Dendritic K^+ channels contribute to spike-timing dependent long-term potentiation in hippocampal pyramidal neurons

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Communicated by Bert Sakmann, Max Planck Institute for Medical Research, Heidelberg, Germany, April 8, 2002 (received for review February 8, 2002)

We investigated the role of A-type K^+ channels for the induction of long-term potentiation (LTP) of Schaffer collateral inputs to hippocampal CA1 pyramidal neurons. When low-amplitude excitatory postsynaptic potentials (EPSPs) were paired with two postsynaptic action potentials in a theta-burst pattern, *N*-methyl-D-aspartate (NMDA)-receptor-dependent LTP was induced. The amplitudes of the back-propagating action potentials were boosted in the dendrites only when they were coincident with the EPSPs. Mitogen-activated protein kinase (MAPK) inhibitors PD 098059 or U0126 shifted the activation of dendritic K^+ channels to more hyperpolarized potentials, reduced the boosting of dendritic action potentials by EPSPs, and suppressed the induction of LTP. These results support the hypothesis that dendritic K^+ channels and the boosting of back-propagating action potentials contribute to the induction of LTP in CA1 neurons.

Pairing subthreshold excitatory postsynaptic potentials (EPSPs) with back-propagating action potentials has been reported to result in the amplification of dendritic action potentials and the induction of long-term potentiation (LTP) in the dendrites of hippocampal CA1 pyramidal neurons (1). Furthermore, the presence of the action potentials in the dendrites was shown to be required for this LTP-induction protocol. These findings led to the suggestion that amplification or boosting of dendritic action potentials might provide the postsynaptic depolarization required for the unblocking of *N*-methyl-D-aspartate (NMDA) receptors to induce Ca influx that is necessary for induction of LTP (2–4).

Several studies have shown that induction of LTP by pairing postsynaptic action potentials with EPSPs requires that the spikes coincide with the EPSPs within a narrow time window (5–8). If the spikes occur about 10–20 ms before the EPSP, long-term depression is induced; if they occur within about 10–20 ms from the beginning of the EPSP, LTP is induced; and if AP and EPSP are separated by more than 100 ms, no plasticity is elicited (5, 6). This precise timing relationship between the postsynaptic action potentials and the EPSPs is somewhat surprising, given that the deactivation rate for NMDA receptors is around 200 ms (9). One possible explanation for this narrow time window is that the boosting of the amplitude of the dendritic action potential by the increased activation of dendritic Na^+ channels (10) and/or inactivation of dendritic K^+ channels (2) is required for LTP induction. Here, we explore the relationship between the boosting of action potential and LTP induction in terms of spike-timing. We also tested the involvement of K^+ channels in this relationship by using the mitogen-activated protein kinase (MAPK) inhibitor U0126, which was found to increase dendritic K^+ currents. The results provide strong support for the hypothesis that spike boosting is required for this form of pairing-induced LTP, and that dendritic K^+ channels play a critical role in this phenomenon.

Materials and Methods

Preparation of Slices and Solutions. Hippocampal slices (350 μm) were prepared from 5- to 8-week-old Sprague–Dawley rats as described (11, 12). All experimental procedures were approved by the Animal Research Committee of the Baylor College of Medicine. A Zeiss Axioskop, fitted with a 40× Zeiss water-immersion objective and differential interference contrast (DIC) optics, was used to view slices. The bathing solution contained 125 mM NaCl, 2.5 mM KCl, 1.25 mM NaH_2PO_4, 25 mM $NaHCO_3$, 2.5 mM $CaCl_2$, 1.5 mM $MgCl_2$, and 10 mM dextrose, and was bubbled with 95% O_2/5% CO_2 at 31–35°C. The γ-aminobutyric acid (GABA) type A ($GABA_A$) receptor antagonist (−)-bicuculline methiodide (10 μM) was present in the bathing solution during recording. Where specified, one or more of the following drugs was included in the media: NMDA-receptor antagonists DL-2-amino-5-phosphonovaleric acid (APV; 50 μM) and MK-801 (20 μM), dissolved directly into the media, and U0126 (Promega), prepared from a stock solution dissolved in DMSO (final concentration of DMSO was 0.2%).

Recording and Stimulating. Area CA3 was removed from each slice before recording. Whole-cell recording pipettes (soma, 2–4 MΩ; dendrite, 7–10 MΩ) were pulled from borosilicate glass and filled with 120 mM potassium gluconate/20 mM KCl/10 mM Hepes/2 mM $MgCl_2$/4 mM disodium adenosine 5′-triphosphate/0.3 mM magnesium guanosine 5′-triphosphate/14 mM phosphocreatine (pH 7.25 with KOH). Tetrapotassium 1,2-bis(2-aminophenoxy)ethane-*N,N,N′,N′*-tetraacetate (BAPTA) (100 μM; Sigma) was added to the recording pipette daily before experiments. Whole-cell patch-clamp recordings were made from the visually identified CA1 pyramidal somata and dendrites with a Dagan IX2–700 amplifier (Dagan Instruments, Minneapolis) in "bridge" modes. The resting membrane potential (V_m) was between −60 and −74 mV. Series resistance for somatic recording was 15–30 MΩ, whereas for dendritic recordings it was 15–70 MΩ. All LTP experiments were done in current clamp. Schaffer collateral fibers were stimulated electrically with a bipolar microelectrode (glass pipette with tip diameter of ≈4 μm, filled with media solution, glued to a fine tungsten rod) and placed in the stratum radiatum (240–260 μm from the edge of the pyramidal cell layer) at a lateral distance of 50–100 μm from the proximal dendrite. Axonally initiated action potentials were elicited with somatic depolarizing current injection, usually 2 nA for 2 ms, or antidromic stimulation by means of a stimulating electrode in the alveus. Test stimuli were delivered every 15 s,

Abbreviations: LTP, long-term potentiation; EPSP, excitatory postsynaptic potential; NMDA, *N*-methyl-D-aspartate; APV, DL-2-amino-5-phosphonovaleric acid; TBP, theta-burst pairing; MAPK, mitogen-activated protein kinase; MEK, MAPK kinase; ERK, extracellular signal-regulated kinase.

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www.pnas.org/cgi/doi/10.1073/pnas.122210599

and a hyperpolarizing current pulse (30 pA, 150 ms) was injected into the cell after the test stimulus to monitor input resistance and series resistance. Stimulus intensity was adjusted to produce EPSPs 2–2.5 mV in amplitude. Slope measurements of EPSP were made from a line fitted to the rising phase of the EPSP. LTP was operationally defined as >50% increase above the baseline for the slope of the EPSP rising phase from 15–17 min after theta-burst pairing (TBP). Data are reported as mean ± SEM. For cell-attached patch recordings, the pipettes (10–20 MΩ) were coated with Sylgard (Dow-Corning), and the tips were visually inspected for tip diameter of ≈1 μm. All neurons exhibited a resting membrane potential between −55 and −73 mV, and this potential was the same for both controls and with the inhibitor of mitogen-activated protein kinase (MAPK) kinase (MEK). A 150-ms pulse from −85 to +55 mV in 20-mV intervals was used to record the total outward current. A 50-ms prepulse to −25 mV was included to inactivate the transient channels, resulting in the isolation of the sustained channels. Digital subtraction of the sustained trace from the total outward current resulted in the isolated transient currents. Sweeps (10–30) were averaged for each test potential. Channel recordings, using an Axopatch 1D amplifier (Axon Instruments, Foster City, CA), were analog filtered at 2 kHz and digitally filtered at 1 kHz. Leakage and capacitive currents were digitally subtracted (12). Activation plots were calculated as described (12, 13). All dendritic recordings were greater than 180 μm from the soma. Data were binned into 10-mV compartments. Significance ($P < 0.05$) was determined by two-sample t tests.

Computer Simulation. All simulations were carried out by using the NEURON simulation environment (14) and a published model of a CA1 pyramidal neuron (15). The model and simulation files are publicly available under the ModelDB section of the Senselab database (http://senselab.med.yale.edu).

Results

Timing Dependence of the Induction of LTP. We first investigated the relative timing between postsynaptic action potentials and EPSPs for LTP induction by using a theta-burst stimulus paradigm. Whole-cell patch-clamp recordings were obtained from visually identified CA1 pyramidal neurons in adult hippocampal slices (Fig. 1*a*). EPSPs ≈2 mV in amplitude were evoked by a glass stimulus electrode placed in stratum radiatum about 240 μm distal to the pyramidal cell layer. We induced LTP with a theta-like stimulation pattern shown in Fig. 1*b*. We used two different protocols for LTP induction (Fig. 1*c*). In the first, there was a 35-ms delay between the onset of the train and the first action potential, with the action potentials coinciding with the approximate peaks of each of the last two EPSPs in the train. With this protocol, LTP was induced with a magnitude and time course that appeared similar to that described in ref. 1 (Fig. 1*d*). Unpaired EPSP bursts and postsynaptic action potentials (1 min apart) did not induce LTP (221% ± 17%, $n = 14$, vs. unpaired 129% ± 23%, $n = 6$, $P < 0.01$; Fig. 1*d*). In the second protocol, we increased the delay by 10 ms so that the first postsynaptic action potential coincided with the last EPSP in the train and the second action potential occurred 10 ms after the last EPSP. Significantly less potentiation was observed with this protocol (135% ± 21%, $n = 7$, $P < 0.01$; Fig. 2). In addition, with the 35-ms delay protocol there was a significantly higher probability of inducing LTP than with the 45-ms protocol (93% vs. 29%; Fig. 2*c*). The LTP with the 35-ms protocol was suppressed by bath application of the NMDA receptor antagonists APV and MK-801 (138% ± 28%, $n = 7$, $P < 0.05$; probability of LTP 43%; Fig. 2 *d* and *e*), suggesting that the LTP was largely dependent on NMDA receptors.

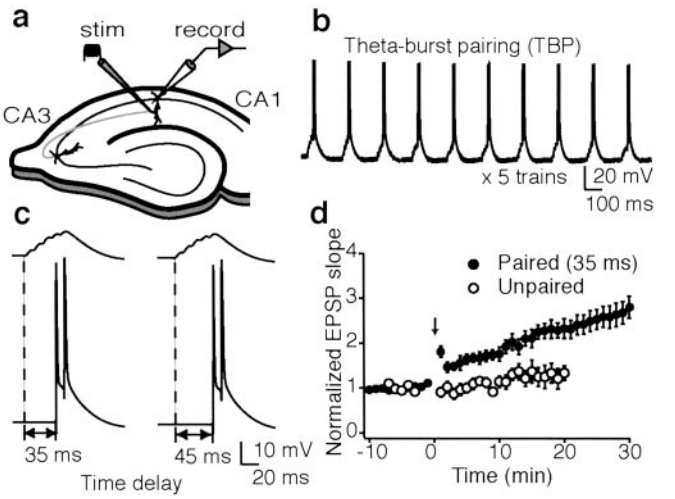

Fig. 1. Pairing of subthreshold synaptic stimulation and action potential trains induces LTP. (*a*) Schematic of a hippocampal slice showing stimulating (stim) and recording (record) sites. (*b*) A representative response evoked by one train of LTP induction protocol (five trains, given every 15 s, each consisting of ten bursts of synaptic stimuli at 5 Hz with back-propagating action potential, each burst consisting of five subthreshold synaptic stimuli at 100 Hz and two action potentials at 100 Hz.) (*c*) Representative traces of subthreshold synaptic response and current-induced back-propagating action potential at a faster time scale. Time delay is defined as the delay between the onset of synaptic input and the first action potential. (*d*) Time course and magnitude of potentiation evoked by pairing (35-ms time delay) and unpairing protocols. The magnitude of LTP induced with 35-ms time delay differed significantly from the potentiation evoked by unpaired protocol [35-ms time delay, 221% ± 17%, $n = 14$; 1-min time delay (unpaired), 129% ± 23%, $n = 6$, $P < 0.01$; both are averages measured at 15–17 min after TBP]. These LTP experiments were done in a saline solution (see *Materials and Methods*) with and without 0.2% DMSO. Because there was no significant difference in the magnitude of LTP between the two groups (LTP without DMSO, 227% ± 21%, $n = 11$; LTP with DMSO, 200% ± 23%, $n = 3$, $P > 0.1$), the data were grouped together into the control group.

Boosting of Action Potentials and LTP Induction. We have shown (1, 2, 13) that local EPSPs can inactivate transient K^+ channels and lead to an increase in the amplitude of dendritic action potentials. If the inactivation of dendritic, A-type K^+ channels contributes to this LTP induction, the boosting of back-propagating action potentials would be expected to depend on the relative timing between spikes and subthreshold EPSPs, in keeping with the rate of inactivation (13) and rate of recovery from inactivation (2) of the dendritic K^+ channels. To test this hypothesis, we recorded from the dendrites (Fig. 3*a*) at about 260 μm from soma to measure the amplitudes of back-propagating action potentials elicited at different time delays with the EPSPs. Back-propagating action potentials were elicited by a stimulating electrode placed in the alveus, and subthreshold EPSPs were evoked by means of a stimulating electrode situated near the recording pipette. In the 35-ms pairing protocol that induced LTP, the amplitudes of both the first and second action potentials were increased or boosted as compared with control (12.8% ± 2.3% and 12.4% ± 6.4%, $n = 7$, $P < 0.0009$ and $P < 0.017$; Fig. 3 *b* and *c*). This boosting was defined as the increase in action potential amplitude found by measuring the action potential amplitudes first without EPSPs, then measuring after pairing with EPSPs, and subtracting an identical train of EPSPs without action potentials. In the 45-ms pairing protocol, the amplitude of only the first action potential was boosted from the unpaired condition, whereas the second action potential, which occurred 10 ms after the last EPSP in the train, was not (17.0% ± 5% and −0.87% ± 1.9%, $P < 0.0008$ and $P = 0.4$; Fig. 3 *b* and *c*). These

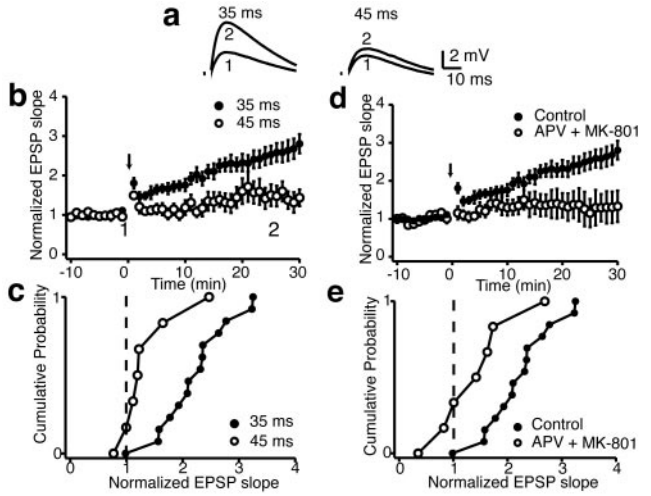

Fig. 2. LTP induction depends on the timing between subthreshold synaptic stimulation and postsynaptic action potentials. (*a*) Representative EPSP responses before (trace 1) and 25 min after (trace 2) TBP. LTP induced with a 35-ms delay is of significantly greater magnitude than with a 45-ms delay. (*b*) Time course and magnitude of potentiation evoked by the different time delay stimulation protocols. The magnitude of synaptic response change induced with a 35-ms time delay protocol differed significantly from synaptic response change with a 45-ms time delay protocol (35-ms time delay, 221% ± 17%, $n = 14$; 45-ms time delay, 135% ± 21%, $n = 7$, $P < 0.01$). (*c*) Cumulative probability plots graphically summarize the data. Each point represents the magnitude of change relative to baseline for a given experiment 15–17 min (average) after TBP (probability of 50% long-term change in EPSP with 35-ms time delay, 93%, with 45-ms time delay, 29%). (*d*) NMDA-receptor antagonists APV (50 μM) and MK-801 (20 μM) suppressed potentiation elicited with the 35-ms protocol compared with control (221% ± 17%, $n = 14$, and 138% ± 28%, $n = 7$, respectively; $P < 0.05$). (*e*) Cumulative probability for control and drug-treated slices (probability of 150% LTP was 43%).

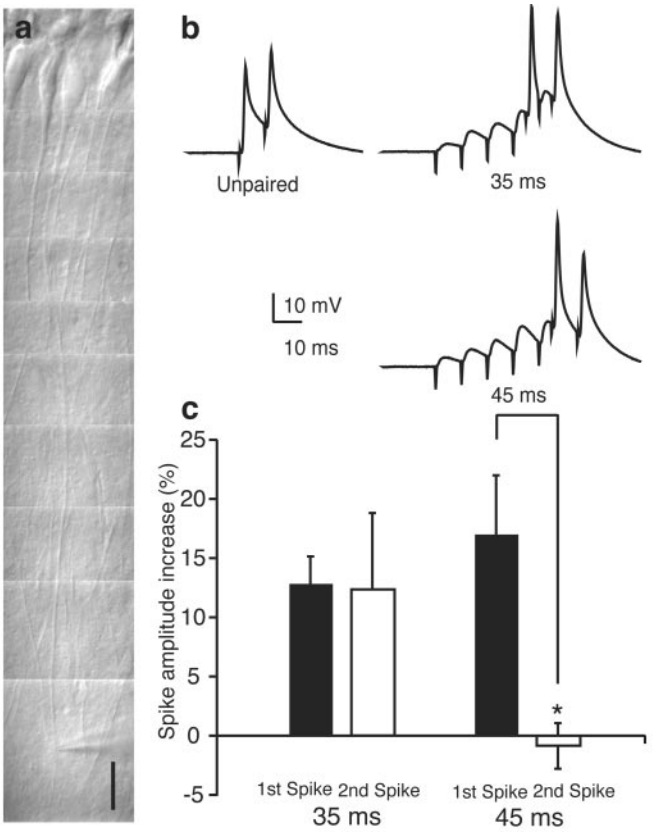

Fig. 3. Boosting of dendritic action potential amplitude paired by subthreshold synaptic response depends on pairing timing. (*a*) IR-differential interference contrast image of CA1 pyramidal neuron and recording site in the dendrites. (Bar = 20 μm.) (*b*) Representative membrane voltage traces from dendritic recording. (*c*) Summary of change in the amplitude of back-propagating action potential paired with EPSPs. There was a supralinear increase in the amplitude of both the first and second action potentials with the 35-ms protocol but only in the first action potential with the 45-ms paradigm (35 ms: first spike 12.8% ± 2.3%, second spike 12.4% ± 6.4%, $n = 7$, both different from unpaired, $P < 0.0009$ and $P < 0.017$, respectively; 45 ms: first spike 17.0% ± 5%, different from unpaired, $P < 0.0008$, second spike −0.87% ± 1.9%, not different from unpaired, $P = 0.4$, $n = 7$; difference between first spike and second spike, $P < 0.01$). The amplitude was normalized by the amplitude of unpaired back-propagating action potentials.

data suggest that the boosting of back-propagating action potentials occurs only when they coincide with EPSPs.

Effect of MEK Inhibitor on K^+ Channel. A means of modulating K^+-channel activity directly would be desirable to support the involvement of the dendritic K^+ channel in these phenomena. Unfortunately, 4-aminopyridine, which blocks these channels at relatively high concentrations, also has major presynaptic effects and cannot be used with intact synaptic transmission. In previous studies, Hoffman and Johnston found that the activity of transient K^+ channels in dendrites is decreased by the activation of protein kinases A and C (PKA and PKC) (12). Moreover, it has recently been demonstrated that the PKA and PKC modulation of these channels is mediated through a common signaling cascade leading to the activation of the extracellular signal-regulated kinase (ERK) isoform of MAPK (16, 17, 36) and that there may be a basal level of ERK activity at rest (18, 19). We therefore tested the effects of inhibitors of ERK activation PD 098059 and U0126 on K^+-channel activity measured with cell-attached patch recordings. Both PD 098059 and U0126 are capable of inhibiting activated MEK1 and preventing endogenously activated MEK1/2 from phosphorylating and activating ERK1/2 MAPK (17, 20). Wash-in of PD 098059 (50 μM) or U0126 (20 μM) both produced small but significant negative shifts in the transient current activation curve of 5–7 mV (PD 098059: $V_{1/2} = -9$ mV vs. −2 mV in control, $n = 7$, $P < 0.0005$; U0126: $V_{1/2} = -7$ mV, $n = 9$, $P < 0.0005$; Fig. 4*a*), suggesting that there is constitutively active MAPK in dendrites. The inhibition of this basal kinase activity thus produced an *increase* in the amplitude of transient K^+ current within the physiological range of membrane potentials (Fig. 4*a Inset*). We also made dendritic whole-cell recordings and measured the amplitudes and the rate of rise of back-propagating action potentials in dendrites before and after applying U0126. We found that the inhibition of MAPK decreased the amplitudes of the action potentials with no change in their initial rate of rise (amplitude: −9.3% ± 2.4%, $P < 0.003$; rate of rise: 1.6% ± 5.3%, $P = 0.18$, $n = 17$; Fig. 4*b*), showing that MAPK inhibition caused an increase in K^+-channel availability without affecting Na^+ currents (12).

Effect of MEK Inhibitor on Spike Boosting and LTP Induction. Based on the above results, we further examined the contribution of transient A-type K^+ channels to spike boosting and LTP induction. We tested whether U0126, which increased the availability of A-type K^+ currents, suppressed the boosting of back-propagating action potentials. Slices were incubated in artificial cerebrospinal fluid (ACSF) containing 20 μM U0126 for more than 20 min before making patch-clamp recordings, and ACSF containing U0126 was kept perfusing throughout the experiment. U0126 significantly reduced the boosting of both the first and second action potentials when paired with the EPSPs in the

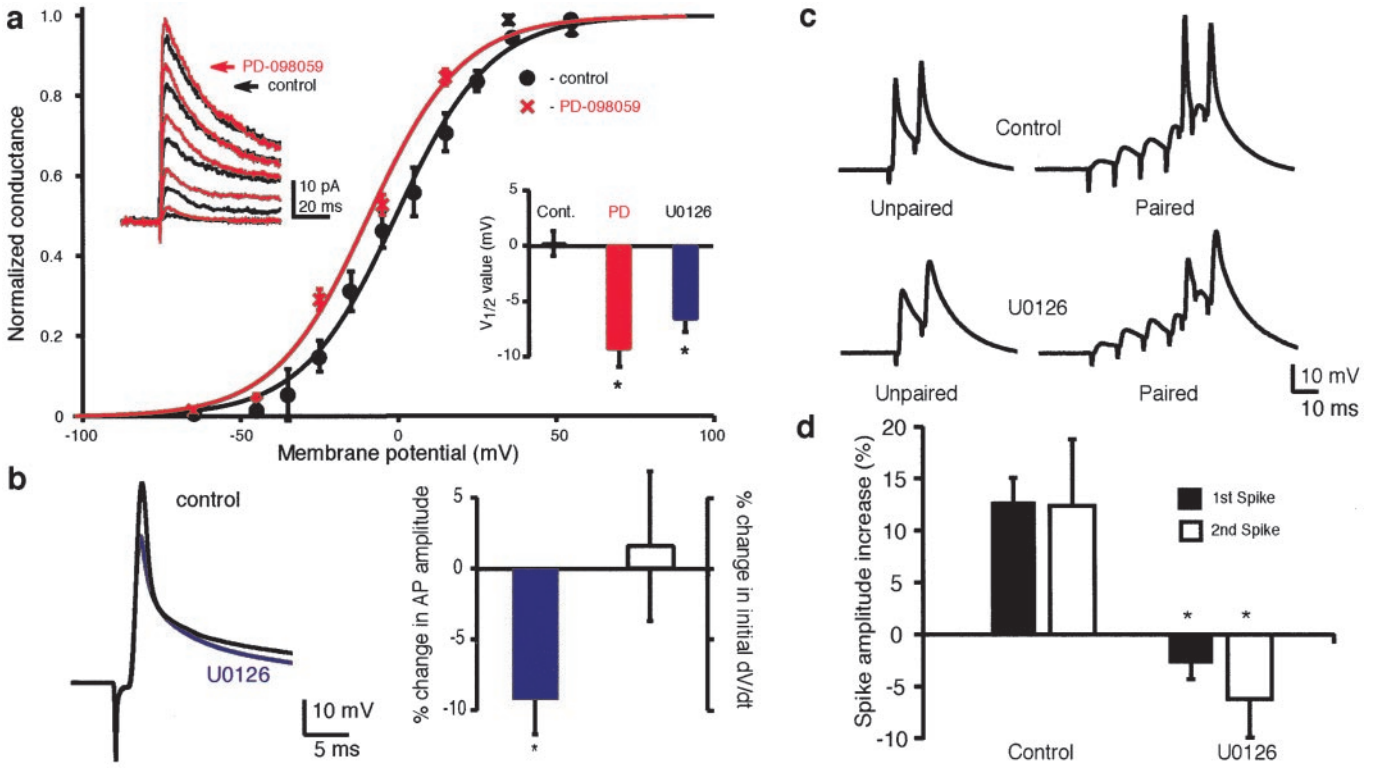

Fig. 4. Inhibition of basal MAPK activity shifts the K^+-channel activation curve, up-regulates dendritic K^+ currents, decreases the amplitude of back-propagating action potentials, and decreases the boosting of dendritic action potential amplitude when paired with subthreshold synaptic responses. (*a*) Steady-state, transient K^+ current activation curves for control and 50 μM PD 098059. Half-activation with PD 098059 ($V_{1/2} = -9$ mV, $n = 7$) was shifted 7 mV in the hyperpolarizing direction compared with controls ($V_{1/2} = -2$ mV, $n = 10$, $P < 0.0005$). In six of seven recordings, PD 098059 was applied to the slice at least 20 min before recording; in one case it was washed in subsequent to construction of a control-activation curve. (*Left Inset*) Family of total outward currents for the experiment where PD 098059 was washed in after recording the control activation curves. Control (black) traces show less transient current at all potentials compared with traces in PD 098059 (red traces). (*Right Inset*) Summary data for change in $V_{1/2}$ for all experiments. Similar results were obtained with another MEK inhibitor, U0126 (20 μM). Half-activation with U0126 ($V_{1/2} = -7$ mV, $n = 9$) was also shifted hyperpolarized as compared with controls ($V_{1/2} = -2$ mV, $n = 10$, $P < 0.0005$). In six of the nine experiments, U0126 was included in the patch pipette, in the other three it was applied to the slice at least 20 min before recording. (*b*) Antidromic action potentials (*Left*) recorded in the dendrites about 180 μm from the soma before and after applying 20 μM U0126. Summary data for change in amplitude and initial rate of rise are shown on the right (decrease in amplitude = 9.3% ± 2.4%, $P < 0.003$; change in initial dV/dt = 1.6% ± 5.3%, $P = 0.18$, $n = 17$). In some experiments, 1 μM 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulfonamide (NBQX) and 50 μM APV were included in the bath to avoid antidromically activated EPSPs. Experiments were done with both potassium gluconate and $KMeSO_4$-based pipette solutions with similar results (see *Materials and Methods*). (*c*) Representative membrane potential traces from dendritic recordings in control (*Upper*) and in a slice pretreated with U0126 (*Lower*). (*d*) Summary of the effects of U0126 on the amplitude of back-propagating action potential paired with EPSPs (Control: first spike 12.8% ± 2.3%, second spike 12.4% ± 6.4%; U0126: first spike −2.8% ± 1.5%, second spike −6.2% ± 3.7%, $n = 5$, both different from control, $P < 0.00025$ and 0.033, respectively; neither different from unpaired in U0126, $P = 0.22$ and 0.11, respectively). The amplitude was normalized by the amplitude of unpaired back-propagating action potentials.

35-ms delay paradigm (12.8% ± 2.3% vs. −2.8% ± 1.5%; 12.4% ± 6.4% vs. −6.2% ± 3.7%, $n = 5$, $P < 0.00025$ and $P < 0.033$; Fig. 4 *c* and *d*). Consistent with the correlation between spike boosting and LTP induction shown above, we also found that U0126 significantly reduced both the magnitude and probability of inducing LTP with this protocol (U0126: 122% ± 6%, $n = 6$, $P < 0.0001$; Fig. 5 *b* and *c*). To test whether U0126 might be suppressing LTP induced with this paradigm through a mechanism downstream of K^+ channels and dendritic spike amplitude, we increased the stimulus protocol by pairing five spikes with the EPSPs instead of two. We found that with this five-spike paradigm, in which presumably there was greater depolarization at the synaptic sites than with just two spikes, LTP was induced (five spikes paired with five EPSPs in U0126: 243% ± 32%, $n = 6$, $P < 0.012$; Fig. 5 *e* and *f*).

Computer Model. To test whether the hypothesis of EPSP-induced inactivation of K^+ channels as a mechanism for spike boosting was consistent with the known biophysical properties of CA1 cells, we used a computer model with a realistic morphology, including Na^+ and DR- and A-type K^+ channels (see ref. 15 for details). A similar theta-burst pattern of EPSP stimulation with two postsynaptic action potentials was used in the model. We found that when the action potentials coincided with EPSPs, both were boosted in a similar manner as found experimentally (Fig. 6, paired 35-ms traces). With the 45-ms delay paradigm, however, only the first action potential was boosted. To model the effect of U0126, we then shifted the activation curve for the transient K^+ channels by −5 mV and found a significant decrease in both spike amplitude and boosting (Fig. 6).

Discussion

These results provide strong support for the hypothesis that spike-timing-dependent LTP in CA1 neurons is mediated in part by the inactivation of A-type K^+ channels in dendrites and that the resulting increase in the amplitude of dendritic action potentials and the subsequent unblocking of NMDA receptors is a major contributor to the induction of LTP in these neurons.

Two different, but not mutually exclusive, mechanisms have been suggested for the pairing-induced boosting of dendritic

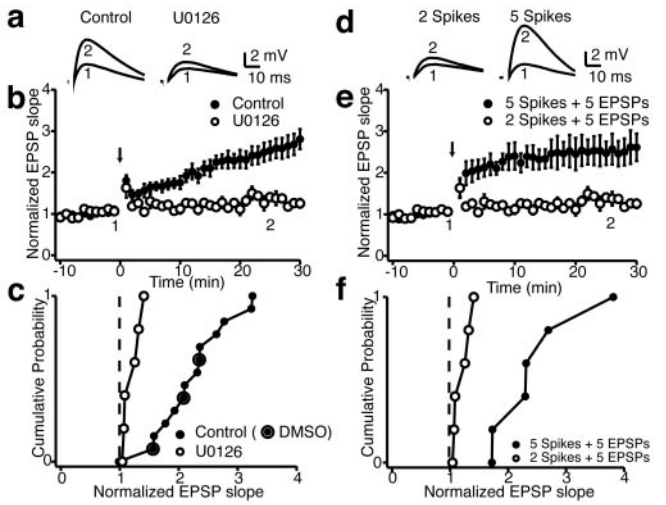

Fig. 5. Effects of MEK inhibitor U0126 on the induction of LTP with TBP. (*a*) Representative synaptic responses before (trace 1) and 25 min after TBP (trace 2) in control condition and in the presence of U0126 (20 μM). Significantly less potentiation occurred in the presence of U0126. (*b*) Summary data from all experiments. U0126 suppressed the induction of LTP by the 35-ms time delay TBP (control, 221% $\pm$ 17%, $n = 14$; U0126, 122% $\pm$ 6%, $n = 6$, $P < 0.0001$). (*c*) Cumulative probability plots summarize the suppression of LTP by U0126 using the 35-ms protocol. (*d*) Representative synaptic responses before (trace 1) and 25 min after TBP (trace 2) in which two spikes or five spikes were paired with EPSPs in the presence of U0126 (20 μM). (*e*) U0126 did not suppress the induction of LTP by TBP in which five spikes instead of two were paired with EPSPs (two spikes paired with five EPSPs, 122% $\pm$ 6%, $n = 6$; five spikes paired with five EPSPs, 243% $\pm$ 32%, $n = 6$, $P < 0.012$). (*f*) Cumulative probability plot summarizes the suppression of LTP by U0126 for the five-spike and two-spike protocols. Probability of LTP (defined as a 50% increase in averaged response amplitude 15–17 min after pairing) was 0% with two spikes and 100% with five spikes.

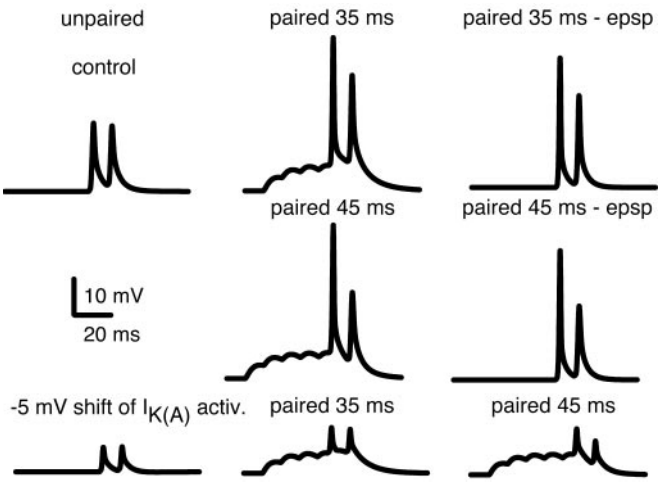

Fig. 6. Computer model for EPSP-induced spike boosting. All traces show membrane potential of a distal dendritic compartment (340 μm from the soma). Each synaptic stimulus was modeled as an alpha function with peak conductance of 1.5 nS, time constant of 6 ms, and reversal potential of 0 mV. (*Top*) Two somatic action potentials were elicited at 100 Hz unpaired (left trace) or paired (center trace) with a burst of five subthreshold synaptic stimuli at 100 Hz with a relative time delay of 35 ms. For comparison purposes, the result obtained in the paired case is shown after subtraction of the depolarization induced by the EPSPs (right trace). (*Middle*) As in top traces, but with somatic action potentials elicited with a relative time delay of 45 ms. (*Bottom*) Effects of a -5-mV shift in the activation curve for the dendritic transient K^+ conductance. Membrane potential is shown when back-propagating action potentials were unpaired (left trace) or paired with synaptic stimuli with a relative time delay of 35 ms (center trace) or 45 ms (right trace).

action potentials: increased activation of dendritic Na^+ channels and/or inactivation of dendritic K^+ channels. Stuart and Hausser showed that in distal dendrites of layer V pyramidal neurons, the pairing of EPSPs and back-propagating action potentials results in action-potential boosting that is largely dependent on an increased activation of dendritic Na^+ channels (10). In layer V neurons, however, there is a smaller gradient of transient K^+ channels in the apical dendrite than in CA1 pyramidal neurons (21–23). Also, Stuart and Hausser made whole-cell recordings at sites in the dendrites where the action potentials were no longer actively propagated. Under these conditions, it would not be expected that dendritic K^+ channels would contribute significantly to the boosting of back-propagating action potentials. In contrast, CA1 neurons in adult animals have an increasing gradient of transient K^+-channel density of at least 5-fold from the soma to about 350 μm into the apical dendrites (13). Furthermore, at the sites we recorded from (250–300 μm), the back-propagating action potential was still regenerative, although suppressed in amplitude by the high K^+-channel activity. Under these conditions, the inactivation of K^+ channels by the depolarization of the EPSPs is more likely to occur and affect dendritic-spike amplitude. It is certainly possible that the EPSPs also increase Na^+-channel activation, which would then contribute to spike-boosting. In an attempt to separate and study these two possible mechanisms independently, we capitalized on our observation that the MAPK inhibitor U0126 increased dendritic transient K^+-channel activity by shifting the voltage range of activation to more negative potentials. An increase in the transient K^+ current also reduced the amplitude of back-propagating action potentials with no change in the initial rate of rise of the action potential. The rate of rise has been used as a measure of the Na^+ current underlying the action potential (12, 24, 37). We then used U0126 as a means of increasing transient K^+ current in the dendrites and found that both the boosting of action potentials by the EPSPs and the induction of LTP were blocked. Although certainly an increase in Na^+-channel activation is possible with such a pairing paradigm, we found that both the spike boosting and the LTP induction could be regulated by changing the properties of the dendritic A-type K^+ channels. Our computer modeling results also supported the hypothesis that K^+-channel inactivation contributed to the spike-boosting and indicated that a small 5-mV shift in voltage activation could have profound effects on dendritic spike amplitude.

Although the results illustrated in Fig. 4 demonstrate an increase in K^+-channel activity and a decrease in dendritic spike amplitude by the bath application of U0126, the suppression of LTP by U0126 could have been due to some unrelated mechanism downstream of its effects on K^+ channels. To test for this possibility, we used a stronger stimulus protocol consisting of five paired spikes instead of two and found that we were able to overcome the block of LTP by U0126 under these conditions. Taken together, these results support the hypothesis that the effects of U0126 on LTP, at least with the more modest protocol of two paired spikes, were indeed the result of reducing the amplitude of the paired spikes through increasing K^+-channel activity in the dendrites.

The relatively narrow time window within which the EPSPs and postsynaptic action potentials must occur for the induction of the NMDA-receptor-dependent LTP found in this and previous studies is quite interesting, given the much longer off rate for glutamate binding to NMDA receptors (9). Bi and Poo suggested that there is a cooperative relationship between Ca^{2+} entry through NMDA receptors and the Ca^{2+}-binding site that leads to LTP induction. They suggested that only near the peak

of the EPSP would there be sufficient Ca^{2+} entry to initiate the LTP process (6).

The LTP induced in our experiments was largely dependent on NMDA receptors and thus the most parsimonious hypothesis is that the boosting of the action potentials by the pairing with EPSPs leads to the unblocking of NMDA receptors and sufficient Ca^{2+} influx to induce LTP. Such a mechanism, however, does not rule out a cooperative relationship with Ca^{2+} and its binding site, such as that suggested by Bi and Poo, but instead emphasizes larger action potentials as the source for enhanced Ca^{2+} entry. It should be noted, however, that the LTP was not completely blocked in all cells even with APV + MK801. It is possible, therefore, that the LTP induced with these pairing paradigms is partly NMDA-receptor independent. This was observed by Magee and Johnston (1), who found that the LTP induced with a similar pairing protocol was partially blocked by Ca^{2+}-channel antagonists. Also, the LTP observed here and previously had a component that appeared immediately following the pairing, and another component that continued to increase with time during the expression phase. This time course is also characteristic of NMDA-receptor-independent LTP (25).

A number of previous studies have shown that MAPK plays a critical role in the induction of LTP and learning. One isoform of MAPK (p42 MAPK or ERK2) is activated in LTP (18), and inhibitors of ERK activation block LTP in a variety of preparations and with several different induction protocols (20, 26–28). Furthermore, inhibitors of ERK activation block certain forms of learning (29–31). Among many potential substrates for ERK, one is the transient K^+ channel Kv4.2 (17), which is expressed at high density in the dendrites of CA1 neurons (32–34). Biochemical studies (18) have also demonstrated basal activity of ERK in CA1 neurons. The inhibitors of ERK activation increased native K^+ current by means of a leftward shift in the voltage-activation curve. The action of U0126 appeared to be selective for K^+ channels, because there was no effect on the initial rate of rise of the dendritic action potential at the same time that the amplitude was significantly decreased.

In summary**,** the present results provide strong support for the hypothesis that the transient K^+ channel in the dendrites of CA1 neurons plays an important role in the induction of LTP at Schaffer collateral synapses. This K^+ channel may help establish the relatively precise timing requirements for induction of an EPSP-spike-timing LTP, and may help regulate the change in amplitudes of the dendritic action potentials that has been reported for different behavioral states (35). Furthermore, the results of MAPK-inhibitor experiments raise the possibility that this K^+ channel may be a substrate for the role of MAPK in learning and memory in the behaving animal.

We thank Rick Gray for help with aspects of these experiments and David Sweatt, Jeff Magee, and Nick Poolos for comments on the manuscript. This work was supported by National Institutes of Health Grants MH48432, MH44754, and NS37444, the Human Frontier Science Program, and the Hankamer Foundation. M.M. was supported in part by the National Institute on Deafness and Other Communication Disorders (Human Brain Project).

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