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Adenylyl Cyclase Activation Modulates Activity-Dependent Changes in Synaptic Strength and Ca^{2+}/Calmodulin-Dependent Kinase II Autophosphorylation

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Activation of the Ca^{2+}- and calmodulin-dependent protein kinase II (CaMKII) and its conversion into a persistently activated form by autophosphorylation are thought to be crucial events underlying the induction of long-term potentiation (LTP) by increases in postsynaptic Ca^{2+}. Because increases in Ca^{2+} can also activate protein phosphatases that oppose persistent CaMKII activation, LTP induction may also require activation of signaling pathways that suppress protein phosphatase activation. Because the adenylyl cyclase (AC)–protein kinase A signaling pathway may provide a mechanism for suppressing protein phosphatase activation, we investigated the effects of AC activators on activity-dependent changes in synaptic strength and on levels of autophosphorylated αCaMKII (Thr^{286}). In the CA1 region of hippocampal slices, briefly elevating extracellular Ca^{2+} induced an activity-dependent, transient potentiation of synaptic transmission that could be converted into a persistent potentiation by the addition of phosphatase inhibitors or AC activators. To examine activity-dependent changes in αCaMKII autophosphorylation, we replaced electrical presynaptic fiber stimulation with an increase in extracellular K^+ to achieve a more global synaptic activation during perfusion of high Ca^{2+} solutions. In the presence of the AC activator forskolin or the protein phosphatase inhibitor calyculin A, this treatment induced a LTP-like synaptic potentiation and a persistent increase in autophosphorylated αCaMKII levels. In the absence of forskolin or calyculin A, it had no lasting effect on synaptic strength and induced a persistent decrease in autophosphorylated αCaMKII levels. Our results suggest that AC activation facilitates LTP induction by suppressing protein phosphatases and enabling a persistent increase in the levels of autophosphorylated CaMKII.

Key words: hippocampal slices; long-term potentiation; Ca^{2+}- and calmodulin-dependent kinase II; protein phosphatase; adenylyl cyclase; calcium

At many excitatory synapses, increases in postsynaptic calcium arising from activation of NMDA-type glutamate receptors induce long-term potentiation (LTP), a persistent enhancement of synaptic strength that may be involved in some forms of learning and memory (Nicoll and Malenka, 1995; Wang et al., 1997). Although the calcium-activated signaling pathways that underlie the induction of LTP at excitatory synapses onto hippocampal CA1 pyramidal cells are only partly understood, there is strong evidence that the multifunctional Ca^{2+}- and calmodulin-dependent protein kinase II (CaMKII) has a central role (Malenka et al., 1989; Malinow et al., 1989; Silva et al., 1992). Two features of CaMKII activity that may be particularly important for LTP induction are CaMKII's ability to both phosphorylate and enhance the activity of postsynaptic AMPA-type glutamate receptors (McGlade-McCulloh et al., 1993; Pettit et al., 1994; Lledo et al., 1995; Barria et al., 1997) as well as to become persistently activated by autophosphorylation (Miller and Kennedy, 1986; Miller et al., 1988; Schworer et al., 1988; Thiel et al., 1988; Lou and Schulman, 1989). Autophosphorylation of CaMKII after a brief increase in postsynaptic Ca^{2+} may thus provide a mechanism by which transient NMDA receptor activation induces a long-lasting biochemical change that enhances synaptic transmission (Miller and Kennedy, 1986; Fukunaga et al., 1993; Lisman, 1994; Barria et al., 1997; Ouyang et al., 1997; Giese et al., 1998).

Although these findings suggest an appealingly simple Ca^{2+}-activated and CaMKII-dependent mechanism for LTP induction, increases in postsynaptic calcium not only activate CaMKII but can also activate protein phosphatases, such as protein phosphatase 1 (PP1) and protein phosphatase 2B (calcineurin), that oppose the induction of LTP (O'Dell and Kandel, 1994; Wyllie and Nicoll, 1994; Coussens and Teyler, 1996; Thomas et al., 1996) and/or induce long-term depression (Mulkey et al., 1993, 1994). Because increases in intracellular Ca^{2+} can activate signaling cascades that have opposing effects on synaptic strength, it seems likely that other factors may determine whether an increase in intracellular Ca^{2+} successfully activates the mechanisms responsible for LTP. Because the activity of PP1 is inhibited by protein kinase A (PKA) phosphorylation of the PP1 regulatory protein inhibitor-1, one possibility is that activation of Ca^{2+}- and calmodulin-sensitive isoforms of adenylyl cyclase (AC) during strong levels of NMDA receptor activation (Chetkovich and Sweatt, 1993) provides a mechanism that allows CaMKII autophosphorylation and AMPA receptor phosphorylation to proceed unopposed by PP1 (Lisman, 1989, 1994). Indeed, a PKA-

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mediated inhibition of PP1 is required for LTP induction under some conditions (Blitzer et al., 1995, 1998; Thomas et al., 1996; Winder et al., 1998).

In the present study we sought to examine the potential role of cross-talk between cAMP–PKA and CaMKII signaling pathways in LTP induction using chemical manipulations that induce short- and long-term changes in synaptic strength. Our results indicate that AC activation not only potently modulates the induction of LTP-like changes in synaptic transmission but also regulates activity-dependent changes in the levels of autophosphorylated αCaMKII.

MATERIALS AND METHODS

Electrophysiology. Standard techniques were used to prepare 400-μm-thick hippocampal slices from hippocampi obtained from halothane-anesthetized 4- to 6-week-old male C57BL/6 mice. The slices were maintained in an interface recording chamber (Fine Science Tools) constantly perfused at 2–3 ml/min with a warm (30°C), oxygenated (95% O_2/5% CO_2) artificial CSF (ACSF) containing 124 mM NaCl, 4.4 mM KCl, 25 mM $NaHCO_3$, 1 mM NaH_2PO_4, 1.2 mM $MgSO_4$, 2 mM $CaCl_2$, and 10 mM glucose. Slices were allowed to recover from slice preparation for at least 1 hr before an experiment. Schaffer collateral–commissural fibers in the CA1 region of the hippocampus were activated with capacity-coupled 0.02-msec-duration stimulation pulses delivered at 0.02 Hz by a Grass S88 stimulator connected to SIU5 stimulus isolators (Astro-Med) using bipolar-stimulating electrodes fabricated from twisted strands of Formvar-coated nichrome wire (A-M Systems). The resulting EPSPs were recorded in the stratum radiatum of the CA1 region with glass microelectrodes filled with ACSF (5–10 MΩ) using either IX2–700 (Dagan) or Axoprobe 1A (Axon Instruments) amplifiers. At the beginning of each experiment the intensity of presynaptic fiber stimulation was adjusted to evoke field EPSPs (fEPSPs) that were ~50% of the maximal fEPSP amplitude evoked at strong stimulation intensities. Data acquisition and analysis were done using the Experimenter's Workbench and Common Processing software package (Data Wave Technologies).

Whole-cell current-clamp recordings were used to record EPSPs from individual CA1 pyramidal cells in slices maintained in a submerged recording chamber. In these experiments low resistance patch-clamp electrodes (3–5 MΩ) were filled with a solution containing 122.5 mM potassium gluconate, 15.5 mM KCl, 8 mM NaCl, 0.2 mM EGTA, 2 mM Mg-ATP, 0.3 mM GTP, and 10 mM HEPES, pH = 7.2 (osmolarity, 280–290 mOsm). Current injection was used to hyperpolarize cells continuously to −85 to −90 mV, and both access and input resistances were monitored with a 40 msec, 0.1 nA current pulse delivered every 20 sec. Only cells with stable access resistances of 20 MΩ or less were used. At the start of an experiment, the strength of synaptic stimulation was set to evoke EPSPs that were 5–10 mV in amplitude (stimulation rate = 0.05 Hz). In experiments with the CaMKII inhibitory peptide AIP (autocamtide-2-related inhibitory peptide; KKALRRQEAVDAL) (Ishida et al., 1995), the peptide was dissolved directly in the electrode-filling solution (final concentration was 1.0 or 1.66 mM) and used within 3–4 hr. The intracellular concentration of AIP is unknown in these experiments. However, because of the limited amount of time allowed for intracellular perfusion (<20 min in the whole-cell mode) and the degradation of peptides introduced into cells through patch-clamp electrodes by intracellular proteases (Otmakhov et al., 1997), the intracellular concentration of AIP is likely to be much less than that present in the recording electrode.

Salts used in the ACSF were purchased from Sigma (St. Louis, MO). D,L-2-Amino-5-phosphonovaleric acid (APV), isoproterenol (ISO), and forskolin (FSK) were purchased from Research Biochemicals (Natick, MA). The CaMKII inhibitory peptide AIP was purchased from Biomol (Plymouth Meeting, PA). APV and ISO were dissolved directly into ACSF or prepared as concentrated stock solutions in H_2O. FSK was prepared as a concentrated stock solution in dimethyl sulfoxide (DMSO) and then diluted to a final concentration of 50 μM (final DMSO concentration = 0.1%) in ACSF just before application. Calyculin A and KN-62 (Alexis Biochemicals, San Diego, CA) were dissolved in DMSO and diluted into ACSF to achieve final concentrations of 750 nM and 3–5 μM, respectively (DMSO concentrations, 0.15 and 0.2%, respectively). KN-04 (Seikagaku America, Rockville, MD) was dissolved in DMSO and diluted into ACSF to achieve a final concentration of 5.0 μM (0.2% DMSO). Slices were exposed to either KN-04, KN-62, or calyculin A for at least 30 min before the start of the experiment (see Mulkey et al., 1993; Wylie and Nicoll, 1994). Results from electrophysiological experiments are EPSP slopes expressed as the percent of baseline (mean ± SEM), and Student's *t* tests were used for statistical comparisons.

Western blot analysis. For each Western blot, three to five slices were chemically treated while an additional three- to five-untreated slices from the same animal served as controls. Chemical treatments consisted of a 5 min application of a high K^+/Ca^{2+} ACSF (30 mM KCl, 10 mM $CaCl_2$, and 0 mM $MgSO_4$) applied either alone or preceded by 50 μM FSK, 50 μM FSK + 100 μM D,L-APV, or 750 nM calyculin A. Control experiments also included an analysis of slices exposed to FSK and calyculin A alone. One hour after the start of perfusion with high K^+/Ca^{2+} ACSF, slices were quickly frozen, and all regions except CA1 were removed. These frozen CA1 slices were then transferred to microcentrifuge tubes in a dry ice/ethanol bath where they remained frozen. The tissue was thawed with 70 μl of ice-cold homogenization buffer (50 mM HEPES, pH 7.4, 10 mM $MgCl_2$, 1 mM EDTA, 1 mM EGTA, 10 mM benzamide, 100 ng/ml leupeptin, 100 ng/ml aprotinin, 0.01% Triton X-100, 0.08 mM sodium molybdate, and 2 mM sodium pyrophosphate) and homogenized by three rounds of 5 sec each with a Micron Ultrasonic Cell Disruptor. We observed negligible differences in immunoreactivity for phospho-αCaMKII (or total CaMKII) in CA1 homogenates prepared in homogenization buffer in either the presence or absence of 10 mM $MgCl_2$. Immediately after homogenization, aliquots were removed for protein analysis, and 70 μl of denaturing protein loading buffer [0.5 M Tris-HCl, pH 6.8, 4.4% (w/v) SDS, 20.0% (v/v) glycerol, 2.0% 2-mercaptoethanol, and bromophenol blue] was added. These homogenates were kept on ice for ~45 min while protein concentrations were determined by the method of Bradford (1976) using a Bio-Rad Protein Assay Kit (Hercules, CA). In a control experiment we confirmed that the denaturing protein loading buffer stopped protein kinase and protein phosphatase activity while samples were kept on ice. Here aliquots from homogenates were electrophoresed immediately after loading buffer was added, and these aliquots were compared with identical volumes of the same homogenate samples kept on ice for 1 hr before electrophoresis. No significant changes were found in the levels of phosphorylated αCaMKII or total αCaMKII between the samples loaded immediately and those that had been left on ice for 1 hr (data not shown).

Treated and untreated homogenates containing 30–50 μg of protein each were electrophoresed on 15% SDS-PAGE gels, transferred to nitrocellulose membranes, and probed with various primary antisera as described previously (Johnson et al., 1997). Autophosphorylated αCaMKII was detected using a monoclonal antibody (clone 22B1; Affinity Bioreagents) that selectively recognizes Thr^{286}-phosphorylated αCaMKII (Patton et al., 1993). Total αCaMKII levels were assessed using a monoclonal anti-αCaMKII antibody (clone 6G9; Boehringer Mannheim, Indianapolis, IN) that recognizes both unphosphorylated and phosphorylated αCaMKII (Erondu and Kennedy, 1985). The membranes were incubated with horseradish peroxidase-conjugated anti-mouse or anti-goat IgG (1:2500), and protein signals were visualized by chemiluminescence (Amersham ECL Western Blotting Analysis System; Arlington Heights, IL). Densitometry data of the exposed film were processed with a Molecular Dynamics (Sunnyvale, CA) Personal Densitometer SI using ImageQuaNT software. Digital resolution was set at 12 bits per pixel, with a 50 μm pixel size. Areas scanned as a single data set included a single experiment from the same animal and the same film exposure time. Protein bands were boxed, and the integrated intensity of all the pixels within that band was calculated above object average background levels of a box of the same size. Percent changes attributable to chemical treatment were calculated relative to the optical density volume of the corresponding untreated protein bands within a single experiment, and Student's *t* tests were used to assess statistical significance.

RESULTS

Synaptic stimulation in elevated extracellular Ca^{2+} induces a transient NMDA receptor- and CaMKII-dependent potentiation of synaptic transmission

Although previous reports have shown that a brief increase in extracellular Ca^{2+} induces an LTP-like potentiation of synaptic transmission in the CA1 region of the hippocampus (Turner et al., 1982; Reymann et al., 1986), we found that under our exper-

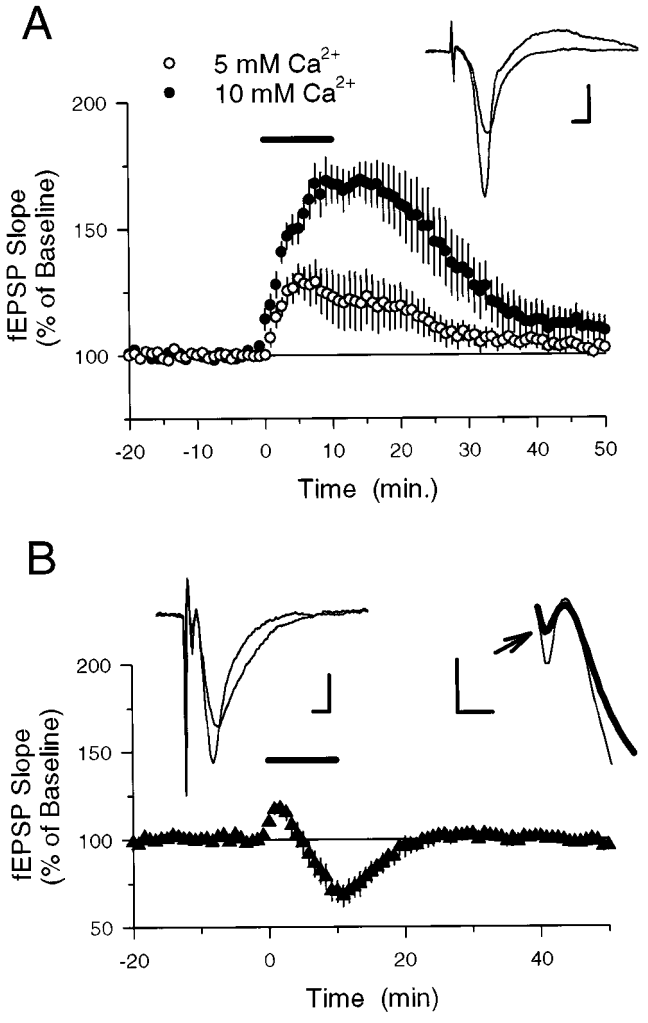

Figure 1. High Ca^{2+} ACSF-induced changes in synaptic transmission in the CA1 region of the hippocampus. *A*, A 10 min bath application of ACSF containing 5 mM $CaCl_2$ (*horizontal bar*) induced only a small, short-term potentiation of synaptic transmission (*open circles*; $n = 6$). Application of a nominally Mg^{2+}-free ACSF containing 10 mM $CaCl_2$ induced a larger, but still transient, potentiation of synaptic transmission (*filled circles*; $n = 7$). fEPSPs in slices exposed to 10 mM Ca^{2+} and 0 mM Mg^{2+} ACSF were potentiated to 168.0 ± 8.1% of baseline 5 min after high Ca^{2+} ACSF application but returned to near baseline levels (109.5 ± 5.3% of baseline) by 40 min after high Ca^{2+} ACSF application. *Inset*, The *traces* show representative fEPSPs recorded during baseline and at the end of the high Ca^{2+} ACSF application (larger response). Calibration: 2 mV, 2 msec. *B*, In slices bathed throughout the experiment in 100 μM D,L-APV (*triangles*; $n = 7$), high Ca^{2+} ACSF (*horizontal bar*) induced a very brief enhancement of synaptic transmission followed by a longer-lasting, but transient, depression. *Left Inset*, The *traces* show fEPSPs recorded in one experiment during baseline (larger response) and after application of high Ca^{2+} ACSF. Calibration: 1 mV, 2 msec. *Right Inset*, The *traces* show the fEPSPs at a higher gain. Calibration: 1 mV, 1 msec. Note the decrease in the fiber volley amplitude (*arrow*) in the presence of high Ca^{2+} ACSF.

imental conditions a 10 min application of ACSF containing 5 mM $CaCl_2$ induced only a small, transient potentiation of synaptic transmission (Fig. 1*A*) (see also Grover and Teyler, 1990). In an attempt to enhance postsynaptic Ca^{2+} influx through NMDA receptors and thus to facilitate the induction of a persistent potentiation, we examined the effects of a 10 min application of nominally Mg^{2+}-free ACSF containing an even higher concentration of $CaCl_2$ (10 mM, hereafter referred to as high Ca^{2+} ACSF). Although this high Ca^{2+} ACSF induced a larger initial potentiation, synaptic strength again gradually returned to near baseline levels when slices were re-exposed to ACSF containing normal levels of extracellular Ca^{2+} and Mg^{2+} (Fig. 1*A*). The short-term enhancement of synaptic transmission induced by high Ca^{2+} ACSF was almost completely blocked by the NMDA receptor antagonist D,L-APV, and in these experiments a transient depression of synaptic transmission was observed (Fig. 1*B*). This short-term depression may be caused by a decrease in cellular excitability arising from the screening of negative surface charges by the high levels of divalent cations (Hille, 1992) that is unmasked when the synaptic potentiation is blocked with APV. Indeed, in these experiments there was a clear decrease in the fiber volley magnitude in the presence of high Ca^{2+} ACSF, indicative of a decrease in presynaptic fiber excitability (Fig. 1*B*).

The CaMKII inhibitor KN-62 (Tokumitsu et al., 1990) also blocked the short-term potentiation induced by high Ca^{2+} ACSF, whereas KN-04, a structural analog of KN-62 that does not inhibit CaMKII (Ishikawa et al., 1990) but shares many of the nonselective effects of KN-62 (Cui et al., 1996; Maurer et al., 1996; Tsutsui et al., 1996), had no effect (Fig. 2). Together, these results suggest that synaptic stimulation in high Ca^{2+} ACSF produces a sufficient NMDA receptor-dependent Ca^{2+} influx to induce a CaMKII-dependent potentiation of synaptic transmission. The transient nature of the potentiation indicates, however, that under these conditions CaMKII activation alone is not sufficient to induce a persistent potentiation of synaptic transmission. Importantly, because KN-62 also inhibits other calcium- and calmodulin-dependent protein kinases (CaM kinases) (Mochizuki et al., 1993; Enslen et al., 1994), these results do not eliminate the possibility that multiple forms of CaM kinase are involved. We thus examined the effects of introducing the highly selective and potent CaMKII inhibitor AIP (Ishida et al., 1995) into individual CA1 pyramidal cells. In these experiments we used whole-cell current-clamp recordings to monitor EPSPs evoked by Schaffer collateral fiber stimulation and bath-applied high Ca^{2+} ACSF for 10 min within 20 min after achieving whole-cell recordings. Under these conditions, high Ca^{2+} ACSF applications reliably evoked a transient potentiation that outlasted the high Ca^{2+} ACSF application in control cells (seven out of eight cells) but had little effect on synaptic strength in interleaved experiments in which the electrode-filling solution contained 1.0 or 1.66 mM AIP ($n = 8$; Fig. 2*B*). Thus activation of postsynaptic CaMKII seems to be required for the potentiation induced by a brief exposure to high Ca^{2+} ACSF.

One reason for the transient nature of the CaM kinase-dependent potentiation observed in our experiments may be that the increase in intracellular Ca^{2+} produced by high Ca^{2+} ACSF application not only activates CaM kinase but also activates protein phosphatases that oppose the induction of a persistent potentiation. To determine whether protein phosphatase activation might contribute to the transient nature of the potentiation induced by high Ca^{2+} ACSF, we examined the effects of high Ca^{2+} ACSF on synaptic transmission in slices pretreated with calyculin A (750 nM), a selective inhibitor of protein phosphatases 1 and 2A. As shown in Figure 3*A*, high Ca^{2+} ACSF induced a robust and persistent potentiation of synaptic transmission in calyculin A-treated slices. Physiologically, the cAMP–PKA signaling pathway may provide a mechanism by which certain patterns of synaptic activity (Blitzer et al., 1995; Winder et al., 1998) or modulatory neurotransmitters (Thomas et al., 1996) can enable

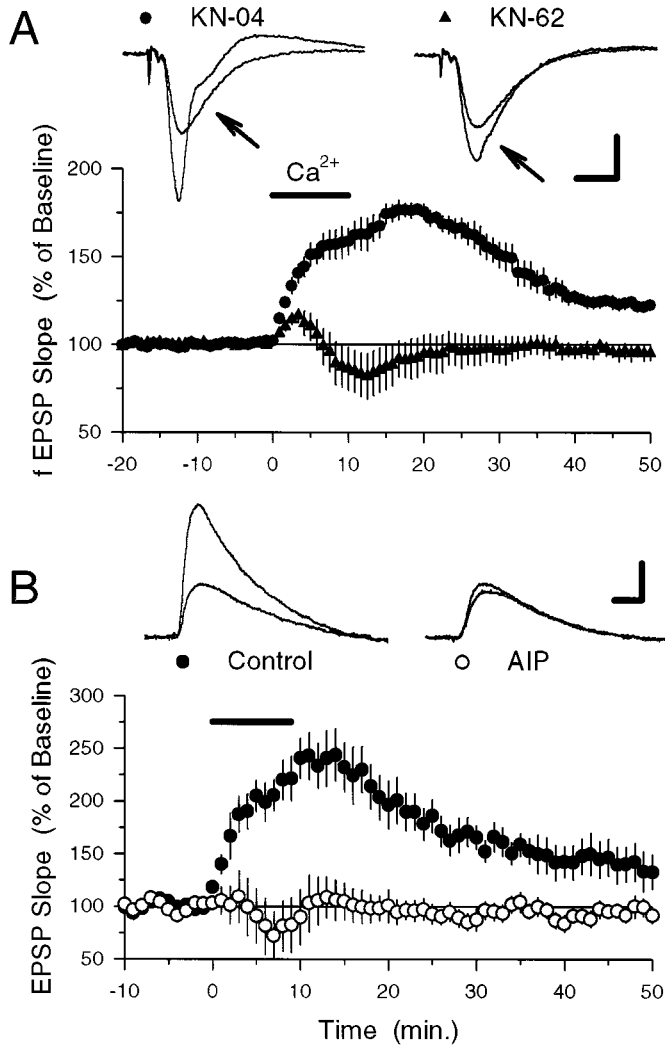

Figure 2. *A*, The CaM kinase inhibitor *KN-62* blocks the short-term synaptic potentiation induced by high Ca^{2+} ACSF. The short-term potentiation induced by high Ca^{2+} ACSF (*horizontal bar*) was blocked in slices pretreated with 3–5 μM *KN-62* (*triangles*; $n = 7$); *KN-62* application began at least 30 min before the start of the experiment and continued throughout the experiment. *KN-04*, an inactive analog of *KN-62*, did not block the potentiation induced by high Ca^{2+} ACSF (*circles*; $n = 5$). *Insets*, The *traces* show fEPSPs recorded during baseline (*arrows*) and at the end of the high Ca^{2+} ACSF application in slices treated with *KN-04* (*left*) or *KN-62* (*right*). Calibration: 2 mV, 2 msec. *B*, The CaMKII inhibitor *AIP* blocks the high Ca^{2+} ACSF-induced potentiation when introduced into CA1 pyramidal cells. The *filled circles* show the potentiation induced by a 10 min application of high Ca^{2+} ACSF (*horizontal bar*) in control cells ($n = 8$). The *open circles* show the results from interleaved experiments in which the electrode-filling solution contained either 1.0 mM (3 cells) or 1.66 mM (5 cells) *AIP*. The results with these two different concentrations of *AIP* were similar and have been combined. At the end of the 10 min high Ca^{2+} ACSF application, EPSPs were potentiated to 240.5 ± 19.1% of baseline in control cells and 89.9 ± 26.3% of baseline in *AIP*-filled cells [$t(14) = 4.64$; $p < 0.005$]. *Insets*, The responses show EPSPs (averages of 3 responses) recorded over the last minutes of baseline and just after the high Ca^{2+} ACSF application in control (*left*) and *AIP*-filled cells (*right*). Calibration: 4 mV, 10 msec.

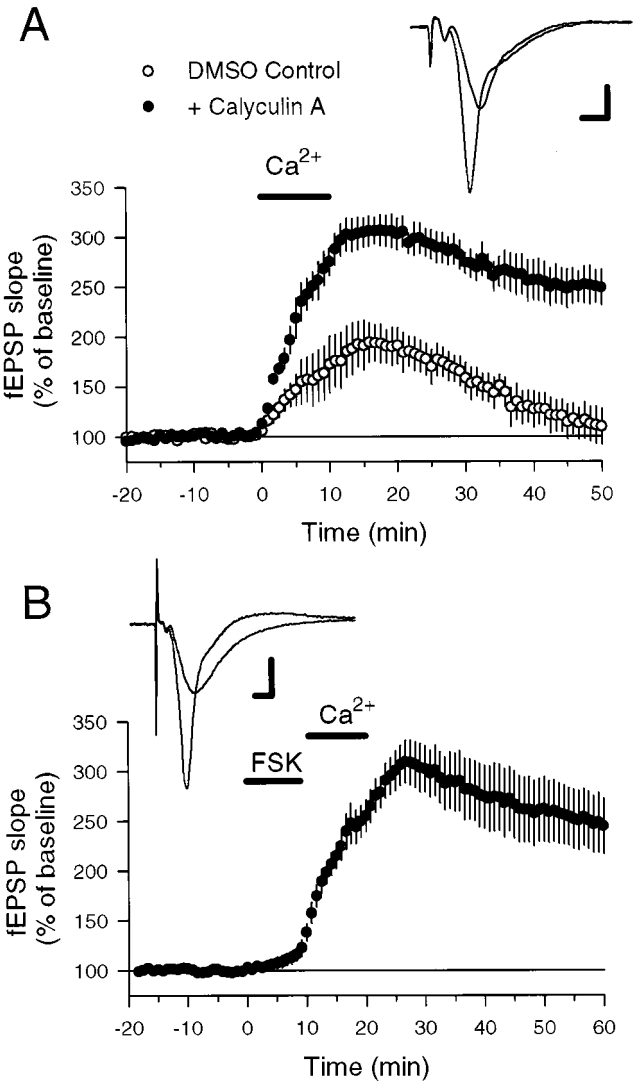

Figure 3. High Ca^{2+} ACSF induces a persistent potentiation in slices treated with the protein phosphatase inhibitor calyculin A or with activators of cAMP signaling. *A*, Slices were pretreated (45–60 min) either with 0.75 μM calyculin A dissolved in 0.2% *DMSO* (*closed circles*) or with 0.2% *DMSO* alone (*open circles*). Forty minutes after bath application of high Ca^{2+} ACSF (*horizontal bar*), fEPSPs were potentiated to 249.0 ± 18.0% of baseline ($n = 5$) in calyculin A-treated slices and were 109.7 ± 9.8% of baseline ($n = 4$) in slices treated with *DMSO* alone. *Inset*, The responses are fEPSPs recorded during baseline (smaller response) and 40 min after high Ca^{2+} ACSF in calyculin A-treated slices. Calibration: 2 mV, 2 msec. *B*, High Ca^{2+} ACSF induces a persistent potentiation of synaptic transmission in slices pretreated with 50 μM *FSK*. *FSK* (50 μM) was applied for 10 min (*lower horizontal bar*) just before application of high Ca^{2+} ACSF (*upper horizontal bar*). Forty minutes after application of high Ca^{2+} ACSF, fEPSPs were potentiated to 248.4 ± 30.0% of baseline ($n = 6$). *Inset*, The superimposed fEPSPs shown were recorded during baseline (smaller response) and 40 min after high Ca^{2+} ACSF in a *FSK*-treated slice. Calibration: 2.0 mV, 2.0 msec.

the induction of LTP by inhibiting protein phosphatases. Thus, we also examined whether activating AC with FSK or by activation of G_s-protein-linked β-adrenergic receptors with ISO could enable the induction of a persistent potentiation by high Ca^{2+} ACSF. As shown in Figures 3*B* and 4, high Ca^{2+} ACSF also induced a large and persistent potentiation of synaptic transmission in slices pretreated with either 50 μM FSK or 1.0 μM ISO.

Synaptic activity is required for the induction of a persistent, high Ca^{2+} ACSF-induced potentiation

Like the transient potentiation induced by high Ca^{2+} ACSF in the absence of activators of cAMP signaling, the persistent potentiation induced by high Ca^{2+} ACSF in the presence of ISO

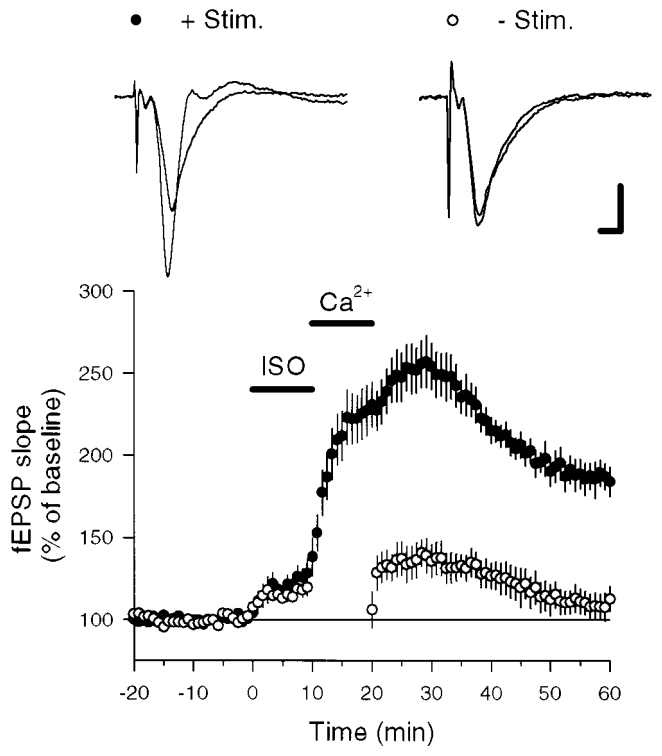

Figure 4. Synaptic stimulation during high Ca^{2+} ACSF application is required for the induction of a persistent potentiation in *ISO*-treated slices. With continuous 0.02 Hz synaptic stimulation, fEPSPs were potentiated to 183.8 ± 9.1% of baseline (*filled circles*; $n = 7$) 40 min after high Ca^{2+} ACSF was applied in the presence of *ISO*. High Ca^{2+} ACSF in *ISO* failed to induce a persistent potentiation of synaptic transmission when presynaptic fiber stimulation was omitted during high Ca^{2+} ACSF application (*open circles*; $n = 5$). In these experiments, 40 min after high Ca^{2+} ACSF application, fEPSP slopes were 112.1 ± 9.6% of baseline [not significantly different from baseline, $t(4) = 1.02$]. *Insets*, The superimposed fEPSPs shown were recorded during baseline and 40 min after high Ca^{2+} ACSF in experiments in which stimulation (*Stim.*) was continued throughout the experiment (*left*) or omitted during high Ca^{2+} ACSF application (*right*). Calibration: 2 mV, 2 msec.

was blocked by APV, suggesting that NMDA receptor activation is required for the induction of a persistent potentiation. In these experiments fEPSPs were 100.4 ± 2.2% of baseline 40 min after high Ca^{2+} ACSF application in ISO + 100 μM D,L-APV ($n = 6$) compared with 183.8 ± 9.1% of baseline after high Ca^{2+} ACSF application in ISO in the absence of APV [$n = 7$; $t(11) = 7.83$; $p < 0.001$]. Because ambient levels of extracellular glutamate in hippocampal slices are sufficient to produce a small, tonic activation of NMDA receptors in CA1 pyramidal cells (Sah et al., 1989), glutamate released by synaptic stimulation might not be required for the induction of a lasting potentiation by high Ca^{2+} ACSF in ISO. If so, briefly exposing slices to high Ca^{2+} ACSF in the presence of ISO or FSK might persistently potentiate a large number of synapses and thus provide an ideal preparation for neurochemical studies of the cellular processes underlying persistent changes in synaptic strength. However, we found that only a transient enhancement of synaptic transmission was induced when presynaptic fiber stimulation was not delivered during application of high Ca^{2+} ACSF in ISO (Fig. 4), indicating that synaptic stimulation is required for the induction of a persistent potentiation. Ambient levels of extracellular glutamate thus do not appear to provide adequate levels of NMDA receptor activation to enable the induction of a persistent potentiation by high Ca^{2+} ACSF.

In an attempt to achieve a protocol for inducing a persistent enhancement in synaptic strength that did not require electrical, presynaptic fiber stimulation, we examined whether depolarizing cells with elevated levels of extracellular K^+ could replace the synaptic stimulation needed for the induction of a persistent potentiation by high Ca^{2+} ACSF in ISO-treated slices. In these experiments, after a 10 min ISO application, slices were exposed to a 5 min application of high Ca^{2+} ACSF containing 30 mM K^+ during which presynaptic fiber stimulation was omitted. When test stimulation was resumed 15 min later, a large, persistent potentiation was observed (fEPSP slopes were 175.6 ± 30.5% of baseline at 50 min after high K^+/Ca^{2+} ACSF). Similar results were obtained in slices pretreated with 50 μM FSK (Fig. 5*A*).

Our rationale for elevating extracellular K^+ during the high Ca^{2+} ACSF application was to depolarize both presynaptic and postsynaptic cells, thus providing the glutamate release and postsynaptic depolarization needed for NMDA receptor activation. However, we noted in these experiments that high K^+/Ca^{2+} ACSF evoked a brief period of spontaneous bursting (Fig. 6*A*). Thus, to determine whether spontaneous bursts were providing the synaptic activity needed to induce a lasting potentiation, we suppressed spontaneous bursting with a low concentration of tetrodotoxin (TTX) and exposed FSK-treated slices to high K^+/Ca^{2+} ACSF. Here we bath applied 250 nM TTX for 10 min [a concentration and duration of application that had no effect on the synaptic transmission elicited by our test pulses (data not shown) (but see Thomas et al., 1998)] at the same time as the FSK application. Under these conditions spontaneous bursting in the presence of high K^+/Ca^{2+} ACSF was dramatically reduced, and only a small persistent potentiation of synaptic transmission was observed (Fig. 6*B*,*D*). We also found that removing the CA3 region from slices prevented both the spontaneous bursting and the persistent potentiation induced by high K^+/Ca^{2+} ACSF in FSK-treated slices (Fig. 6*C*,*D*). Thus, high extracellular potassium appears to alleviate the need for electrical presynaptic fiber stimulation in the induction of a persistent potentiation by high Ca^{2+} ACSF by inducing a spontaneous bursting mode of neuronal activity that is dependent on synaptic connections between CA3 and CA1.

The persistent potentiation induced by high K^+/Ca^{2+} ACSF in FSK-treated slices was inhibited by the NMDA receptor antagonist APV (Fig. 5*A*), suggesting that this potentiation may arise from signaling pathways similar to those underlying the induction of LTP by more conventional means. To examine this possibility in more detail, we compared the amount of LTP induced by high frequency synaptic stimulation in control, untreated slices with that induced in slices that were treated first with FSK and then with high K^+/Ca^{2+} ACSF. Although robust high frequency stimulation-induced LTP was observed in control slices, high frequency synaptic stimulation had only a small effect on synaptic transmission in slices exposed to high K^+/Ca^{2+} ACSF in FSK 90–120 min earlier (Fig. 5*B*). Moreover, when the persistent potentiation induced by high K^+/Ca^{2+} ACSF in FSK-treated slices was inhibited with APV (100 μM), subsequent high frequency synaptic stimulation delivered after APV washout induced normal levels of LTP (fEPSP slopes were 205.1 ± 5.7% of baseline 60 min after high frequency stimulation; $n = 5$). Because the induction of a persistent potentiation of synaptic transmission

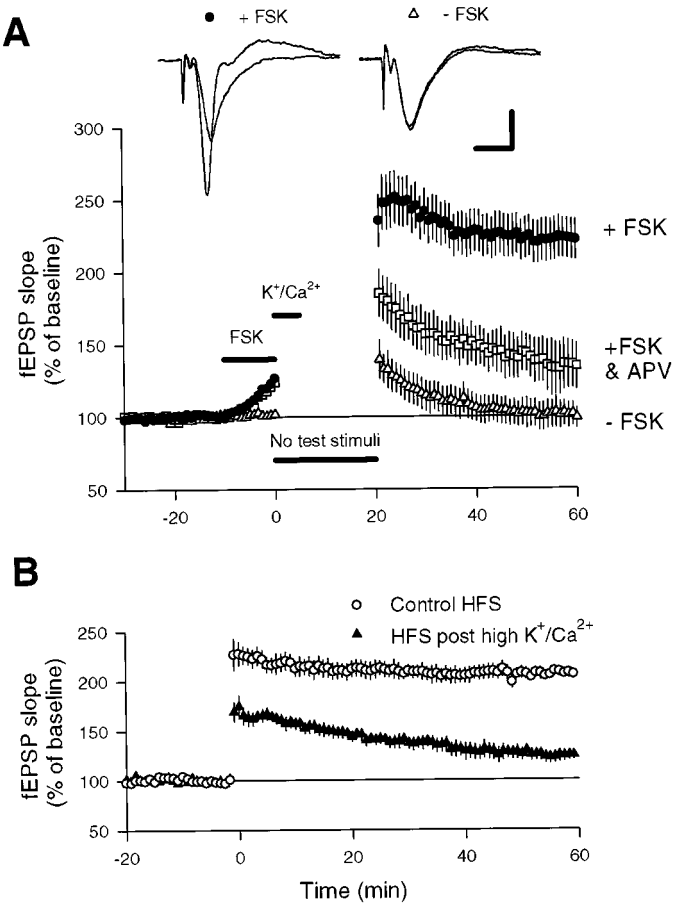

Figure 5. In the absence of electrical presynaptic fiber stimulation, high K^+/Ca^{2+} ACSF induces an LTP-like, persistent potentiation of synaptic transmission in *FSK*-treated slices. *A*, Treatment of slices with 50 μM *FSK* (*lower horizontal bar*) followed by a 5 min perfusion with high K^+/Ca^{2+} ACSF containing 30 mM K^+, 10 mM Ca^{2+}, and no Mg^{2+} (*upper horizontal bar*) induces a persistent potentiation of synaptic transmission (*filled circles*). fEPSP slopes were 222.0 ± 14.1% of baseline at 60 min. Test stimulation was turned off for 20 min beginning at the start of perfusion with high K^+/Ca^{2+} ACSF. The NMDA receptor antagonist *APV* (100 μM; present throughout the experiment) significantly attenuates the persistent potentiation induced by high K^+/Ca^{2+} ACSF in *FSK*-treated slices [*open squares*; $t(20) = 3.16$; $p < 0.005$ compared with treatment in the absence of *APV*]. fEPSP slopes were 134.5 ± 15.8% of baseline at 60 min ($n = 5$). High K^+/Ca^{2+} ACSF fails to induce a persistent potentiation of synaptic transmission in slices not first exposed to *FSK* (*open triangles*). fEPSP slopes were 98.8 ± 7.0% of baseline at 60 min [$n = 8$; $t(23) = 5.77$; $p < 0.0001$ compared with the potentiation observed in slices pretreated with *FSK*]. *Insets*, Responses shown are fEPSPs recorded during baseline and 50 min after high K^+/Ca^{2+} ACSF application in slices treated with (*left*) and without (*right*) *FSK*. Calibration: 2 mV, 5 msec. *B*, High frequency synaptic stimulation (*HFS*; 2 1-sec-long trains of 100 Hz stimulation; intertrain interval = 10 sec) induces large LTP in control slices (*open circles*) but has little effect on synaptic strength in slices previously exposed to *FSK* and high K^+/Ca^{2+} ACSF (*filled triangles*). In control slices, 60 min after high frequency stimulation, fEPSPs were potentiated to 206.1 ± 5.1% of baseline ($n = 6$), whereas fEPSPs were potentiated to only 124.4 ± 4.0% of baseline in slices exposed to high K^+/Ca^{2+} ACSF in *FSK* [$n = 6$; $t(9) = 12.8$; $p < 0.0001$ comparing tetanus-induced LTP in treated vs untreated slices].

by high K^+/Ca^{2+} ACSF in FSK-treated slices is NMDA receptor-dependent and significantly occludes tetanus-induced LTP, the potentiations induced by these two different protocols most likely share overlapping signaling pathways.

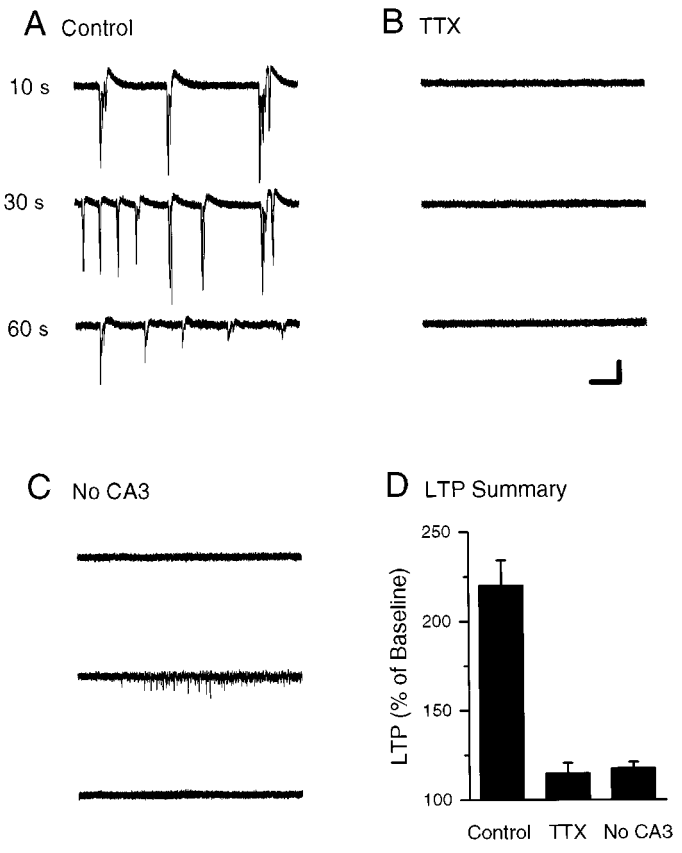

Figure 6. Spontaneous bursting is required for the induction of a persistent potentiation by high K^+/Ca^{2+} ACSF. *A*, *Traces* from a representative experiment show 4-sec-long sweeps of spontaneous bursting recorded extracellularly 10 sec (*top*), 30 sec (*middle*), and 60 sec (*bottom*) after slices were exposed to high K^+/Ca^{2+} ACSF. Bursting typically persisted for between 30 and 90 sec. *B*, Spontaneous bursting was blocked in slices exposed to 250 nM *TTX* for 10 min before high K^+/Ca^{2+} ACSF application. *Traces* correspond to the same time points indicated in *A*. *C*, High K^+/Ca^{2+}-induced spontaneous bursting was also prevented in slices in which the *CA3* region of the slice had been removed. However, in some experiments (2 out of 5), an increase in noise levels that may reflect single-unit activity was present (see *middle trace*). Calibration for *A–C* in *B*: 0.5 mV, 0.5 sec. *D*, Summary of the amount of *LTP* induced in FSK-treated slices exposed to high K^+/Ca^{2+} ACSF is shown. In control experiments (*CA3* intact and no *TTX*), fEPSPs were potentiated to 220.0 ± 14.1% of baseline 50 min after high K^+/Ca^{2+} ACSF application (*left bar*). In slices pre-exposed to 250 nM *TTX* (*middle bar*), fEPSPs were 114.6 ± 5.9% of baseline 50 min after high K^+/Ca^{2+} ACSF application ($n = 5$; $p < 0.001$ compared with control). In slices in which the *CA3* region was removed (*right bar*), fEPSPs were 117.4 ± 3.8% of baseline ($n = 3$; $p < 0.01$ compared with control).

Activators of cAMP signaling enable both persistent CaMKII autophosphorylation and a persistent synaptic potentiation by high K^+/Ca^{2+} ACSF

One hour after FSK-treated slices were exposed to high K^+/Ca^{2+} ACSF, a time point in which synaptic transmission is potentiated by more than twofold (Fig. 5*A*), we processed slices for Western immunoblot analysis to determine whether the persistent potentiation of synaptic transmission observed in these experiments was accompanied by an increase in the levels of phospho-αCaMKII. In addition to measuring αCaMKII phosphorylation with primary antisera directed specifically against the Thr^{286} autophosphorylation site of αCaMKII (Patton et al., 1993), we also investigated possible changes in the levels of total αCaMKII

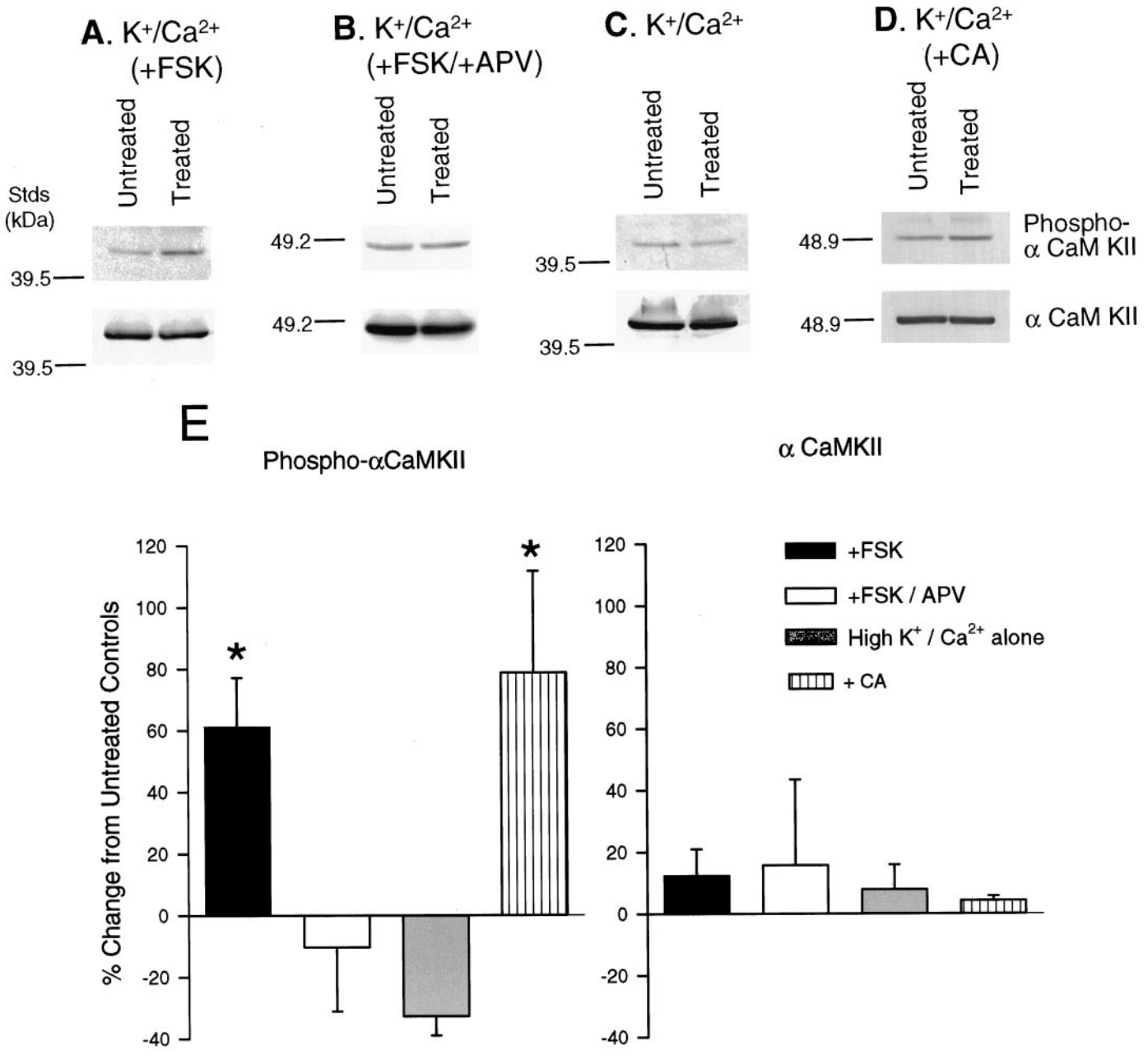

Figure 7. Western immunoblots of high K^+/Ca^{2+} ACSF-induced changes in αCaMKII Thr^{286} phosphorylation. *A–D*, The protein bands visualized with antibodies to phospho-αCaMKII or total αCaMKII are identified on the *right*. Molecular weight standards (*Stds*) are indicated on the *left* in each *panel*. CA1 regions were harvested from hippocampal slices at 60 min after the beginning of each regimen of pharmacological treatment, quick frozen, and immediately prepared as protein homogenates for SDS-PAGE and Western immunoblotting (also see Materials and Methods). *Lanes* marked *untreated* show proteins visualized in homogenates from slices exposed only to normal ACSF. Untreated and treated bands in *A–D* were obtained from cut segments of the same Western blot within a single experiment using paired slices from the same animal. *A*, Treated slices were exposed to high K^+/Ca^{2+} ACSF after a 10 min application of 50 μM FSK. A total of 40 μg of protein was loaded into each *lane*. *B*, Treated slices continuously bathed in 100 μM APV were exposed to 50 μM FSK followed by high K^+/Ca^{2+} ACSF. A total of 50 μg of protein was loaded into each *lane*. *C*, Treated slices were exposed to high K^+/Ca^{2+} ACSF alone. Each *lane* was loaded with 40 μg of protein. *D*, Treated slices were pre-exposed to 750 nM calyculin A (*CA*) before high K^+/Ca^{2+} ACSF application. Each *lane* contains 35 mg of protein. *E*, The *graphs* show the percent changes in the levels of Thr^{286}-phosphorylated (*left*) and total (*right*) αCaMKII in treated slices relative to that in paired, untreated control slices. Values are reported as percent changes of optical density from protein bands visualized by Western immunoblot using αCaMKII antibodies. In slices pre-exposed to FSK there was a significant increase in phospho-αCaMKII (61.2 ± 16.0% increase compared with the level in untreated slices; $n = 5$). When slices were exposed to FSK and high K^+/Ca^{2+} ACSF in the presence of 100 μM APV, no significant change in phospho-αCaMKII levels was detected (10.3 ± 20.8% decrease compared with the level in untreated slices; $n = 5$). High K^+/Ca^{2+} ACSF induced a significant decrease in phosphorylated αCaMKII (32.8 ± 6.2% decrease compared with that in untreated slices; $n = 4$). In slices exposed to CA before high K^+/Ca^{2+} ACSF application, there was a significant increase in phospho-αCaMKII levels (78.6 ± 33.0% increase compared with that in untreated slices; $n = 5$). An ANOVA of the results shown on the *left* revealed a significant effect of treatment ($F = 5.25$; $p < 0.02$) on the levels of phospho-αCaMKII. Follow-up multiple pairwise comparisons (Student–Newman–Keuls) revealed that, compared with high K^+/Ca^{2+} ACSF application, both FSK and CA treatment significantly increased phospho-αCaMKII levels ($p < 0.05$). No significant changes in the levels of total αCaMKII (*right*) were detected in any of these experiments (*+FSK*, 12.5 ± 8.5% and $n = 5$; *+FSK* and *+APV*, 15.7 ± 27.8% and $n = 4$; high K^+/Ca^{2+} ACSF alone, 7.8 ± 8.1% and $n = 5$; *+CA*, 4.3 ± 1.4% and $n = 5$).

using an antibody that recognizes both unphosphorylated and phosphorylated forms (Erondu and Kennedy, 1985). In agreement with previous reports (Molloy and Kennedy, 1991; Ocorr and Schulman, 1991; Fukunaga et al., 1993, 1995), we detected phospho-αCaMKII in untreated hippocampal slices (Fig. 7*A–D*, *lanes* marked *untreated*). However in FSK-treated slices exposed to high K^+/Ca^{2+} ACSF, there was a significant increase in phospho-αCaMKII levels ($p < 0.01$) compared with that in paired control slices exposed only to normal ACSF (Fig. 7*A*,*E*). In these same experiments there was no significant change in total αCaMKII levels (Fig. 7*A*,*E*). Blocking NMDA receptors with APV, which inhibits the persistent potentiation of synaptic transmission induced by high K^+/Ca^{2+} ACSF in FSK (Fig. 5*A*), also prevented the increase in phospho-αCaMKII levels seen in FSK-treated slices exposed to high K^+/Ca^{2+} ACSF (Fig. 7*B*,*E*). Levels of both phospho-αCaMKII and total αCaMKII were also unchanged in experiments in which slices were exposed to FSK alone. In these experiments ($n = 4$) levels of phospho-αCaMKII were $-10.3 \pm 17.4\%$ of those in control, untreated slices [difference not significant, $t(3) = 0.59$], while levels of total αCaMKII were $+2.9 \pm 3.9\%$ [not significant, $t(3) = 0.74$]. In previous experiments we have found that under our experimental conditions 50 μM FSK alone produces only a small, persistent enhancement of synaptic transmission (Thomas et al., 1996) that may be primarily caused by a presynaptic effect on transmitter release (Chavez-Noriega and Stevens, 1994). Together with these previous findings, the present results thus show that activating AC with FSK has only a small effect on synaptic transmission and does not significantly alter levels of Thr^{286}-phosphorylated αCaMKII. However, FSK enables the induction of both a persistent, LTP-like potentiation of synaptic transmission and a persistent increase in phospho-αCaMKII levels when coupled to a brief application of high K^+/Ca^{2+} ACSF.

In agreement with our results showing that AC activation enables the induction of a persistent potentiation by synaptic stimulation combined with high Ca^{2+} ACSF (Figs. 3*B*, 4), high K^+/Ca^{2+} ACSF had no lasting effect on synaptic strength in slices not treated with FSK (Fig. 5*A*). Immunoblot analysis revealed that in the absence of FSK, high K^+/Ca^{2+} ACSF significantly reduced phospho-αCaMKII levels ($p < 0.01$ compared with that in untreated slices) in the absence of any change in total αCaMKII levels (Fig. 7*C*,*E*). Thus, in the absence of FSK, high K^+/Ca^{2+} ACSF may activate protein phosphatases that dephosphorylate αCaMKII. Consistent with this notion, the substitution of calyculin A for FSK enabled both the induction of a persistent potentiation of synaptic transmission by high K^+/Ca^{2+} (fEPSPs were potentiated to $208.2 \pm 19.2\%$ of baseline 60 min after starting a 5 min application of high K^+/Ca^{2+} ACSF; $n = 4$) and a significant increase in the levels of phospho-αCaMKII ($p < 0.05$ compared with that in untreated control slices; Fig. 7*D*,*E*). In slices in which the high K^+/Ca^{2+} application was omitted, calyculin A had a small but nonsignificant effect ($p > 0.05$) on the basal levels of both phospho-αCaMKII and total αCaMKII ($n = 3$); levels of phospho-αCaMKII were increased $20.7 \pm 10.6\%$, and levels of total αCaMKII were increased $2.9 \pm 2.4\%$ relative to that of untreated controls.

DISCUSSION

Although an increase in postsynaptic Ca^{2+} is necessary for the induction of LTP at excitatory synapses onto pyramidal cells in the CA1 region of the hippocampus (Lynch et al., 1983; Malenka et al., 1992), under some experimental conditions a simple increase in postsynaptic Ca^{2+} does not appear to be sufficient for LTP induction (Kauer et al., 1988; Kullman et al., 1992). Similarly, we found that low frequency synaptic stimulation in the presence of elevated levels of extracellular Ca^{2+} induced an NMDA receptor-dependent but transient potentiation of synaptic transmission. In agreement with a previous study showing that levels of Ca^{2+}-independent CaMKII activity are enhanced immediately after hippocampal slices are exposed briefly to a high Ca^{2+} ACSF like that used in our experiments (Ocorr and Schulman, 1991), we also found that the potentiation induced by high Ca^{2+} ACSF was blocked by the CaM kinase inhibitor KN-62. Thus, although low frequency synaptic stimulation in high Ca^{2+} ACSF produces sufficient Ca^{2+} influx through NMDA receptor ion channels to activate CaM kinase and potentiate synaptic transmission, it does not induce a persistent potentiation of synaptic transmission. This suggests that, just as increases in intracellular Ca^{2+} are not always sufficient for LTP induction, activation of CaMKII can also occur without inducing a persistent potentiation of synaptic transmission.

Why might CaM kinase activation not be sufficient for the induction of a persistent potentiation by synaptic stimulation high Ca^{2+} ACSF? One possibility, suggested by our observation that protein phosphatase inhibitors convert the short-term potentiation induced by synaptic stimulation in high Ca^{2+} ACSF into a persistent potentiation, is that increases in intracellular Ca^{2+} not only activate CaM kinases but can also activate protein phosphatases that oppose CaM kinase activity. Indeed, although Ca^{2+} initially increases CaMKII autophosphorylation in isolated postsynaptic densities, it also produces a delayed, phosphatase-dependent decrease in the levels of autophosphorylated CaMKII (Dosemeci and Reese, 1993), an effect most likely mediated by PP1 (Shields et al., 1985; Dosemeci and Reese, 1993; Strack et al., 1997). Moreover, protein phosphatase activation may prevent LTP induction by repetitive activation of voltage-sensitive calcium channels (Kullman et al., 1992) or long trains of low frequency synaptic stimulation (Thomas et al., 1996), because these same patterns of stimulation induce a persistent potentiation of synaptic transmission in the presence of protein phosphatase inhibitors (Wyllie and Nicoll, 1994; Coussens and Teyler, 1996; Thomas et al., 1996). Finally, inhibiting calcineurin activity in hippocampal slices from adult animals is sufficient to induce a PKC and CaM kinase-dependent, LTP-like potentiation of excitatory synapses onto CA1 pyramidal cells (Wang and Kelly, 1997). Our results, together with these findings, suggest that in addition to CaMKII activation, downregulation of protein phosphatase activity also has an important role in LTP induction.

The Lisman model of LTP (Lisman, 1994) proposes that patterns of synaptic activity that produce low levels of NMDA receptor activation and small increases in intracellular Ca^{2+} depress synaptic strength via a cascade of protein phosphatase activation (Mulkey and Malenka, 1992; Mulkey et al., 1993, 1994). This cascade of protein phosphatase activation is thought to entail a Ca^{2+}- and calmodulin-dependent activation of calcineurin that dephosphorylates the PP1 regulatory protein inhibitor-1 (Mulkey et al., 1994). Dephosphorylation of inhibitor-1 activates PP1 that in turn dephosphorylates CaMKII. In contrast, stronger levels of NMDA receptor activation and larger increases in intracellular Ca^{2+} induce LTP by increasing levels of autophosphorylated CaMKII via a simultaneous activation of CaMKII and downregulation of PP1. In the model, a large increase in Ca^{2+} is thought to suppress PP1 activation by stimulating Ca^{2+}- and calmodulin-sensitive isoforms of AC and by

activating PKA that suppresses PP1 activation by opposing calcineurin-mediated dephosphorylation of inhibitor-1. Because high Ca^{2+} ACSF only induces a transient potentiation in the absence of phosphatase inhibitors, our results suggest that low frequency synaptic stimulation in the presence of elevated extracellular Ca^{2+} does not activate this PKA-dependent mechanism for suppressing protein phosphatases. However, in agreement with the notion that cAMP–PKA signaling contributes to the induction of long-lasting, NMDA receptor-dependent forms of synaptic plasticity (Blitzer et al., 1995, 1998; Thomas et al., 1996; Winder et al., 1998), the AC activators FSK and ISO enabled the induction of a persistent potentiation by synaptic stimulation in the presence of high Ca^{2+} ACSF.

By using a combination of AC activators and a modified ACSF containing elevated levels of K^+ and Ca^{2+}, we developed a protocol that could, in the absence of electrical presynaptic fiber stimulation, induce a persistent, NMDA receptor-dependent potentiation of synaptic transmission that occludes tetanus-induced LTP. Because our chemically induced LTP (chemLTP) shares common signaling pathways with tetanus-induced LTP, we used this technique to examine the potential role of cross-talk between the cAMP–PKA and CaMKII pathways in the induction of persistent changes in synaptic strength. In agreement with the notion that protein phosphatase activation prevents LTP induction by dephosphorylating αCaMKII, high K^+/Ca^{2+} ACSF applied in the absence of FSK had little lasting effect on synaptic strength and decreased phospho-αCaMKII levels. Phospho-αCaMKII levels and synaptic strength were both persistently elevated, however, when high K^+/Ca^{2+} ACSF was applied in the presence of the AC activator FSK, which alone had little persistent effect on basal levels of autophosphorylated CaMKII. Together, these results show that AC activation dramatically modulates activity-dependent changes in αCaMKII autophosphorylation and synaptic strength, thus providing experimental support for the idea that PKA activation modulates LTP induction by regulating the activity of protein phosphatases that oppose CaMKII autophosphorylation. In contrast to a recent report suggesting that increases in phospho-αCaMKII levels in pyramidal cell dendrites after LTP induction are secondary to a rapid increase in total αCaMKII levels (Ouyang et al., 1997), we did not observe significant changes in the levels of total αCaMKII in slices exposed to high K^+/Ca^{2+} ACSF in FSK. One possibility is that our chemLTP protocol does not mimic all aspects of the signaling pathways underlying the induction of LTP by synaptic stimulation (however, see Barria et al., 1997). We cannot rule out, however, that some of the increase in αCaMKII autophosphorylation seen in our experiments may be attributable, in part, to localized protein synthesis in either dendrites or soma (see Ouyang et al., 1997). We are currently addressing this question with subcellular studies that include measurements of changes in αCaMKII autophosphorylation and synthesis as a function of time after chemLTP is induced.

Although PKA was initially identified as having an important signaling role in the protein synthesis-dependent late stages of LTP (Frey et al., 1993; Matthies and Reymann, 1993), more recent evidence suggests that PKA also provides a mechanism for suppression of protein phosphatase activation in the early stages of LTP induction (Blitzer et al., 1995, 1998; Thomas et al., 1996; Winder et al., 1998). Consistent with this notion, our results show that activation of the cAMP–PKA signaling pathway regulates both activity-dependent changes in synaptic strength and CaMKII phosphorylation in a chemLTP induction protocol. Moreover, similar results have also been found after the induction of LTP by high frequency synaptic stimulation (Blitzer et al., 1998). However, PKA inhibitors do not block the early phases of LTP induced by all forms of synaptic stimulation (Weisskopf et al., 1994; Blitzer et al., 1995; Thomas et al., 1996). In addition, PKA inhibitors also do not block the ability of direct injections of Ca^{2+} and/or calmodulin into CA1 pyramidal cells to induce a CaM kinase-dependent, LTP-like potentiation of synaptic transmission (Wang and Kelly, 1995). PKA regulation of activity-dependent changes in CaMKII autophosphorylation may thus have an important modulatory, but not obligatory, role in the early stages of LTP.

Is the increase in phospho-αCaMKII levels enabled by FSK directly responsible for the potentiation of synaptic transmission observed in our experiments? Although CaMKII is required for the induction of LTP (Malenka et al., 1989; Malinow et al., 1989; Silva et al., 1992), attempts to block the maintenance of LTP by introducing CaMKII inhibitors into pyramidal cells after LTP induction have produced conflicting results (Feng, 1995; Otmakhov et al., 1997). These disparate findings may indicate that an increase in the levels of autophosphorylated, Ca^{2+}-independent CaMKII is only one of several biochemical changes that can persistently potentiate synaptic transmission. Thus, although our results are consistent with the view that PKA activation regulates the induction of LTP by inhibiting protein phosphatases that oppose CaMKII activity, they do not eliminate the possibility that AC activation modulates other components of the signaling pathways involved in LTP induction. Indeed, PKA activation also opposes the effects of calcineurin on NMDA receptor activity (Raman et al., 1996). Moreover, multiple serine/threonine kinases may be involved in the induction and maintenance of LTP (Bliss and Collingridge, 1993; Wang et al., 1997), and a PKA-mediated suppression of PP1 might provide a general mechanism for enhancing the effects of these kinases by inhibiting substrate dephosphorylation. The chemLTP protocol developed in our studies should provide a useful tool for examining how activation of cAMP–PKA signaling might regulate these other components of the signaling processes responsible for the induction and maintenance of LTP.

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