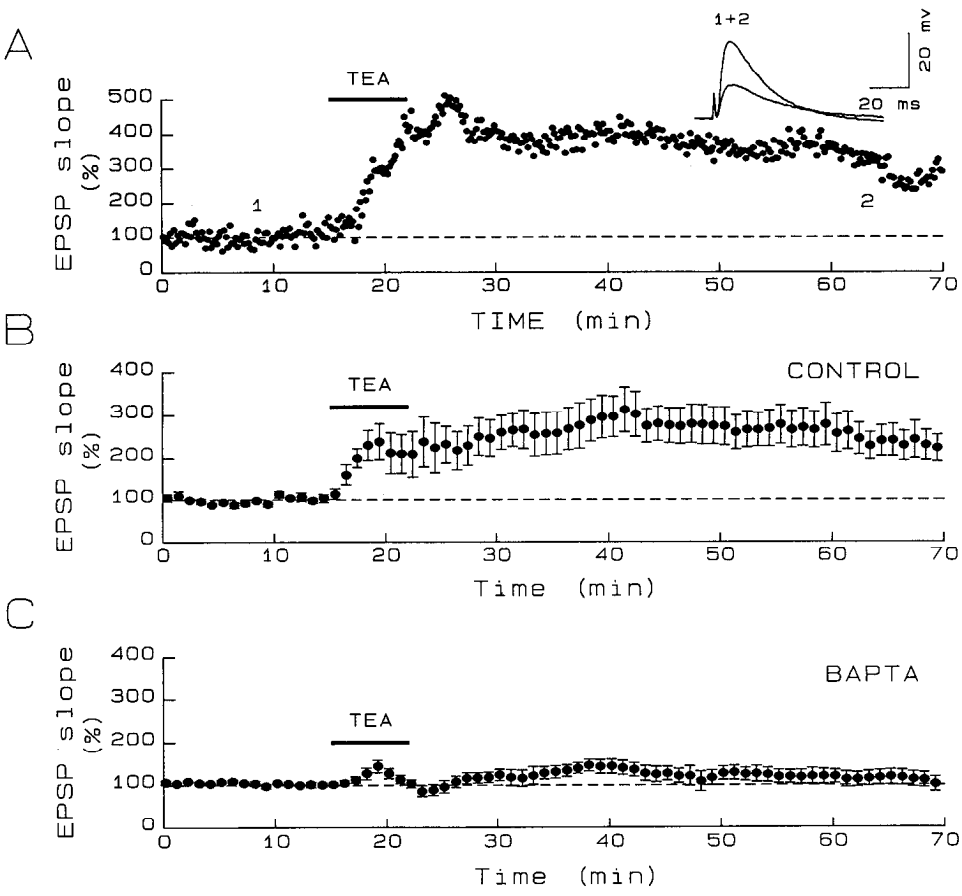

Figure 3. The TEA-induced synaptic enhancement is blocked by intracellular injection of the calcium chelator BAPTA. *A*, Example of an experiment showing the TEA-induced synaptic enhancement recorded with an intracellular electrode (3 M K-acetate). The *inset* shows raw data traces taken at the times indicated by the *numbers* on the graph. *B*, Summary of experiments ($n = 6$) like that shown in *A*. *C*, Summary of experiments ($n = 7$) like that in *A* except intracellular electrodes were also filled with the Ca^{2+} chelator BAPTA (50 mM).

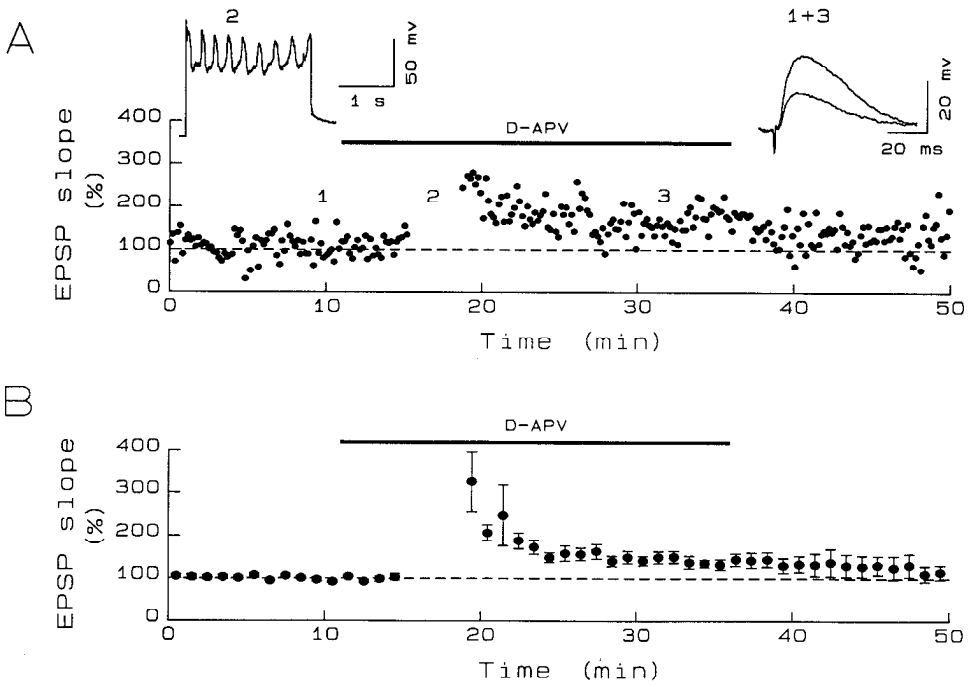

Figure 4. Direct activation of Ca^{2+} spikes causes synaptic enhancement. *A*, Example of experiment in which synaptic enhancement was elicited in the presence of D-APV (25 μM) by applying 2 sec depolarizing current pulses (1.0 nA) every 3 sec. Afferent stimulation was stopped during the period when Ca^{2+} spikes were elicited. *Insets* show raw data traces taken at the times indicated by the *numbers* on the graph. *B*, Summary of experiments ($n = 7$) like that shown in *A*.

paled with electrodes filled with the Ca^{2+} chelator 1,2-bis(2-aminophenoxy)ethane *N,N,N′,N′*-tetra-acetic acid (BAPTA; 50 mM). In control cells, TEA application caused a significant, long-lasting increase in synaptic transmission (Fig. 3*A,B*; $n = 6$) that outlasted an associated small depolarization (5 ± 2 mV) and the occurrence of synaptically evoked Ca^{2+} spikes (Anikszteijn and Ben-Ari, 1991). Examination of the effects of TEA on action potential duration (Lancaster and Nicoll, 1987; Storm, 1987) indicated that following the 7 min application, it took no longer than 15–20 min to wash TEA out of the slice completely. In contrast, in cells filled with BAPTA ($n = 7$), TEA application caused a transient increase in EPSP slope but had no long-lasting effects on synaptic transmission (Fig. 3*C*) even though synaptic stimulation still evoked Ca^{2+} spikes.

The results presented thus far are consistent with the proposal that TEA application causes a long-lasting enhancement of synaptic transmission primarily due to activation of postsynaptic L-type VDCCs (Anikszteijn and Ben-Ari, 1991). To test whether direct activation of postsynaptic L-type VDCCs could produce synaptic enhancement, long (2 sec) depolarizing current pulses (0.8–1.4 nA) were applied repetitively (every 3 sec for 3–4 min) to CA1 cells recorded with electrodes filled with 3 M CsCl. This was done in the presence of D-APV (25 μM) and after synaptic stimulation had been stopped to ensure that NMDA receptors were not activated. These pulses caused repetitive discharge of Ca^{2+} spikes (Fig. 4*A*, inset) and, consistent with a previous report (Perkel et al., 1991), caused a synaptic enhancement that often decayed back to control over 10–30 min (Fig. 4*A,B*; $n = 7$).

It appears clear that repetitive activation of L-type VDCCs either by application of TEA or by direct depolarizing current pulses can result in synaptic enhancement. A critical question is whether activation of L-type VDCCs contributes to the generation of "normal" NMDA receptor–dependent LTP induced either with a tetanus (50–100 Hz) or by pairing synaptic stimulation with postsynaptic depolarization. Previous work using a number of organic blockers of L-type VDCCs demonstrated that VDCCs are likely not required to generate LTP (Taube and Schwartzkroin, 1986). However, based on the observation that the Ca^{2+} channel agonist BAY K8644 enhances LTP, it has been suggested that Ca^{2+} influx through L-type VDCCs may contribute to LTP (Mulkeen et al., 1987). Because nifedipine was capable of completely inhibiting the TEA-induced synaptic enhancement (Fig. 2), its effects on LTP were examined using extracellular recording. Figure 5 shows that nifedipine (10–12 μM) had no discernible effect on the magnitude or duration of NMDA receptor–dependent LTP measured 30 min following the final tetanus (potentiation in nifedipine = 158 ± 26%, $n = 6$; control potentiation = 143 ± 39%, $n = 8$).

The ineffectiveness of nifedipine indicates that L-type VDCCs likely do not contribute to the generation of normal tetanus-induced NMDA receptor–dependent LTP. The question remains, however, of whether the synaptic enhancement induced by activation of L-type VDCCs is mechanistically distinct from NMDA receptor–dependent LTP or rather reflects the same synaptic modification. This issue can be addressed by performing so-called "occlusion" experiments in which the mechanisms responsible for NMDA receptor–dependent LTP are saturated using repetitive tetani (100 Hz, 1 sec) prior to TEA application. To reduce inherent variability as much as possible, within-slice comparisons were made between the effect of TEA application on synaptic transmission in two pathways, only one of which had received prior tetanic stimulation. Figure 6*A* shows an example of such an experiment in which saturation of NMDA receptor–dependent LTP resulted in a significant reduction in the TEA-induced synaptic enhancement as compared to that induced in a control pathway. Before application of TEA, stimulus strength in the test path was reduced so that the test EPSP approximately matched the control EPSP. This ensured that any reduction in the magnitude of the TEA-induced synaptic enhancement was not due to the large differences in the size of the test and control EPSPs. This was of significant concern because as the depolarization caused by the EPSP becomes greater, the driving force for the evoked synaptic currents becomes less

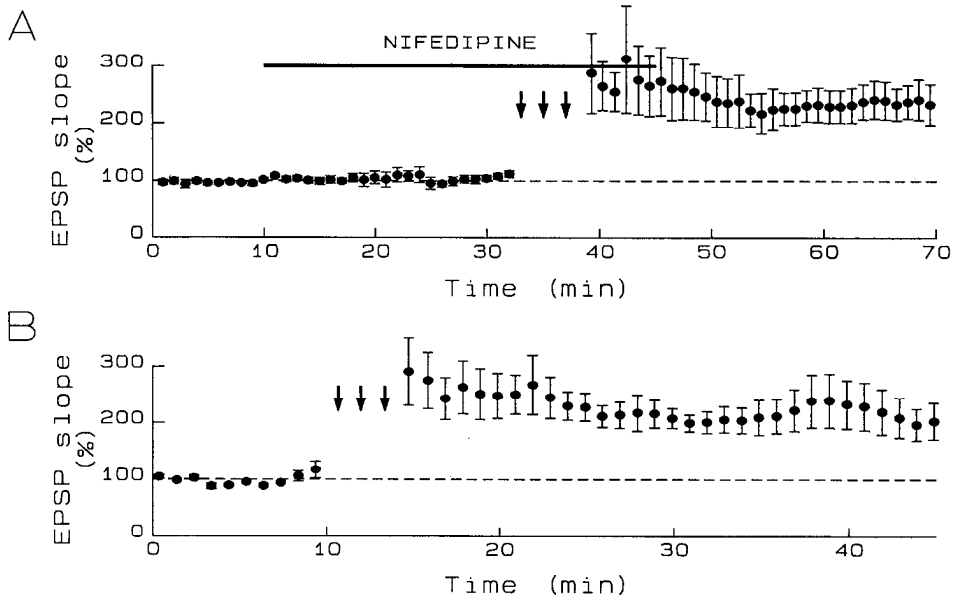

Figure 5. Nifedipine has no effect on tetanus-induced LTP. *A*, Summary of experiments ($n = 6$) in which LTP was elicited in the presence of nifedipine (10–12 μM). *B*, Summary of experiments ($n = 8$) showing LTP elicited in normal bathing solution. At the *arrows*, a 100 Hz, 1 sec tetanus was given.

such that the same percentage change in synaptic conductance will cause less of a change in a large EPSP than in a small one. A summary of these experiments (Fig. 6*B*; $n = 9$) indicates that following saturation of NMDA receptor–dependent LTP, the TEA-induced synaptic enhancement, measured 40 min after TEA washout, was reduced by 55% (control potentiation = 60 ± 8%; test potentiation = 27 ± 12%; $p < 0.001$).

The reduction in the synaptic enhancement induced by activation of VDCCs by prior saturation of NMDA receptor–dependent LTP suggests that the two forms of synaptic potentiation may activate some common mechanisms. To test this possibility further, another occlusion experiment was performed, but in this case we examined the effects of prior TEA-induced synaptic potentiation on NMDA receptor–dependent

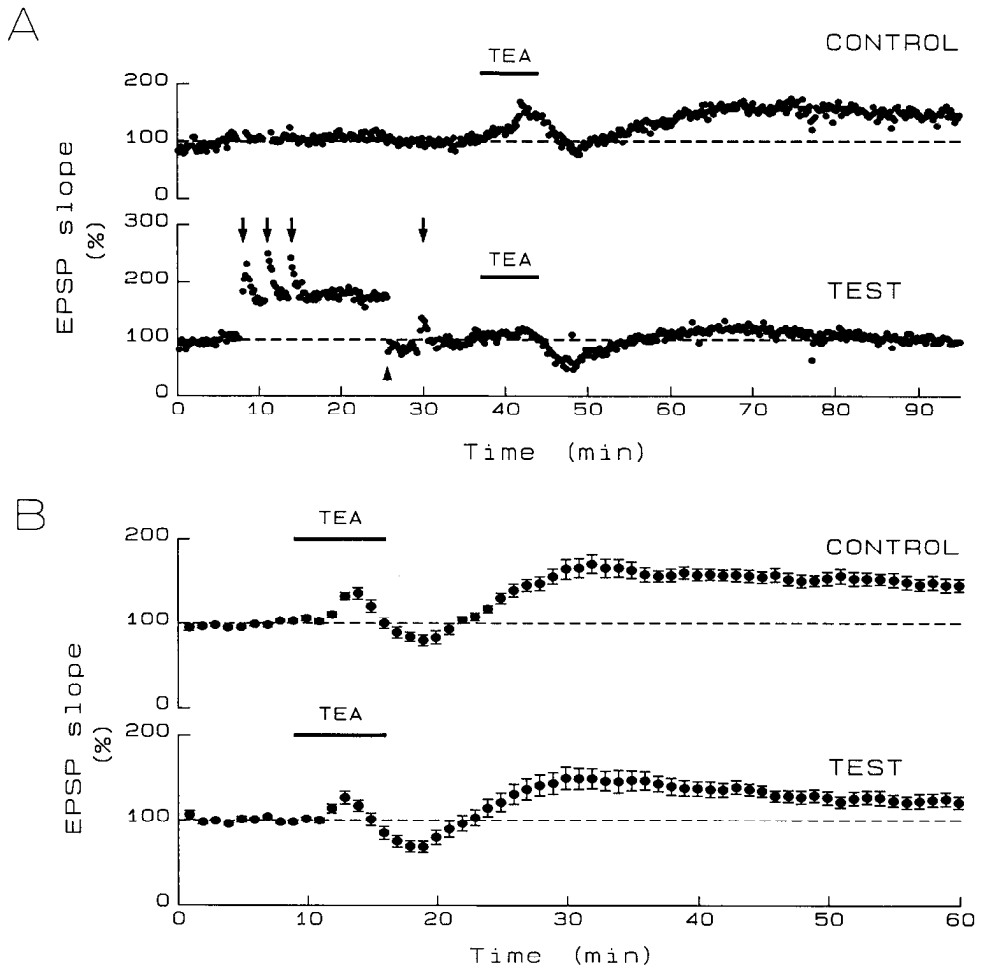

Figure 6. Tetanus-induced LTP partially occludes the TEA-induced synaptic enhancement. *A*, Example of experiment in which two independent pathways were alternately stimulated. At the *arrows*, the test pathway received a 100 Hz, 1 sec tetanus. At the *arrowhead*, test pathway stimulation strength was reduced so that the field EPSPs evoked by the control and test pathway stimulation were approximately the same amplitude. Note that after the reduction of stimulation strength, a tetanus caused no further potentiation in the test pathway. Subsequent application of TEA caused a larger synaptic enhancement in the control pathway than in the test pathway. *B*, Summary of experiments ($n = 9$) like that shown in *A*.

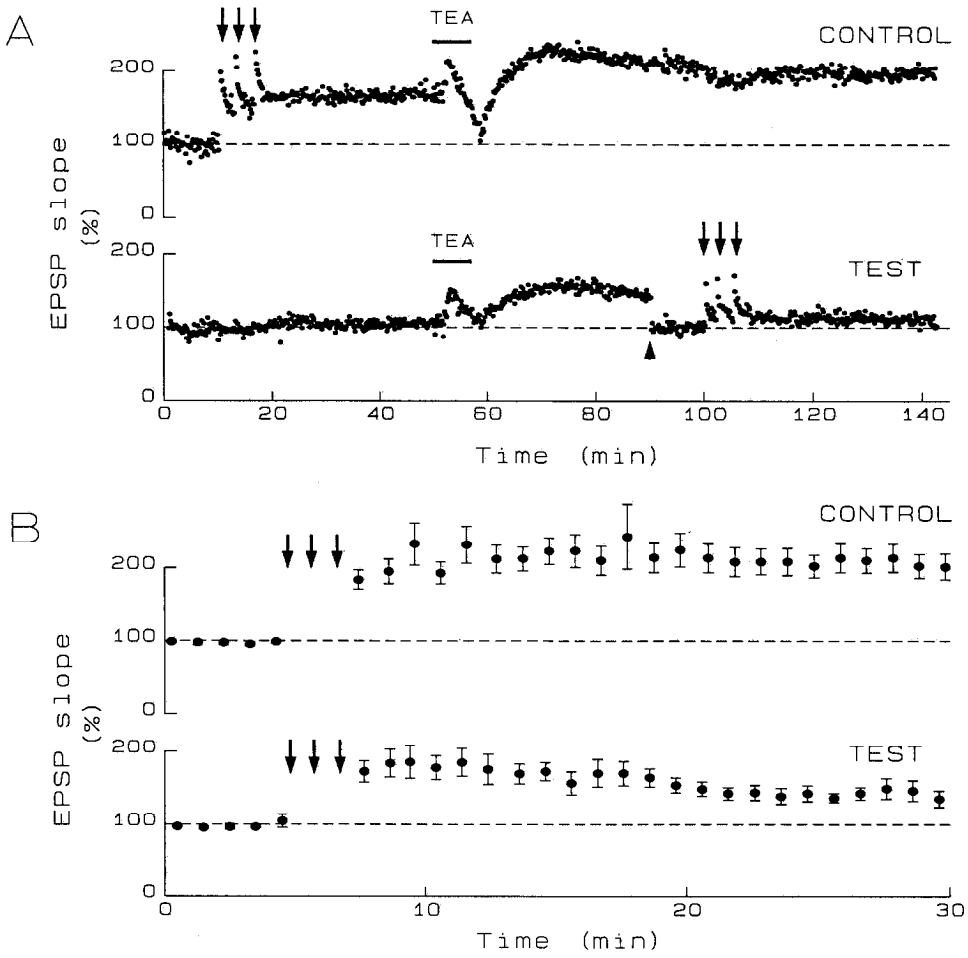

Figure 7. TEA-induced synaptic enhancement partially occludes tetanus-induced LTP. *A*, Example of experiment in which two independent pathways were alternatively stimulated. At the *arrows*, the pathway received a 100 Hz, 1 sec tetanus. Following application of TEA, stimulation strength in the test pathway was reduced (*arrowhead*) so that the EPSP was approximately the same size as the control EPSP prior to LTP induction. *B*, Summary of experiments ($n = 7$) like that shown in *A*.

LTP. Again, within-slice comparisons were made to reduce variability as much as possible. Figure 7*A* shows a typical experiment in which LTP was first induced in the control pathway and, following application of TEA, the same number of LTP-inducing tetani were given to the test pathway. In this case, potentiating synaptic transmission by application of TEA significantly reduced NMDA receptor–dependent LTP. Before applying tetani to the test pathway, stimulation strength was reduced to match the test EPSP with the control EPSP. As was the case in the preceding experiment, this was done to ensure that any differences in the magnitude of LTP were not due to differences in the size of the baseline responses. A summary of these experiments (Fig. 7*B*; $n = 7$) shows that TEA application reduced the magnitude of NMDA receptor–dependent LTP (measured 30 min after the final tetanus) by 61% (control potentiation = 119 ± 19%; test potentiation = 46 ± 11%, $p < 0.005$). Since the EPSPs evoked by test and control path stimulation were matched before inducing LTP, the magnitude of depolarization induced by the tetanus in either pathway should be comparable and the decrease in LTP following TEA application cannot be attributed to the lower stimulus strength used in the test path.

Several groups have demonstrated that the processes responsible for NMDA receptor–dependent LTP do not interact with those activated during PPF (McNaughton, 1982; Muller and Lynch, 1989; Zalutsky and Nicoll, 1990). If LTP and the synaptic enhancement elicited by application of TEA share common mechanisms, no effect on PPF should be observed. Figure 8, *A* and *B*, shows the effects on PPF (50 msec interstimulus interval) of application of TEA. Although the large increase in the field EPSP induced by TEA reduced PPF, when the EPSP was matched to the original control by reducing stimulation strength, the magnitude of PPF no longer was significantly affected. The decrease in PPF seen before controlling for EPSP size is therefore likely due to the synaptic conductance increase resulting from PPF causing less of a change in EPSP size in a large unclamped EPSP than in a smaller one. This occurs because of the decrease in driving force for synaptic currents (i.e., nonlinear summation) (Martin, 1955). Figure 8*C* is a summary of these experiments ($n = 8$) showing that the magnitude of PPF during the initial 10 min baseline period was not significantly different ($p > 0.05$) than its magnitude 60 min following TEA application and reduction of stimulation strength to match the potentiated EPSP initial slope with that of the control EPSP.

Discussion

We have confirmed that repetitive synaptic activation of Ca^{2+} spikes due to extracellular application of TEA causes a long-lasting increase in the strength of synaptic transmission in CA1 pyramidal cells (Aniksztejn and Ben-Ari, 1991). Several lines of evidence indicate that this synaptic enhancement is due to activation of postsynaptic, dihydropyridine-sensitive VDCCs. First, the TEA-induced synaptic enhancement is blocked by the VDCC antagonists nifedipine or flunarizine (Aniksztejn and Ben-Ari, 1991). Second, it is blocked by intracellular injection of the Ca^{2+} chelator BAPTA. Third, direct repetitive activation of

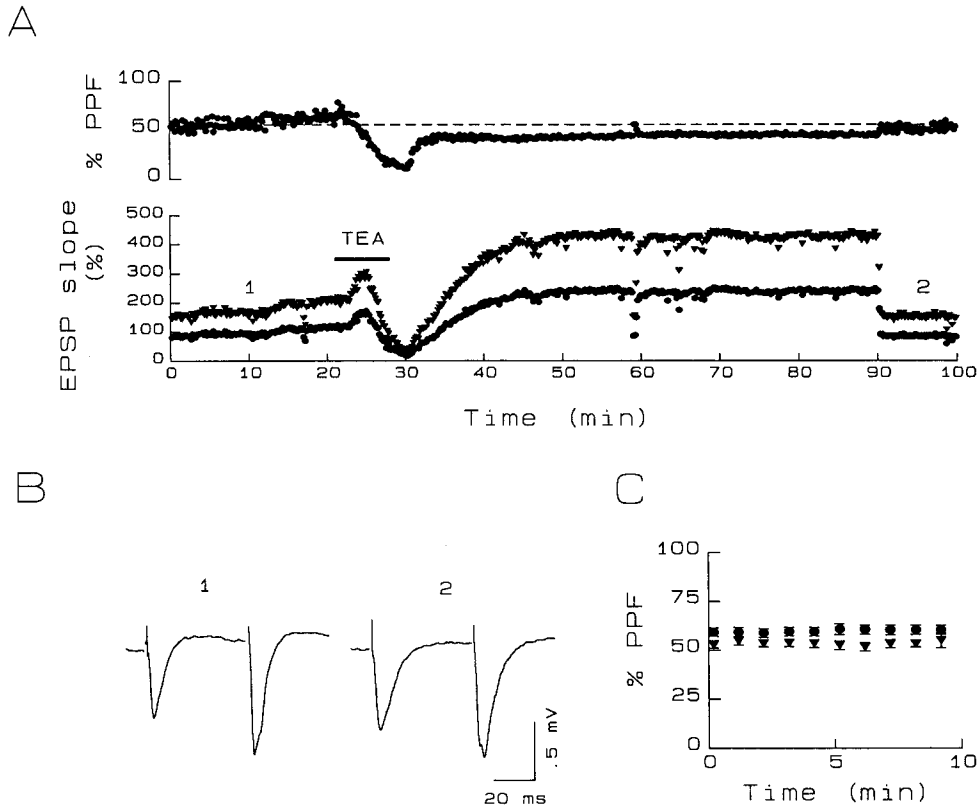

Figure 8. PPF is not significantly affected during the TEA-induced synaptic enhancement. *A*, Example of an experiment in which PPF (50 msec interstimulus interval) was monitored continuously before and after TEA application. *Top graph* shows the percentage facilitation during the course of the experiment. *Bottom graph* shows EPSP slope in response to the first (*circles*) or second (*triangles*) of the paired stimuli. At 90 min, stimulation strength was reduced so that the EPSP matched the control EPSP prior to TEA application. Note that reducing stimulus strength returned the percent facilitation to control values. *B*, Raw data traces taken at the times indicated by the *numbers* on the graph in *A*. *C*, Summary of experiments ($n = 8$) like that shown in *A*. *Circles* show mean percentage facilitation during the 10 min prior to TEA application. *Triangles* show mean percentage facilitation 60 min following TEA application and following reduction of stimulation strength. Points on the two graphs were not significantly different ($p > 0.05$).

VDCCS with depolarizing current pulses causes synaptic enhancement (Perkel et al., 1991). A previous examination of the effects on synaptic transmission of applying repetitive depolarizing current pulses to evoke Ca^{2+} spiking failed to observe a synaptic enhancement (Malenka et al., 1989a), likely because VDCCs were not activated sufficiently and thus the net change in intracellular Ca^{2+} did not reach a critical level. Recent Ca^{2+} imaging experiments in hippocampal slices have demonstrated that depolarizing current pulses delivered to the soma do not cause significant increases in dendritic Ca^{2+} whereas Ca^{2+} spikes evoked in the presence of TEA result in large increases in intracellular Ca^{2+} throughout the dendritic tree (Jaffe et al., 1992).

Although Ca^{2+} entry through L-type VDCCs can result in a potentiation of synaptic transmission, this source of Ca^{2+} does not appear to contribute to the rise in postsynaptic Ca^{2+} that is required for NMDA receptor–dependent LTP. Nifedipine, which was shown to block TEA-induced synaptic enhancement completely, had no effect on tetanus-induced LTP. If dihydropyridine-sensitive VDCCs contributed to LTP induction, some effect on either the magnitude or duration (Malenka, 1991) of LTP should have been observed. The effects of nifedipine were not tested on LTP induced by pairing synaptic stimulation with postsynaptic depolarization. However, the mechanisms responsible for NMDA receptor–dependent LTP appear to be independent of the method of induction (Gustafsson et al., 1987), and it therefore seems safe to conclude that L-type VDCCs also do not contribute to LTP when it is induced by pairing. The well-documented observation that D-APV completely blocks any synaptic enhancement except posttetanic potentiation (Collingridge et al., 1983; Gustafsson et al., 1987; Malenka, 1991) also is consistent with the proposal that L-type VDCCs do not contribute to the induction of LTP except when very high-frequency (200 Hz) tetani are applied (Grover and Teyler, 1990). Since L-type VDCCs do not contribute to the induction of LTP under most circumstances, it seems reasonable to continue to propose that they either are absent from dendritic spines or, if present, do not contribute significantly to the requisite rise in Ca^{2+} (Malenka et al., 1989a). However, some sort of contributory role of N-type VDCCs or T-type VDCCs in LTP cannot be ruled out by our experiments.

Saturation of NMDA receptor–dependent LTP significantly reduced the subsequent synaptic enhancement produced by activation of L-type VDCCs. Similarly, potentiating synaptic transmission with TEA reduced subsequent NMDA receptor–dependent LTP. These results suggest that activation of some common intracellular process or some common synaptic modification occurs following a rise in postsynaptic Ca^{2+} either due to Ca^{2+} influx solely through VDCCs or through the NMDA receptor ionophore. One proposal that could account for these results is that during normal LTP induction, Ca^{2+}-dependent biochemical processes localized within the spine are activated by a rise in Ca^{2+} solely due to Ca^{2+} entry via the NMDA receptor ionophore. Under these circumstances, any rise in Ca^{2+} due to entry via L-type VDCCs is buffered and does not significantly affect Ca^{2+} levels within spines. Only when L-type VDCCs are repetitively activated for many seconds does this rise in Ca^{2+} overcome endogenous buffering mechanisms and activate the processes within the spine that are responsible for LTP. Consistent with this proposal are recent Ca^{2+} imaging experiments in which application of APV blocked the synaptically evoked accumulation of Ca^{2+} in "hot spots" believed to be dendritic spines while the simultaneous increase in dendritic shaft Ca^{2+} was minimally affected by APV (Muller and Connor, 1991). The finding that repetitive activation of VDCCs with current pulses often resulted in short-term potentiation (Fig. 4) also is consistent with this proposal since this manipulation would be

expected to elicit less of an increase in intraspine Ca^{2+} than activation of NMDA receptors (Malenka, 1991). However, since stimulation of presynaptic fibers was stopped during application of the repetitive depolarizing current pulses, an alternative explanation is that presynaptic activity contributes some ingredient required for a stable, long-lasting increase in synaptic efficacy (Kauer et al., 1988).

The lack of complete occlusion or inhibition of one form of synaptic enhancement by the other does not negate the presented hypothesis but does suggest that there may also be some differences between TEA-induced potentiation and LTP. This would occur if application of TEA has additional effects on synaptic transmission not directly due to the processes activated during LTP. For example, it has been reported that TEA application increases the phosphorylation of synapsin I at the site normally phosphorylated by CaMKII (Dudek et al., 1991), raising the possibility that TEA may have direct presynaptic actions. Because VDCCs can result in large increases in dendritic Ca^{2+} throughout the dendritic tree (Regehr et al., 1989; Jaffe et al., 1992), TEA application may also result in potentiating synaptic transmission at a much higher percentage of the synapses contributing to the evoked EPSP than occurs during LTP. This could explain why saturating tetanus-induced LTP only partially reduced the TEA-induced synaptic enhancement. A final intriguing possibility is that activation of NMDA receptors, but not VDCCs, causes stimulation of some process that results in an inhibition of LTP induction (Huang et al., 1992). This inhibitory process may normally contribute to the saturation of LTP seen with repetitive tetani (Bliss and Lomo, 1973; McNaughton et al., 1978) and may not have been activated during the TEA-induced synaptic enhancement.

Recent experiments in which Ca^{2+} changes within dendritic spines were directly observed (Guthrie et al., 1991; Muller and Connor, 1991) demonstrated that spines can function as isolated compartments in which Ca^{2+} levels are independently regulated. The results presented in this article support the hypothesis that synapse specificity in LTP is achieved by providing each spine with a private source of Ca^{2+} that is due to NMDA receptor activation (Wigström and Gustaffson, 1985) and is not normally influenced by Ca^{2+} entry via VDCCs. However, these and previous results (Grover and Teyler, 1990; Anikstejn and Ben-Ari, 1991; Perkel et al., 1991) also demonstrate that intracellular increases in Ca^{2+} due to VDCCs can cause significant changes in synaptic efficacy in CA1 cells and may contribute to long-lasting changes at other synapses (Johnston et al., 1992). Such changes may occur most prominently in pathological hyperexcitable states and thus may play a significant role in such phenomena as kindling and epileptogenesis.

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