Attenuation of Paired-Pulse Facilitation Associated With Synaptic Potentiation Mediated by Postsynaptic Mechanisms

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Wang, Jin-Hui and Paul T. Kelly. Attenuation of paired-pulse facilitation associated with synaptic potentiation mediated by postsynaptic mechanisms. J. Neurophysiol. 78: 2707–2716, 1997. The relationship between paired-pulse facilitation (PPF) and synaptic potentiation induced by various protocols and their cellular and molecular mechanisms were examined by extracellular field potential and current- or voltage-clamp recordings at CA1 synapses in rat hippocampal slices. Microelectrodes were used for both intracellular recordings and injections of modulators of calcium (Ca$^{2+}$) and Ca$^{2+}$/calmodulin (CaM) signaling pathways into postsynaptic neurons. Basal synaptic transmission was not accompanied by changes in PPF. Tetanic stimulation induced long-term potentiation (LTP) of synaptic transmission and attenuated PPF. Experiments stimulating two independent Schaffer collateral/commisural (S/C) pathways showed that PPF attenuation and tetanus-LTP were pathway specific. Postsynaptic injections of pseudosubstrate inhibitors of CaM-dependent protein kinase II and protein kinase C (CaM-KII/PKC), [Ala$^{286}$]CaMKII$^{286-302}$ plus PKC$^{19-31}$, almost completely attenuated tetanus-LTP and reversed PPF attenuation but did not affect synaptic transmission and PPF under basal conditions. Postsynaptic injections of heparin and dantrolene (inhibitors of IP$_3$, and ryanodine receptors at intracellular Ca$^{2+}$ stores) prevented tetanus-LTP induction and PPF attenuation. Postsynaptic injections of calcineurin (CaN) inhibitors, CaN autoinhibitory peptide (CaN-AIP) or FK-506, enhanced synaptic transmission and decreased PPF. CaN-inhibited synaptic potentiation and PPF attenuation were unaffected by d(-)-a-Amino-5-phosphonopentanoic, but blocked by coinjecting 1,2-bis(2-aminophenoxy)ethane-N$_2$N$_2$N$_2$N$'$-tetraacetic acid, heparin plus dantrolene, calmodulin-binding peptide, or [Ala$^{286}$]CaMKII$^{281-302}$ plus PKC$^{19-31}$. PPF attenuation associated with tetanus-LTP or CaN-inhibited synaptic potentiation resulted from smaller increases in the potentiation of the first responses (R1) compared with the potentiation of the first responses (R1). Our results indicate that PPF attenuation is associated with synaptic potentiation mediated by postsynaptic mechanisms, and postsynaptic Ca$^{2+}$/CaM signaling pathways play a dual role in synaptic plasticity. CaN activity limits synaptic transmission under basal conditions, whereas the activation of Ca$^{2+}$-dependent protein kinases enhances synaptic transmission and attenuates PPF at central synapses.

INTRODUCTION

Synaptic facilitation, a short-term synaptic plasticity (Magleby 1987; Zucker 1989), has been observed at many chemical synapses (Feng 1941; Katz and Miledi 1968; Kuno 1964; Magleby 1973; Martin and Pilar 1964; McNaughton 1982; Porter 1970; Zengel et al. 1980). It has been shown that synaptic facilitation is associated with increases in presynaptic Ca$^{2+}$ levels and transmitter release (Charlton et al. 1982; Dudel and Kufferli 1961; Katz and Miledi 1967; Llinas et al. 1981; Mallart and Martin 1967, 1968; Miledi and Par-ker 1981; Miledi and Thies 1971), which indicates that presynaptic residual calcium is responsible for synaptic facilitation, i.e., residual calcium hypothesis (Magleby 1987; Zucker 1989). This hypothesis is used traditionally to explain paired-pulse facilitation (PPF) of central synaptic transmission (Christie and Abraham 1994; Creager et al. 1980; Manabe et al. 1993; McNaughton 1982; Muller and Lynch 1989; Schulz et al. 1994). It is noteworthy that presynaptic residual Ca$^{2+}$ as the sole mechanism for PPF has been questioned (Blundon et al. 1993; Winslow et al. 1994), alternative mechanisms may be involved in regulating synaptic facilitation (Chapman et al. 1995; Nathan and Lambert 1991; Nathan et al. 1990), and postsynaptic Ca$^{2+}$/CaM signaling pathways that alter Ca$^{2+}$/CaM signaling pathways and AMPA receptor properties appear to regulate the magnitude of synaptic facilitation (Wang and Kelly 1996b). Thus a complete understanding of the mechanisms for synaptic facilitation should include that increases in transmitter release by residual Ca$^{2+}$ generate PPF and the responsiveness of AMPA receptors modified by postsynaptic Ca$^{2+}$ and Ca$^{2+}$/CaM signaling pathways regulate PPF magnitude.

Long-term potentiation (LTP) of synaptic transmission triggered by electrical stimuli (Bliss and Gardner-Medwin 1973; Bliss and Lomo 1973) or biochemicals (Kang and Schultman 1995; Malenka et al. 1986; Wang and Kelly 1995) is believed to be one of the cellular bases of learning and memory in mammalian. While addressing the location of LTP expression (pre- or postsynaptic), a frequent criteria is to identify if PPF is changed. Synthetic potentiation followed by a change in PPF is attributed to presynaptic mechanisms (Christie and Abraham 1994; Kuhnt and Voronin 1994; Schulz et al. 1994, 1995; Voronin and Kuhnt 1990), which is based on the presynaptic origin for PPF. A few reports have shown no PPF change during LTP and argued that postsynaptic mechanisms are responsible for LTP (Gustafsson et al. 1988; Manabe et al. 1993). If the magnitude of PPF also is regulated by postsynaptic mechanisms, as previously shown (Wang and Kelly 1996b), the conclusion that synaptic potentiation with PPF changes are due to presynaptic mechanisms should be made with caution. In other words, changes in PPF may not serve as a criteria for judging the location of LTP expression. We have shown that postsynaptic Ca$^{2+}$/CaM signaling pathways and AMPA receptor responsiveness regulate synaptic transmission and synaptic facilitation (Wang and Kelly 1995, 1996a,b). To provide further evidence for our notion as well as to better understand how postsynaptic mechanisms regulate the facilitation and potentiation of synaptic transmission, we conducted a variety
of postsynaptic manipulations to reevaluate the relationship between synaptic potentiation and synaptic facilitation and to address the cellular location of PPF regulation.

METHODS

Transverse hippocampal slices were prepared from male Harlan Sprague-Dawley rats (6–7 wk) with a McIlwain tissue cutter in ice-cold standard medium (gassed with 95% O2-5% CO2) as previously described (Wang and Kelly 1995, 1996b). Slices were incubated in standard medium at 25°C for >1 h and then transferred to a submersion chamber (31°C; 2 ml/min perfusion rate) for electrophysiological experiments. Standard medium contained (in mM) 124 NaCl, 3 KCl, 1.3 NaH2PO4, 26 NaHCO3, 2.4 MgCl2, 2.4 CaCl2, 10 dextrose, and 10 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (pH 7.25). To reduce the effects of γ-aminobutyric acid (GABA), γ-mediated inhibitory components on excitatory synaptic transmission, experiments were conducted in the presence of bicucullin plus picrotoxin (10 μM each). In addition, orthodromic stimulating electrodes were placed as far away from recording sites as possible to avoid evoking monosynaptic GABAergic synaptic activities due to directly stimulating interneurons. Certain manipulations were used to prevent neuronal hyperexcitability in GABAergic antagonists: 1) “isolation” of CA1 area was achieved by cutting presynaptic axons in stratum radiatum while leaving oriens/aleuoch of hippocampal slices intact; this optimizes the integrity of CA1 neurons (Wang and Kelly 1996a), and 2) the concentration of Mg2+ was 2.4 mM to limit overactivity of synapses. Under these conditions, seizure activity was never observed during basal synaptic transmission and postsynaptic injections of agents; complex waveforms only occurred after tetanic stimulation in a few experiments.

Bipolar tungsten electrodes (12 MΩ) were positioned in stratum radiatum of CA1 area for orthodromically stimulating schaffer collateral/commisural (S/C) fibers. Certain experiments were conducted with stimulating two independent S/C pathways, in which two electrodes were used (1 was positioned on one area each for the recording site in stratum radiatum of CA1 area for orthodromic stimulation), and stimulation was alternated between these two pathways every 2 min. Independent pathways were verified by the observation that excitatory postsynaptic potential (EPSP) or excitatory postsynaptic current (EPSC) amplitudes measured from simultaneous stimulation of both pathways (data points in brackets in Figs. 2, 4, and 5) approximated the algebraic sum of EPSPs or EPSCs when P1 and P2 were stimulated individually (Wang and Kelly 1995). The frequency of test stimuli was 0.05 Hz; high-frequency tetanic stimulation was composed of 10 trains with 5-s intervals (each train contained 10 pulses with 200-Hz frequency) at the same stimulus intensity as test stimulation. Glass microelectrodes (60–85 MΩ) were filled with 2 M potassium acetate (KAc) or 2 M KAc plus various agents [calcineurin autoinhibitory peptide (CaN-AIP), FK-506, rapamycin, Ca2+/CaM, 1.2-bis (2-amino-phenoxy) -ethane-N,N’,N’ ,N’-tetraacetic acid (BAPTA), heparin, dantrolene, a calmodulin-binding peptide (CBP), or CaM-dependent protein kinase II and protein kinase C (CaM-KII/PKC) pseudosubstrate inhibitory peptides [Ala309-CaM-KII381-392 plus PKC9–31)]. Synaptic responses in CA1 pyramidal neurons were recorded under current- and voltage-clamp conditions in each set of studies to reduce the limitation inherent to each recording mode (e.g., current clamp is less effective in reducing the shunting effect caused by GABA receptor-channel activity, whereas voltage clamp is limited by space clamp). Pipettes containing 1 M NaCl for field potential recordings were placed in stratum radiatum near intracellular recording sites to verify the viability of tissue slices and status of synaptic activity. Experimental results are analyzed only from neurons in which stable recordings were obtained within 2 min after impalement and that exhibited stable membrane potentials at 70–73 mV throughout the experiment. Certain experiments were conducted by discontinuous single electrode voltage-clamp (dSEVC) recordings with 3-kHz sampling rate, and membrane potentials were held at −78 mV (i.e., near the GABA, reversal potential), which may weaken the shunting effect of GABAergic activity on EPSCs. During tetanic stimulation, the dSEVC recording mode was changed briefly to current-clamp mode. Synaptic responses (R) were computed as the initial slopes of EPSPs, EPSCs, or field EPSPs (efEPSP). The control (baseline) values of EPSPs, EPSCs, and efEPSPs are 8–10 mV, 200–250 pA, and 0.8–1 mV, respectively (~40% of maximal values). The values of PPF were calculated as (R2-R1)/R1 (i.e., relative facilitation). Baseline values (the first data point in each figure) of EPSPs, EPSCs, efEPSPs, or PPF were calculated from synaptic responses during the first minute after stable intracellular recordings were established and defined as 100%. Values of CaN- inhibited synaptic potentiation and PPF changes were from data points 1–3 min before tetanic stimulation compared with baseline, and values of LTP with PPF attenuation were from data points 20 min after tetanus. Synaptic responses are represented as means ± SE. Series and input resistances were monitored throughout all voltage- and current-clamp experiments by measuring responses to 4 mV and 0.1 nA injections (50 ms). Data were obtained with pClamp 5.5 and analyzed with custom software to compute initial EPSP, EPSC, and fEPSP slopes. Student’s t-tests were used for statistical comparisons. Waveforms were averaged from six consecutive responses and waveforms in each figure were selected from a representative experiment.

CaN-AIP (3 mM stock in distilled water) was diluted to a final concentration of 300 μM in 2 M KAc. Ca2+/CaM mixtures were prepared from CaCl2 and CaM stock solutions and mixed with CaN-AIP and then diluted to final concentrations of 20/80 μM and 300 μM in 2 M KAc. FK-506 and rapamycin were dissolved in 100% ethanol (50 mM stock solutions) and diluted to a final concentration of 50 μM in 2 M KAc. BAPTA was dissolved in 3 M KOH (400 mM stock, pH 7.2) and diluted to a final concentration of 20 mM in 2 M KAc plus 50 μM FK-506. Heparin and dantrolene were dissolved in 2 M KAc at final concentrations of 300 μM and 80 μM or plus 50 μM FK-506. Stock solutions of CBP or [Ala309]CaM-KII381–392/PKC19–31 in distilled water were diluted to final concentrations of 100 μM or 200 μM/100 μM in 2 M KAc. Microelectrodes were filled completely using these solutions. Bicucullin and BAPTA were purchased from Sigma; heparin, dantrolene, Δ(−)-2-amino-5-phosphonopentanoic acid (Δ-AP5) and picrotoxin were from RBI; CaM was gift from Dr. J. Aronowski; CaN-AIP was gift from Dr. Randall Kincaid (Veritas, Rockville, MD); FK-506 and rapamycin were gifts from Dr. Stan Stepkowski (University of Texas Medical School at Houston).

RESULTS

Tetanus-induced LTP is accompanied by PPF attenuation

It is controversial whether tetanus-induced LTP is accompanied by a change (Christie and Abraham 1994; Kuhnt and Voronin 1994; Schulz et al. 1994, 1995; Voronin and Kuhnt 1990) or no change in PPF (Gustafsson et al. 1988; Manabe et al. 1993). We reexamined this issue at CA1 synapses in rat hippocampal slices. First, the dynamic process of PPF during LTP was observed under field potential recordings. As shown in Fig. 1, after recording stable synaptic transmission and PPF for 32 min, tetanic stimulation induced nondecremental LTP (R1, 166 ± 7%; R2, 154 ± 8% relative to baseline 100%) and attenuated PPF (66 ± 5% relative to baseline 100%, n = 14/14 (i.e., 14 of 14 experiments); Fig.
Transmission and induced potentiation of synaptic responses (response R1; attenuation, and the changes are synapse-specific. It is noteworthy that simultaneous stimulation to these two independent pathways (i.e., pathway 1, P1; pathway 2, P2), and results are shown in Fig. 2. After tetanus was given to P1, synapses in P1 expressed robust LTP (R1, 172 ± 9%; R2, 153 ± 6%) and PPF attenuation (56 ± 2%; n = 15/15), whereas P2 did not display changes in basal synaptic transmission (R1, 101 ± 3%; R2, 102 ± 4%) and PPF (109 ± 8%; n = 14/14); both values were obtained at 40–44 min. Tetanic stimulation given to P2 (n = 7/7) at 100 min induced LTP (R1, 165 ± 6%; R2, 159 ± 7%) and PPF attenuation (77 ± 9%). These results indicate that tetanus induces simultaneous changes in synaptic plasticity, i.e., LTP and PPF attenuation, and the changes are synapse-specific. It is noteworthy that simultaneous stimulation to these two independent pathways results in decreased PPF during field potential recordings (brackets in Fig. 2).

We further examined these results under intracellular recordings (dSEVC mode) with electrodes containing 50 μM rapamycin. Basal synaptic transmission and PPF were not changed. Tetanic stimulation at 60 min induced robust LTP (R1, 184 ± 10%; R2, 157 ± 7%) and PPF attenuation (53 ± 10%; n = 5/5; Fig. 3). Rapamycin binds CaN-anchoring proteins (FK-506 binding proteins, FKBP5) without affecting CaN activity (MacKintosh and MacKintosh 1994; Schreiber and Crabtree 1992; Wiederrecht et al. 1989) and was used as a control for FK-506 experiments (see below). These results further indicate that tetanus-induced LTP is associated with PPF attenuation under a variety of experimental conditions.

Postsynaptic injections of CaM-KII and PKC pseudosubstrate inhibitors suppress LTP maintenance and reverse PPF attenuation

Experiments were conducted by sequentially recording two nearby CA1 neurons with KAc- and [Ala268]CaM-KII281–302/PKC19–31-containing pipettes and alternatively stimulating two independent S/C pathways. Figure 4 shows effects of postsynaptic injections of [Ala268]CaM-KII281–302 plus PKC19–31 on established LTP and PPF attenuation. In the first part of experiments, a 2 M KAc pipette was used to impale a CA1 pyramidal neuron. After getting stable control values of synaptic transmission and verifying two independent pathway stimulation (see METHODS), tetanus was delivered to P1. Synaptic responses displayed typical LTP (R1 196 ± 21% relative to baseline 100%; R2, 152 ± 19%; Fig. 4A) and PPF attenuation (39 ± 12%; n = 7/7; Fig. 4B) in P1, but little if any change of basal synaptic transmission (R1, 118 ± 10%; R2, 117 ± 8%) and PPF (103 ± 11%) in P2. After 40
min, the KAc pipette was withdrawn carefully, and another pipette containing 200 \( \mu \)M \([\text{Ala}^{286}]\text{CaM-KII}_{281-302}\) and 100 \( \mu \)M PKC\(_{19-31}\) was used to impale a second CA1 neuron near the first one (this manipulation was accomplished within 20 min). Synaptic responses in P1 and P2 were monitored continuously for an additional 120 min and normalized relative to the first data point of basal synaptic responses recorded from the first neuron in P1 and P2.

The second part of the experiments shows the effect of pseudosubstrate inhibitors of CaM-KII and PKC on synaptic transmission and PPF in potentiated and basal pathways. The postsynaptic injections of \([\text{Ala}^{286}]\text{CaM-KII}_{281-302}\) and PKC\(_{19-31}\) significantly decreased prepotentiated synaptic transmission (R1, 133 \( \pm \) 22% relative to 254 \( \pm \) 37%; R2, 130 \( \pm \) 21% relative to 204 \( \pm \) 26%; \( n = 7/7 \); \( P < 0.002 \); Fig. 4A, ● and ■) and reversed PPF attenuation close to baseline values within 90 min (102 \( \pm \) 12%; \( n = 7/7 \); \( P < 0.01 \); Fig. 4B, ◇) in P1 but had little effect on basal synaptic transmission (R1, 107 \( \pm \) 12%; R2, 114 \( \pm \) 12%) and PPF (122 \( \pm \) 13%) in P2. These results indicate that postsynaptic CaM-KII/PKC activities triggered by tetanus are responsible for maintaining synaptic potentiation and PPF attenuation. It is noteworthy that simultaneous stimulation to these two independent pathways results in decreased PPF during intracellular recording with 2 M KAc (Fig. 4, [●]).

**Postsynaptic injections of heparin plus dantrolene prevent LTP induction and PPF attenuation**

Our results showed that the increases in postsynaptic Ca\(^{2+}\), Ca\(^{2+}/\text{CaM}\), or Ca\(^{2+}\)-dependent protein kinase activities (e.g., CaM-KII and PKC) play an important role in synaptic potentiation and PPF attenuation (Wang and Kelly 1996) (Fig. 4). To define the source of Ca\(^{2+}\) for these changes, we examined whether postsynaptic Ca\(^{2+}\) increases are due to N-methyl-D-aspartate (NMDA) receptor activation (see Fig. 8), release from intracellular Ca\(^{2+}\) stores by inositol-1,4,5-triphosphate (IP\(_3\)) and ryanodine (Ry) receptor-channels (Ghosh and Greenberg 1995) or both. Heparin and dantrolene were selected for inhibiting activities of IP\(_3\)R and RyR (Ohta 1990; Smith and Gallagher 1994). Experiments were conducted under the condition of simultaneous intracellular and field potential recordings as shown in Fig. 5. Postsynaptic injections of heparin plus dantrolene had little if any effect on basal synaptic transmission but prevented tetanus-induced LTP (R1, 112 \( \pm \) 1%; R2, 117 \( \pm \) 9%; Fig. 5A, ● and ■) and PPF attenuation (115 \( \pm \) 17%; Fig. 5B, ◇; \( n = 5/5 \)) compared with the values of LTP and PPF in simultaneous field potential recordings (R1, 159 \( \pm \) 2.5%; R2, 130 \( \pm \) 1%; PPF, 46 \( \pm \) 5%). These results indicate that tetanus triggers postsynaptic Ca\(^{2+}\) release from intracellular stores by IP\(_3\)R and RyR, which is responsible for LTP induction and PPF attenuation. Interestingly, the decreases in PPF by simultaneously stimulating two independent pathways in field potential recordings (◇, □, and ◄) were not observed under intracellular recordings with heparin/dantroline-containing electrodes (Fig. 5B, [●]).

**FIG. 3.** Tetanus LTP is accompanied by PPF attenuation under voltage-clamp recordings. After recording stable synaptic transmission for 60 min, tetanic stimulation induced LTP (R1, ◇; R2, □) and PPF attenuation (◇; \( n = 5 \)). Pipettes contained 50 \( \mu \)M rapamycin in 2 M potassium acetate (KAc; control for FK-506 experiments). Inset: representative excitatory postsynaptic current (EPSC) waveforms; calibration is 250 pA/30 ms; →, tetanic stimulation.

**FIG. 4.** Effects of postsynaptic injections of CaM-KII plus PKC inhibitors on basal synaptic transmission, tetanus-induced LTP and PPF attenuation. A: \([\text{Ala}^{286}]\text{CaM-KII}_{286-302}/\text{PKC}_{19-31}\) attenuates LTP without significantly affecting basal synaptic transmission. After stable EPSP recordings with 2 M KAc electrodes for 10 min, tetanic stimulation given to pathway 1 (P1, ● and ■) induced LTP (R1, ●; R2, ■), and synaptic transmission in pathway 2 (P2, ◇ and ◄) was not significantly changed. KAc electrodes were replaced by the second electrodes containing 200 \( \mu \)M \([\text{Ala}^{286}]\text{CaM-KII}_{281-302}\) and 100 \( \mu \)M PKC\(_{19-31}\) in 2 M KAc for impaling a second nearby neuron within 20 min. Manipulations were done very carefully to prevent movement of electrodes, and stimulation intensity was not changed. During subsequent recordings for \( > \)100 min, synaptic responses in P1 (R1 and R2, ● and ■) returned to near baseline values (○, ○, ○) and EPSPs in P2 (R1 and R2, ◇ and ◄) were not significantly changed. Inset: representative EPSPs showing the effect of \([\text{Ala}^{286}]\text{CaM-KII}_{286-302}/\text{PKC}_{19-31}\) on LTP in P1; calibration is 20 mV/30 ms. B: \([\text{Ala}^{286}]\text{CaM-KII}_{286-302}/\text{PKC}_{19-31}\) reverses PPF attenuation induced by tetanus. Left: tetanus-induced PPF attenuation in P1 (●) recorded with 2 M KAc electrodes, but PPF in pathway 2 (P2, ◇) was not significantly changed. Right: postsynaptic injections of \([\text{Ala}^{286}]\text{CaM-KII}_{286-302}\) (200 \( \mu \)M in 2 M KAc electrodes) plus PKC\(_{19-31}\) (100 \( \mu \)M) into a second nearby neuron reversed tetanus-induced PPF attenuation in P1 (●) without significantly affecting PPF in P2 (○). ---: inhibitor or KAc diffusion into neurons; ●: tetanus; and [ ] enclose data points with simultaneous stimulation in P1 and P2.
CaN-inhibited synaptic potentiation is accompanied by PPF attenuation

Ca\textsuperscript{2+}/CaM-induced synaptic potentiation and tetanus LTP, which share similar postsynaptic mechanisms (Wang and Kelly 1995), are associated with PPF attenuation (Wang and Kelly 1996b) (Figs. 1–3). Are other types of synaptic potentiation initiated by postsynaptic mechanisms, e.g., CaN-inhibited synaptic potentiation (Wang and Kelly 1996a), also associated with PPF attenuation? We first examined the effect of CaN-AIP (Hashimoto et al. 1990) on synaptic transmission and PPF. Figure 6A shows that postsynaptic injections of CaN-AIP (300 \(\mu\)M in pipette) induced synaptic potentiation (R1, 202 \(\pm\) 14%; R2, 172 \(\pm\) 12%) and PPF attenuation (60 \(\pm\) 8%; \(n = 6/6\)). Interestingly, subsequent tetanus induced synaptic depotentiation in R1 (153 \(\pm\) 13%) but not R2 (168 \(\pm\) 6%) and reversed PPF attenuation (95 \(\pm\) 10%; \(n = 6/6\)). We also examined the effect of postsynaptic injections of CaN-AIP plus Ca\textsuperscript{2+}/CaM on synaptic transmission and PPF (Fig. 6B). Intracellular recordings with pipettes containing 300 \(\mu\)M CaN-AIP and 80/20 \(\mu\)M Ca\textsuperscript{2+}/CaM displayed synaptic potentiation (R1, 183 \(\pm\) 10%; R2, 138 \(\pm\) 10%) and PPF attenuation (40 \(\pm\) 7%; \(n = 7/7\)). Subsequent tetanus induced synaptic depotentiation in R1 (165 \(\pm\) 8%) but not R2 (154 \(\pm\) 2%) and reversed PPF attenuation (99 \(\pm\) 11%; \(n = 7/7\)). These results demonstrate that CaN-AIP–induced synaptic potentiation is accompanied by PPF attenuation. It is noteworthy that PPF attenuation by CaN-AIP plus Ca\textsuperscript{2+}/CaM (40 \(\pm\) 7%; Fig. 6B) is significantly larger than that by CaN-AIP (60 \(\pm\) 8%, Fig. 6A; \(P < 0.017\)), which may be due to the synergistic effect of CaN-AIP plus Ca\textsuperscript{2+}/CaM on PPF.

To further examine the effect of postsynaptic CaN activity on synaptic transmission and PPF, we used another CaN inhibitor, FK-506, which inhibits CaN activity by binding to FKBP\textsubscript{5} (e.g., FKBP-12) and is a much more potent (IC\textsubscript{50} \(\sim\) 30 nM) (Dawson et al. 1994; MacKintosh and Mackintosh 1994; Schreiber and Crabtree 1992; Steiner et al. 1992; Wiederrrecht et al. 1989) than CaN-AIP (IC\textsubscript{50} \(\sim\) 10 \(\mu\)M) (Hashimoto et al. 1990). Figure 7 shows the result of injecting FK-506 (50 \(\mu\)M in pipette) into postsynaptic neurons under voltage-clamp recordings. Postsynaptic injections of FK-506 induced a gradual synaptic potentiation (R1, 207 \(\pm\) 13%; R2, 169 \(\pm\) 12% at 60 min) and decreased PPF (32 \(\pm\) 6%; \(n = 6/6\); Fig. 7A). This result together with CaN-AIP results indicate that CaN-inhibited synaptic potentiation is associated with PPF attenuation. CaN-inhibited synaptic potentiation with PPF attenuation further indicates that postsynaptic CaN activity limits synaptic transmission and facilitates paired-pulse responses under basal conditions. The effect of tetanus on FK-506–induced synaptic potentiation is shown in Fig. 7B. After synaptic potentiation (R1, 220 \(\pm\) 16%; R2, 183 \(\pm\) 11% at 32 min) and PPF attenuation (31 \(\pm\) 7%), tetanic stimulation produced synaptic depotentiation (R1, 189 \(\pm\) 17%; R2, 168 \(\pm\) 18%; \(P = 0.021\) for R1...
and R2 potentiation, respectively; L required postsynaptic Ca\(^{2+}\) CaN-inhibited synaptic potentiation and PPF attenuation. The larger synaptic depotentiation in R1 relative to R2.

Inhibited PPF attenuation by tetanus appeared to result from if certain manipulations simultaneously prevented CaN-inhibited synaptic potentiation and PPF attenuation. As CaN-inhibited synaptic potentiation occludes tetanus LTP (Figs. 6 and 7) and LTP induction requires NMDA receptor activation (Collingridge et al. 1983a,b) and postsynaptic Ca\(^{2+}\)/CaM signaling pathways. First, the role of postsynaptic Ca\(^{2+}\)/CaM signaling pathways was tested. Simultaneous intracellular (dSEVC) and extracellular recordings were conducted in presynaptic stores) was examined. Heparin and dantrolene, which inhibit IP\(_3\)R and RyR activities, respectively (Ohta 1990; Smith and Gallacher 1994), were coinjected with FK-506 into CA1 neurons. Figure 9B shows that heparin/dantrolene significantly decrease FK-506–induced synaptic potentiation (R1, 124 \pm 5\% relative to 207 \pm 11\%; \(P = 0.0009\)) and PPF attenuation (113 \pm 10\% relative to 32 \pm 6\%; \(P = 0.0008\); n = 8/8). This result indicates that Ca\(^{2+}\) comes from intracellular release by IP\(_3\) and ryanodine receptors.

We further examined the role of Ca\(^{2+}\) and CaM downstream targets in CaN-inhibited synaptic potentiation and PPF attenuation. A high-affinity CBP (Hanley et al. 1988; Kelly et al. 1989) was used. Pipettes containing 100 \mu M CBP plus 50 \mu M FK-506 in 2 M KAc were used to impale CA1 neurons for intracellular recordings and postsynaptic coinjections. Figure 10A shows that CBP significantly decreased FK-506–induced synaptic potentiation (R1, 101 \pm 10\% at 40 min) and prevented PPF attenuation (60 \pm 8\%; n = 5/5; Fig. 10A).

Next, the role of postsynaptic Ca\(^{2+}\) signaling pathways was examined. Pipettes containing 50 \mu M FK-506 with 40 mM BAPTA in 2 M KAc were used for intracellular recordings (dSEVC) in CA1 neurons. Figure 9A shows that postsynaptic coinjections of BAPTA significantly decreased FK-506–induced synaptic potentiation (R1, 117 \pm 5\% relative to 207 \pm 11\%; \(P = 0.0008\)) and PPF attenuation (101 \pm 10\% relative to 32 \pm 6\%; \(P = 0.0004\); n = 6/6). This result indicates that postsynaptic Ca\(^{2+}\) increases are responsible for CaN-inhibited synaptic potentiation and PPF attenuation. Because D-AP5 did not affect the result of FK-506 injections (Fig. 8), another source for postsynaptic Ca\(^{2+}\) increases (e.g., intracellular stores) was examined. Heparin and dantrolene, which inhibit IP\(_3\)R and RyR activities, respectively (Ohta 1990; Smith and Gallacher 1994), were coinjected with FK-506 into CA1 neurons. Figure 9B shows that heparin/dantrolene significantly decrease FK-506–induced synaptic potentiation (R1, 124 \pm 5\% relative to 207 \pm 11\%; \(P = 0.0009\)) and PPF attenuation (113 \pm 10\% relative to 32 \pm 6\%; \(P = 0.0008\); n = 8/8). This result indicates that Ca\(^{2+}\) comes from intracellular release by IP\(_3\) and ryanodine receptors.

CaN-inhibited synaptic potentiation and PPF attenuation require postsynaptic Ca\(^{2+}\) signaling pathways

Further evidence for the associated changes in CaN-inhibited synaptic potentiation and PPF attenuation was obtained by testing if certain manipulations simultaneously prevented CaN-inhibited synaptic potentiation and PPF attenuation. As CaN-inhibited synaptic potentiation occludes tetanus LTP (Figs. 6 and 7) and LTP induction requires NMDA receptor activation (Collingridge et al. 1983a,b) and postsynaptic Ca\(^{2+}\) (Lynch et al. 1983), we examined the effects of blocking NMDA receptor activation or postsynaptic Ca\(^{2+}\) signaling pathways. First, the role of NMDA receptors was tested. Simultaneous intracellular (dSEVC) and extracellular recordings were conducted in presence of 40 \mu M D-AP5; pipettes contained 50 \mu M FK-506. As shown in Fig. 8, FK-506 induced synaptic potentiation (R1, 211 \pm 12\%; R2, 186 \pm 13\% at 40 min) and PPF attenuation (66 \pm 6\%). Subsequent tetanus did not decrease CaN-inhibited synaptic potentiation (R1 237 \pm 20\%; R2 200 \pm 14 at 72 min) and PPF attenuation (60 \pm 8\%; n = 5/5; Fig. 8A). However, compared with results in Figs. 1 and 2, D-AP5 attenuated LTP magnitude in field potential recordings (R1, 117 \pm 5\%; R2, 113 \pm 4\%; Fig. 8B) and prevented PPF attenuation (85 \pm 22\%; \(P = 0.09\)). This result indicates that NMDA receptor activation does not contribute to CaN-inhibited synaptic potentiation and PPF attenuation. The small tetanus-LTP of fEPSPs in D-AP5 (Fig. 8B) may be similar to the NMDA-independent LTP reported previously (Grover and Tyler 1990).
and PPF attenuation. As Ca\(^{2+}\) a functional element in CaN-inhibited synaptic potentiation. Pseudosubstrate inhibitors of CaM-KII and PKC, [Ala\(^{286}\)]-CaM-KII attenuates the effect of FK-506. Pipettes containing 40 mM BAPTA with 50 \(\mu\)M FK-506 were used for intracellular recordings; coinjection results R1 (●) and PPF (○) compared with the effects of FK-506 alone on synaptic transmission (△) and PPF (○). Inset: representative EPSCs from coinjecting FK-506 plus BAPTA; calibration is 250 pA/30 ms. 

9% relative to 207 ± 11%; \(P = 0.00004\) and PPF attenuation (119 ± 16% relative to 32 ± 6%; \(P = 0.0008\); \(n = 7/7\)). This result indicates that active Ca\(^{2+}\)/CaM serves as a functional element in CaN-inhibited synaptic potentiation and PPF attenuation. As Ca\(^{2+}\)/CaM-induced synaptic potentiation and PPF attenuation require CaM-KII and PKC activities (Wang and Kelly 1995, 1996), we tested if CaN-inhibited synaptic effects require CaM-KII and PKC activities. Pseudosubstrate inhibitors of CaM-KII and PKC, [Ala\(^{286}\)]-CaM-KII\(_{381-392}\) (100 \(\mu\)M) and PKC\(_{19-31}\) (100 \(\mu\)M) (Hanson et al. 1994; Kemp 1994) plus FK-506 (50 \(\mu\)M) in 2 M KAc were co-injected into postsynaptic neurons. Figure 10B shows that [Ala\(^{286}\)]-CaM-KII\(_{381-392}\) and PKC\(_{19-31}\) significantly decreased FK-506-induced synaptic potentiation (R1, 123 ± 11% relative to 207 ± 11%; \(P = 0.0008\)) and PPF attenuation (110 ± 10% relative to 32 ± 6%; \(P = 0.0009\); \(n = 7/7\)). This result indicates that postsynaptic CaM-KII/PKC activities are involved in CaN-inhibited synaptic potentiation and PPF attenuation.

**DISCUSSION**

Our results show that tetanus LTP is accompanied by PPF attenuation in extracellular or intracellular recordings (Figs. 1–3); this supports previous reports (Christie and Abraham 1994; Kuhnt and Voronin 1994; Schulz et al. 1994, 1995; Voronin and Kuhnt 1990). Based on the hypothesis that PPF is generated by presynaptic residual Ca\(^{2+}\) (Magleby 1987; Zucker 1989), previous authors suggested that the mechanisms of LTP expression are presynaptic. However, we found that postsynaptic injections of IP\(_{3}\)/RyR inhibitors prevented tetanus-LTP induction and PPF attenuation (Fig. 5), and CaM-KII/PKC pseudosubstrate inhibitors significantly attenuated LTP maintenance and reversed PPF attenuation (Fig. 4). These results strongly suggest that PPF attenuation associated with LTP is regulated by postsynaptic mechanisms. We reported that postsynaptic Ca\(^{2+}\)/CaM-induced synaptic potentiation is accompanied by PPF attenuation (Wang and Kelly 1996b). We now show that PPF attenuation is associated with synaptic potentiation induced by inhibiting postsynaptic CaN activity (Figs. 6–10). These associated changes in synaptic potentiation and PPF attenuation rely on postsynaptic Ca\(^{2+}\) and Ca\(^{2+}\)/CaM signaling mechanisms. Taking all of these results together, we conclude that postsynaptic mechanisms contribute to both LTP expression and PPF modulation and suggest that changes in PPF should not be a criteria for assigning a presynaptic location for LTP expression because postsynaptic biochemical manipulations alter PPF.

Ca\(^{2+}\)/CaM-induced or CaN-inhibited synaptic potentiation occlude tetanus LTP (Wang and Kelly 1995, 1996a) (Figs. 6 and 7). All three forms of synaptic potentiation and

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**FIG. 9.** Postsynaptic co-injections of 1,2-bis(2-aminophenoxy)-ethane-N,N,N',N'-tetraacetic acid (BAPTA) or heparin/dantrolene block FK-506–induced synaptic potentiation and PPF attenuation. A: BAPTA attenuates the effect of FK-506. Pipettes containing 40 mM BAPTA with 50 \(\mu\)M FK-506 were used for intracellular recordings; coinjection results R1 (●) and PPF (○) compared with the effects of FK-506 alone on synaptic transmission (△) and PPF (○). Inset: representative EPSCs from coinjecting FK-506 plus BAPTA; calibration is 250 pA/30 ms. B: heparin/dantrolene attenuates the effects of FK-506. Pipettes containing 300 \(\mu\)M heparin and 80 \(\mu\)M dantrolene plus 50 \(\mu\)M FK-506 were used for intracellular recording; coinjection results R1 (●) and PPF (○) compared with FK-506 alone (△ and ○). Inset: representative EPSCs from co-injecting heparin/dantrolene with FK-506 at the time points of 1 and 2; calibration is 250 pA/30 ms.

**FIG. 10.** Postsynaptic co-injections of CBP, or [Ala\(^{286}\)]-CaM-KII\(_{381-392}\)/PKC\(_{19-31}\) block FK-506–induced synaptic potentiation and PPF attenuation. A: calmodulin-binding peptide (CBP) attenuates the effects of FK-506. Pipettes containing 100 \(\mu\)M CBP with 50 \(\mu\)M FK-506 were used for intracellular recordings; coinjection results R1 (●) and PPF (○) compared with the effects of FK-506 alone on synaptic transmission (△) and PPF (○). Inset: representative EPSCs from co-injecting FK-506 plus CBP; calibration is 250 pA/30 ms. B: [Ala\(^{286}\)]-CaM-KII\(_{381-392}\)/PKC\(_{19-31}\) attenuates the effects of FK-506. Pipettes containing 200/100 \(\mu\)M CaM-KII\(_{381-392}\)/PKC\(_{19-31}\) with 50 \(\mu\)M FK-506 were used for intracellular recordings; coinjection results R1 (●) and PPF (○) compared with FK-506 alone (△ and ○). Inset: representative EPSCs from co-injecting [Ala\(^{286}\)]-CaM-KII\(_{381-392}\)/PKC\(_{19-31}\) with FK-506 at the time points of 1 and 2; calibration is 250 pA/30 ms.
associated PPF attenuation require postsynaptic Ca\(^{2+}\)/CaM signaling pathways (Wang and Kelly 1996b) (Figs. 4, 5, 9, and 10). Thus they may share common mechanisms, i.e., increases in postsynaptic Ca\(^{2+}\)/CaM levels and CaM-KII/PKC activities. Moreover, synaptic potentiation and associated PPF attenuation may require a positive feedback mechanism to maintain persistent expression. In this study, we found that PPF attenuation associated with CaN-inhibited synaptic potentiation and tetanus-LTP required postsynaptic Ca\(^{2+}\) release from intracellular stores by IP\(_{3}\)R and RyR (Figs. 5 and 9). Together with biochemical studies showing that IP\(_{3}\)R and RyR are phosphorylated by CaM-KII/PKC and dephosphorylated by CaN (Cameron et al. 1995; Furuchi and Mikoshiba 1995; Hain et al. 1995; Snyder and Saba-tini 1995), the phosphorylation of IP\(_{3}\)R, IP\(_{3}\)R, and RyR (Cameron et al. 1995), and the phosphorylation of RyR by CaM-KII activates this receptor (Takasawa et al. 1995), we hypothesize that postsynaptic Ca\(^{2+}\) stores are a critical site for this positive feedback, i.e., the phosphorylation of IP\(_{3}\)R and RyR facilitates further increases in Ca\(^{2+}\) and Ca\(^{2+}\)/CaM signaling pathways.

Our previous results indicate that Ca\(^{2+}\)/CaM-induced PPF attenuation is due to an increase in AMPA receptor desensitization through CaM-KII/PKC activity (Wang and Kelly 1996b). As tetanus LTP and CaN-inhibited synaptic potentiation share common mechanisms with Ca\(^{2+}\)/CaM-induced synaptic potentiation, PPF attenuation associated with CaN-inhibited synaptic potentiation and tetanus-LTP may be due to an increase of AMPA receptor desensitization. If each one of these postsynaptic manipulations enhances CaM-KII/PKC activity and then increases AMPA receptor desensitization while increasing receptor sensitivity, their combination may produce higher levels of AMPA receptor desensitization than each alone. The final read-out of receptor responsiveness, which reflects the equilibrium between receptor sensitivity and desensitization (Wang and Kelly 1996b), may account for why postsynaptic injections of Ca\(^{2+}\)/CaM plus CaN-AIP did not induce additive synaptic potentiation but induced a larger PPF attenuation than CaN-AIP alone (Fig. 6) and why tetanus-induced synaptic depotentiation after CaN-inhibited or Ca\(^{2+}\)/CaM-induced synaptic potentiation (Wang and Kelly 1995) (Figs. 6 and 7).

Certain reports seem to argue against a role of affecting postsynaptic AMPA receptors in modulating synaptic facilitation, in which the partial block of AMPA receptor activity by 6-cyano-7-nitroquinoxaline-2,3-dione did not change PPF (Manabe et al. 1993; Schulz et al. 1994) and PPF of EPSC amplitude was independent of membrane potentials (Clark et al. 1994; Manabe et al. 1993). These manipulations either reduce the number of active AMPA receptors or change the electrical driving force for ions through channels but are not believed to modify biochemical properties of an individual receptor; this may explain why these manipulations produce parallel changes in R1 and R2. Our protocols involved activating postsynaptic Ca\(^{2+}\)/CaM signaling pathways or inhibiting CaN, which enhance protein phosphorylation (e.g., the biochemical modification of receptors) such that receptor sensitivity and desensitization are increased, which together are responsible for synaptic potentiation and PPF attenuation.

During the assessment of pathway independence in two-pathway experiments, we observed that additive synaptic responses by simultaneous stimulation of two independent S/C pathways was associated with a decrease in PPF (Figs. 2, 4, and 5, [ ] ) that was due to the decreased summation of R2 compared with R1. This observation may be similar to the result in which PPF decreased during increasing stimulus intensity in one pathway (Dumas and Foster 1995). It is difficult to explain this PPF decrease, which is caused by activating two independent groups of synapses, by presynaptic mechanisms if there is no change in postsynaptic receptor properties. Because of the role of presynaptic residual Ca\(^{2+}\) during the second stimulation, the increases in transmitter release at the two groups of synapses should result in linear summation in R2 similar to R1. Alternatively, this PPF decrease may result from interactions among synapses. If the interaction is presynaptic in origin (e.g., presynaptic inhibition), it is difficult to understand why postsynaptic injections of heparin plus dantrolene prevented this PPF decrease (Fig. 5B, [ ] ), in which heparin and dantrolene appear to attenuate R1 summation with less effect on R2 summation. The mechanism for this phenomenon remains to be elucidated.

Our results show that synaptic potentiation and PPF attenuation induced by tetanus, activation of postsynaptic Ca\(^{2+}\)/CaM signaling pathways, or inhibition of postsynaptic CaN activity require postsynaptic Ca\(^{2+}\) mobilization and CaM-KII/PKC activities. One could argue that our postsynaptic manipulations may induce the production of retrograde messengers [e.g., nitric oxide (NO)], which in turn enhance presynaptic transmitter release (Garthwaite 1991). Although our results cannot rule out this possibility, the phosphorylation of NO synethase (NOS) by PKC or CaM-KII reduces its catalytic activity and decreases NO production (Steiner et al. 1996), i.e., weakening the index of NO function. Thus the precise role of retrograde messengers in regulating PPF remains to be further explored. We conclude that postsynaptic Ca\(^{2+}\) and Ca\(^{2+}\)/CaM signaling pathways play an important role in regulating synaptic strength and synaptic facilitation. As Ca\(^{2+}\)/CaM and/or Ca\(^{2+}\) activate CaN and CaM-KII/PKC, we suggest that these postsynaptic signaling pathways play a dual role in synaptic strength and facilitation, i.e., CaN activity limits synaptic strength (Wang and Kelly 1996a) associated with larger synaptic facilitation, whereas increased CaM-KII/PKC activities enhance synaptic strength with attenuated synaptic facilitation. As the magnitude of PPF is regulated by postsynaptic Ca\(^{2+}\) and Ca\(^{2+}\)/CaM signaling pathways, changes in PPF should not be a criteria for judging the location of mechanisms responsible for the expression of synaptic potentiation.
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