Roles of CaMKII, PKA, and PKC in the Induction and Maintenance of LTP of C-Fiber–Evoked Field Potentials in Rat Spinal Dorsal Horn

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INTRODUCTION

Long-term potentiation (LTP) in hippocampus referring to a long-lasting enhancement in efficacy of synaptic transmission is believed to be a synaptic model of learning and memory (Bliss and Collingridge 1993; Bliss and Lømo 1973). LTP of C-fiber–evoked field potentials in spinal dorsal horn is a form of activity-dependent increase in excitatory synaptic transmission between afferent C-fibers and neurons in superficial laminae of spinal dorsal horn (Liu and Sandkühler 1995, 1997). In response to intense noxious stimulation, neurons in spinal dorsal horn become hypersensitive to subsequent stimuli (Ma and Woolf 1995; Woolf 1983). The phenomenon that is termed central sensitization is considered as a central mechanism underlying hyperalgesia, an increased response to noxious stimuli (Woolf and Salter 2000). Just like hyperalgesia, LTP of C-fiber–evoked field potentials in spinal dorsal horn can be induced by electrical stimulation of afferent C-fibers (Liu and Sandkühler 1995, 1997) as well as by natural noxious stimulation on peripheral tissue or acute nerve injury (Sandkühler and Liu 1998). Pharmacologically, the spinal LTP is prevented by blockage of spinal N-methyl-d-aspartate (NMDA) receptors (Liu and Sandkühler 1995) and neurokinin receptors (Liu and Sandkühler 1997). Our primary work showed that LTP-inducing tetanic stimulation delivered to the sciatic nerve produced mechanical and thermal hyperalgesia that lasted for several days (Zhang et al. 2002). Accordingly, the spinal LTP is believed as an attractive cellular model of injury-induced hyperalgesia (Sandkühler 2000; Zimmermann 2001). However, the molecular mechanisms underlying this form of synaptic plasticity are still unknown.

NMDA receptor-dependent LTP has been intensively studied in hippocampus (Bliss and Collingridge 1993; Nicoll and Malenka 1999). Several lines of evidence indicate that calcium/calmodulin-dependent protein kinase II (CaMKII), protein kinase A (PKA), and protein kinase C (PKC) are important for LTP in hippocampus. In this study, the roles of these three protein kinases in the induction and maintenance of LTP of C-fiber–evoked field potentials were evaluated by application of specific inhibitors of CaMKII (KN-93 and AIP), PKA (Rp-CPT-cAMPS), and PKC (chelerythrine and Gö6983) at the recording segments before and after LTP induction in urethane-anesthetized Sprague-Dawley rats. We found both KN-93 and AIP, when applied at 30 min prior to tetanic stimulation, completely blocked LTP induction. At 30 min after LTP induction, KN-93 and AIP reversed LTP completely, and at 60 min after LTP induction, they depressed spinal LTP in most rats tested. Three hours after LTP induction, however, KN-93 or AIP did not affect the spinal LTP. Rp-CPT-cAMPS, chelerythrine, and Gö6983 blocked the spinal LTP when applied at 30 min before tetanic stimulation and reversed LTP completely at 15 min after LTP induction. In contrast, at 30 min after LTP induction, the drugs never affected the spinal LTP. These results suggest that activation of CaMKII, PKA, and PKC may be crucial for the induction and the early-phase but not for the late-phase maintenance of the spinal LTP.

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LTP induction. We found that all of them blocked induction and depressed the early phase but not the late phase of the spinal LTP.

METHODS

Surgical preparation

Experiments were performed on male Sprague-Dawley rats (250–280 g body wt). Urethane (1.5 g/kg, ip) was used to induce and maintain anesthesia. Additional doses of the drug (0.5 g/kg) were given if needed. Surgical level of anesthesia was verified by the stable mean arterial blood pressure and constant heart rate during noxious stimulation. The trachea was cannulated, and the animal breathed spontaneously. One carotid artery was cannulated to continuously monitor the mean arterial blood pressure, which was maintained from 80 to 120 mmHg. A laminectomy was performed to expose the lumbar enlargement of spinal cord, and the dura mater was incised longitudinally. The left sciatic nerve was dissected free for bipolar electrical stimulation with platinum hook electrodes. All exposed nervous tissues were covered with warm paraffin oil, except for the spinal lumbar enlargement, onto which the drugs will be applied. Core rectal temperature was kept constant (37–38°C) by means of a feedback-controlled heating blanket. At the end of the experiments, animals were killed with an overdose of urethane. The local animal care committee has approved all experiments.

Electrophysiological recordings and nerve stimulation

Electrophysiological recording of C-fiber–evoked field potentials in spinal dorsal horn was described previously (Liu and Sandkühler 1995, 1997). Briefly, following electrical stimulation of the sciatic nerve, field potentials were recorded in the spinal dorsal horn (L4 and L5 segments) at the depth of 50–500 μm from the dorsal surface with a tungsten microelectrode (World Precision Instruments; impedance final concentration of 100 μM immediately before administration. Final DMSO concentration in the diluted working solution was 0.5%. AIP (Sigma), chelethyrine (Sigma), or Rp-CPT-cAMPS (Sigma) was dissolved in 0.9% NaCl to make stock solutions at the concentrations of 10, 10, or 25 mM, respectively, and the stock solutions were further diluted as needed with artificial cerebrospinal fluid. To directly apply the drugs on the dorsal surface of spinal cord, a small well was formed on the cord dorsum at the recording segments with 1.5% agar dissolved with 0.9% NaCl.

Data analysis and statistics

The area of C-fiber–evoked field potentials was determined off-line by parameter extraction (Fig. 1Dd), which was implemented by DataWave. In each experiment, responses to five consecutive test stimuli were averaged. The mean area of C-fiber–evoked field potentials before drug application or tetanic stimulation served as baseline. The summary data from different animals are expressed as means ± SE. Nonparametric Friedman test or Wilcoxon signed-rank test was used for statistic analysis (when appropriate), since the data from some groups were not normally distributed. P < 0.05 was considered significant.

RESULTS

KN-93 or AIP blocked the induction of LTP of C-fiber–evoked field potentials

To investigate the role of CaMKII in the induction of spinal LTP, stable C-fiber–evoked field potentials were recorded for ≥30 min and served as baseline, and KN-93 (100 μM) was applied directly to the recording segments. Thirty minutes afterward, a tetanic stimulation was delivered to the sciatic nerve. The C-fiber–evoked potentials had no significant alteration 30 min after drug application (101.5 ± 6.7 vs. 98.6 ± 5.1%, n = 6, P > 0.05, Wilcoxon signed-rank test). However, the induction of LTP was completely blocked in all six rats tested. The mean area of C-fiber–evoked field potentials did not change after tetanic stimulation compared with baseline (P > 0.05, n = 6, Friedman test, Fig. 1A).

To exclude the possibility that KN-93 may block spinal LTP through nonspecific effects other than inhibition of CaMKII, we next examined the effect of the same concentration (100 μM) of KN-92, an inactive analog of KN-93, on the induction of spinal LTP with the same experimental procedure in other five rats. KN-92 had no effect on the induction of the spinal LTP when applied 30 min before tetanic stimulation (P > 0.05, Friedman test, Fig. 1B).

To further confirm the role of CaMKII in the spinal LTP induction, another specific and potent CaMKII inhibitor AIP was tested. The results showed that spinal application of AIP (200 μM) at 30 min before tetanic stimulation also completely blocked the induction of spinal LTP in six rats tested (P < 0.05, Friedman test, Fig. 1C).

Both KN-93 and AIP reversed established LTP of C-fiber–evoked field potentials in a time-dependent manner

To evaluate the role of CaMKII in the maintenance of LTP of C-fiber–evoked field potentials, we applied KN-93 or AIP at the recording segments at different time points following LTP induction.

Spinal application of KN-93 (100 μM) at 30 min after LTP induction decreased the spinal LTP significantly at 105 min after drug application (from 223.2 ± 16.1 to 175.6 ± 17.7%, n = 6, P < 0.05, Wilcoxon signed-rank test, Fig. 2A). Afterward, the area of C-fiber–evoked field potentials decreased gradually, and at 3 h after drug application reached to 124.3 ± 16.8%, which was no longer different from baseline (P > 0.05, Wilcoxon signed-rank test, Fig. 2A), indicating the spinal LTP was totally reversed. KN-92 at the same concentration, however, never affected the maintenance of the spinal LTP as tested in the other six rats. At 30 min after tetanic stimulation, potentiation was 219.5 ± 18.0% and did not change as observed during ≥4 h after KN-92 application (P > 0.05, Friedman test, Fig. 2B).
FIG. 1. KN-93 and AIP, but not KN-92, completely blocked the induction of long-term potentiation (LTP) of C-fiber–evoked field potentials in spinal dorsal horn when applied at 30 min prior to tetanic stimulation. Mean response of C-fiber–evoked field potentials before drugs application serves as baseline. Summary data, expressed as means ± SE, are plotted vs. time. Downward arrows indicate time points at which the drugs were applied onto the dorsal surface of spinal cord at recording segments, and upward arrows indicate time points when tetanic stimulation (100 Hz, 40 V, 0.5-ms duration, given in 4 trains of 1-s duration at 10-s interval) to the sciatic nerve was delivered. A: KN-93 (100 μM) blocked the induction of the spinal LTP. B: KN-92 (100 μM) failed to block the induction of the spinal LTP. C: AIP (200 μM) blocked the induction of the spinal LTP. D: representative original recordings taken at time points as indicated are shown. Area of C-fiber–evoked field potential as shown in D (filled with oblique lines) is determined automatically by parameter extraction. Baseline, indicated by dashed line, is determined by 2 highest points within the time range defined manually in either sides of C-fiber response. Therefore small change in latency of C-fiber–evoked field potentials does not affect the measurement of the area.
FIG. 2. KN-93 reversed LTP of C-fiber–evoked field potentials in a time-dependent manner. Mean areas are plotted vs. time and vertical bars indicate SE. Upward arrows represent tetanic stimulation (100 Hz, 40 V, 0.5 ms), and downward arrows indicate onset of drug application. A: KN-93 (100 μM) reversed the spinal LTP when applied at 30 min after tetanic stimulation. B: KN-92 (100 μM) didn’t affect the established LTP. C: KN-93 (100 μM) depressed the spinal LTP when applied at 1 h after tetanic stimulation. D: KN-93 (100 μM) did not affect the spinal LTP when applied at 3 h after the LTP induction.
At 60 min after LTP induction, spinal application of KN-93 (100 μM) reversed LTP in five of seven rats tested. In these five rats, the mean area of C-fiber–evoked field potentials was potentiated to 208.4 ± 17.3% of baseline at 60 min after tetanic stimulation. KN-93 depressed LTP significantly (to 142.7 ± 14.3%, \( P < 0.05 \), Wilcoxon signed-rank test) at 100 min after drug application and at 145 min after KN-93 to 118.9 ± 15.5%, which was not different from baseline (\( P > 0.05 \), Wilcoxon signed-rank test, Fig. 2C). KN-93 didn’t affect LTP in the remaining two animals (data not shown).

Our experimental model of recording C-fiber–evoked field potentials in intact rats provides the possibility to study LTP for a long time. This allows us to investigate if CaMKII is involved in the maintenance of late-phase LTP. KN-93 (100 μM), when applied at 3 h after LTP induction, did not affect LTP of C-fiber–evoked field potentials in all five rats tested. The potentiation was 239.3 ± 19.6% immediately before KN-93 and did not change over ≥3.5 h after drug application (\( P > 0.05 \), Friedman test, Fig. 2D).

AIP (200 μM), when applied at 30 min after LTP induction, depressed the spinal LTP more quickly than KN-93. At 30 min after application, LTP decreased significantly from 232.1 ± 16.6 to 187.6 ± 12.4% (\( P < 0.05 \), \( n = 6 \), Wilcoxon signed-rank test, Fig. 3A). At 105 min after AIP application, the mean area of C-fiber–evoked field potentials decreased to 136.1 ± 18.9%, which was not different from baseline (\( P > 0.05 \), \( n = 6 \), Wilcoxon signed-rank test), and remained at this level during resting time of each experiment.

When applied 60 min after LTP induction, AIP depressed spinal LTP in 7 of 10 rats tested. In these seven rats, the potentiation was 220.3 ± 16.1% of baseline as measured immediately before AIP application and decreased significantly to 186.5 ± 16.2% at 40 min after drug application (\( P < 0.05 \), Wilcoxon signed-rank test). However, the mean area of C-fiber–evoked field potentials was still higher compared with baseline (\( P < 0.05 \), Wilcoxon signed-rank test) and kept at this level until the end of the experiments (Fig. 3B).

We next investigated if CaMKII is involved in the maintenance of late-phase LTP. AIP (200 μM), when applied at 3 h after LTP induction, did not affect LTP of C-fiber–evoked field potentials in all six rats tested. The potentiation was 219.4 ± 15.8% immediately before AIP and did not change over ≥3 h after drug application (\( P > 0.05 \), \( n = 6 \), Friedman test, Fig. 3C).

We also tested if AIP by itself could affect baseline synaptic transmission over a long period of recording time. In four other rats, we observed that spinal application of AIP (without tetanic stimulation) had no effect on the basal synaptic transmission throughout experiment time (for ≥5 h, data not shown).

**Rp-CPT-cAMPS blocked the induction of spinal LTP and reversed established LTP of C-fiber–evoked field potentials in a time-dependent manner**

To address the role of PKA in the spinal LTP, cAMP analog Rp-CPT-cAMPS, a membrane-permeable inhibitor of PKA, was tested before and after LTP induction. Rp-CPT-cAMPS (1 mM), when applied at 30 min before tetanic stimulation, did not affect the baseline of C-fiber–evoked field potentials, but blocked LTP induction completely as tested in all six rats. The mean area of C-fiber–evoked potentials did not change after tetanic stimulation compared with baseline (\( P > 0.05 \), \( n = 6 \), Friedman test, Fig. 4A).

To evaluate the role of Rp-CPT-cAMPS in the maintenance of LTP of C-fiber–evoked field potentials, we applied Rp-CPT-cAMPS at the recording segments after LTP induction. At 15 min after LTP induction, spinal application of Rp-CPT-cAMPS (1 mM) decreased LTP significantly at 25 min after drug application (from 219.8 ± 12.2 to 184.7 ± 11.8%, \( P < 0.05 \), \( n = 7 \), Wilcoxon signed-rank test, Fig. 4B). At 105 min after drug application, the mean area of C-fiber–evoked field potentials decreased to 136.2 ± 15.8%, which was no longer different from baseline (\( P > 0.05 \), Wilcoxon signed-rank test, Fig. 4B).

In contrast, at 30 min after LTP induction, spinal application of Rp-CPT-cAMPS (1 mM) did not affect LTP of C-fiber–evoked field potentials in all eight rats tested. The potentiation was 238.6 ± 16.2% immediately before Rp-CPT-cAMPS and did not change over ≥3.5 h after drug application (\( P > 0.05 \), Friedman test, Fig. 4C).

**Chelerythrine or Gö 6983 blocked the induction of spinal LTP and reversed established LTP of C-fiber–evoked field potentials in a time-dependent manner**

To assess the role of PKC in the induction and maintenance of spinal LTP, chelerythrine, a potent and selective PKC inhibitor (IC\(_{50} = 660 \) nM) acting on the catalytic domain of PKC (Herbert et al. 1990), was tested. Spinal application of chelerythrine (200 μM) at 30 min before tetanic stimulation completely blocked LTP induction in all six rats tested (\( P > 0.05 \), Friedman test, Fig. 5A).

Fifteen minutes after LTP induction, chelerythrine (200 μM) depressed LTP significantly 20 min after drug application (from 225.5 ± 11.2 to 178.2 ± 12.4%, \( n = 6 \), \( P < 0.05 \), Wilcoxon signed-rank test, Fig. 5B), and at 75 min after drug application, the spinal LTP was completely reversed (to 119.7 ± 10.8%, \( P > 0.05 \), Wilcoxon signed-rank test, Fig. 5B). At 30 min after LTP induction, the same concentration of chelerythrine failed to affect LTP of C-fiber–evoked field potentials in all five rats tested. The potentiation was 230.4 ± 8.8% immediately before chelerythrine and did not change as monitored for ≥3 h after drug application (\( P > 0.05 \), \( n = 5 \), Friedman test, Fig. 5C).

To further confirm the role of PKC in the induction and maintenance of spinal LTP, another highly specific and potent PKC inhibitor Gö 6983 was examined with the same experimental procedure. Gö 6983 (100 μM), when applied at 30 min before tetanic stimulation, also completely blocked the induction of spinal LTP (\( P > 0.05 \), \( n = 6 \), Friedman test, Fig. 6A). Fifteen minutes after LTP induction, Gö 6983 (100 μM) inhibited spinal LTP significantly 20 min after drug application (from 232.9 ± 11.9 to 187.8 ± 12.6%, \( n = 5 \), \( P < 0.05 \), Wilcoxon signed-rank test, Fig. 6B), and at 100 min after drug application, mean area of C-fiber–evoked field potentials returned to baseline (122.9 ± 11.8%, \( P > 0.05 \), Wilcoxon signed-rank test, Fig. 6B). When applied at 30 min after LTP induction, Gö 6983 (100 μM) did not affect LTP of C-fiber–evoked field potentials in all seven rats tested (\( P > 0.05 \), \( n = 7 \), Friedman test, Fig. 6C).

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A recent work with patch-clamp recording technique has demonstrated that electrical stimulation of the primary afferent C-fibers induces LTP of C-fiber–evoked excitatory postsynaptic currents (EPSCs) in projection neurons, but not in unidentified neurons in lamina I of spinal cord slice of young rats (Ikeda et al. 2003). Therefore the potentiation of the synapses between afferent C-fibers and projection neurons in the spinal dorsal horn may contribute to the LTP of C-fiber–evoked field potentials in the spinal dorsal horn.

**Role of CaMKII in the induction and maintenance of LTP in hippocampus and in spinal dorsal horn**

Our results demonstrated that spinal application of either KN-93 or AIP, two different kinds of CaMKII inhibitors, blocked the induction of LTP of C-fiber–evoked field poten-
tials in spinal dorsal horn when applied before tetanic stimulation and reversed both the early phase and the late phase of the spinal LTP when applied shortly after LTP induction. It is unlikely that the unspecific effects of KN-93 or DMSO (0.5%) in solution contributes to these results, because the same concentration of KN-92, an inactive analog of KN-93, with the same dose of DMSO never affected the induction and maintenance of spinal LTP. It has been shown that in vitro AIP at high concentrations (>10 μM) also inhibits activities of PKA and PKC (Ishida et al. 1995). The concentration of AIP used in this work was 200 μM; therefore the inhibition of PKA and PKC may also contribute to the effect of AIP on the spinal LTP. In our experiments, AIP was applied via an agar well on the cord dorsum. A previous work (Beck et al. 1995) has shown that following superfusion with neurokinin A (NKA) on the dorsal surface of spinal cord via silicon well for 15 or 30 min, the concentration gradient of NKA in the superficial spinal cord (0–500 μM) is 25–70 times lower than that in the superfusate.

**FIG. 4.** Rp-CPT-cAMPS blocked the induction of LTP of C-fiber–evoked field potentials and reversed the spinal LTP in a time-dependent manner. Mean responses of C-fiber–evoked field potentials before Rp-CPT-cAMPS application or tetanic stimulation served as baseline. Downward arrows indicate time points at which the Rp-CPT-cAMPS was applied onto the dorsal surface of spinal cord at recording segments, and upward arrows indicate time points when tetanic stimulation (0.5 ms duration, 40 V, 100 Hz, given in 4 trains of 1-s duration at 10-s interval) to the sciatic nerve was delivered. Rp-CPT-cAMPS (1 mM) blocked the induction of spinal LTP completely when applied 30 min before tetanic stimulation (A) and reversed spinal LTP when applied at 15 min after LTP induction (B) but failed to affect established LTP when applied at 30 min after LTP induction (C).
AIP contains 13 amino acid residues, while NKA consists of 10 residues. Their molecular weight is very similar. Accordingly, AIP concentration at the recording site (50–500 μM from dorsal surface) is likely only about 3–8 μM. More importantly, our results revealed that spinal application of AIP at 30 and 60 min after LTP induction reversed the spinal LTP, but highly specific PKA inhibitor (Rp-CPT-cAMPS) and PKC inhibitors (chelerythrine or Gö 6983) never affected the spinal LTP when applied at 30 min after LTP induction. Taken together, it is reasonable to believe that the effects of AIP on the induction and maintenance of the spinal LTP observed in this study do not result from inhibition of PKA or PKC but from inhibition of CaMKII.

KN-93 and AIP inhibit CaMKII in different ways. KN-93 inhibits CaMKII by competitively interfering with calmodulin binding to the enzyme (Sumi et al. 1991), whereas AIP inhibits CaMKII by binding to the substrate-binding site for autophosphorylation site, and its inhibition on CaMKII is not affected.

FIG. 5. Chelerythrine blocked the induction of LTP of C-fiber–evoked field potentials and reversed the spinal LTP in a time-dependent manner. Mean responses of C-fiber–evoked field potentials before drug application or tetanic stimulation served as baseline. Chelerythrine (200 μM) blocked the induction of spinal LTP completely when applied 30 min before tetanic stimulation (A) and reversed spinal LTP when applied at 15 min after LTP induction (B) but did not affect established LTP when applied at 30 min after LTP induction (C).
by Ca²⁺/calmodulin (Ishida et al. 1995). In this study, spinal application of either of them yielded similar results. This strengthens our confidence that CaMKII plays a key role in the induction and early-phase maintenance of LTP in spinal dorsal horn.

CaMKII is a main protein in the postsynaptic density (PSD), a cytoskeletal structure beneath the postsynaptic membrane in hippocampus (Kelly et al. 1984; Suzuki et al. 1994). Convergent evidence has shown that hippocampal LTP is blocked by CaMKII inhibitors (Chen et al. 2001; Feng 1995; Malinow et al. 1989; Otmakhov et al. 1997) and by genetic deletion of this enzyme (Fukunaga and Miyamoto 2000). Furthermore, introduction of CaMKII or its activator Ca²⁺/calmodulin into postsynaptic cell results in synaptic potentiation (Wang and Kelly 1995). CaMKII also exists in spinal dorsal horn neurons (Bruggemann et al. 2000; Fang et al. 2002). Recently, it has been reported that the capsaicin-induced central sensitization, which is considered as another form of spinal LTP, is pre-

**FIG. 6.** Gö 6983 blocked the induction of LTP of C-fiber–evoked field potentials and reversed spinal LTP in a time-dependent manner. Mean responses of C-fiber–evoked field potentials before drug application or tetanic stimulation served as baseline. Gö 6983 (100 μM) completely blocked the induction of spinal LTP (A) and reversed spinal LTP when applied at 15 min after LTP induction (B) but did not affect established LTP when applied at 30 min after LTP induction (C).
vented by pretreatment with KN-93 (Fang et al. 2002). Our results further demonstrated that spinal application of KN-93 or AIP prior to tetanic stimulation completely blocked the induction of LTP of C-fiber–evoked field potentials in the spinal dorsal horn. It seems undoubted that activation of CaMKII is crucial for LTP induction in both hippocampus and spinal dorsal horn.

In the hippocampus, it has been shown that CaMKII activity is persistently elevated for at least 1 h after LTP induction (Fukunaga et al. 1993, 1995). Recently, it has been demonstrated that intradermal injection of capsaicin, which selectively activates afferent C-fibers, induces a persistent increase in expression and phosphorylation of CaMKII in spinal dorsal horn (Fang et al. 2002). The persistent activity is proposed to be a biochemical memory trace of the previous Ca\(^{2+}\) elevation and is possibly responsible for LTP maintenance (Bliss and Collingridge 1993; Lisman 1985, 1994; Malenka and Nicoll 1999; Miller and Kennedy 1986). Several studies have attempted to investigate the role of the kinase in LTP maintenance in hippocampus (Chen et al. 2001; Feng 1995; Lisman 1994; Malinow et al. 1989; Otmakhov et al. 1997). However, in all studies except one (Feng 1995), postsynaptic application of CaMKII inhibitors after LTP induction does not affect established LTP. In contrast, our results demonstrated that KN-93, applied at 30 or 60 min after LTP induction, reversed LTP of C-fiber–evoked field potentials in the spinal dorsal horn, and KN-92, an inactive analog of KN-93, never affected spinal LTP. Furthermore, our results showed that AIP, a highly specific inhibitor of CaMKII, also inhibited the spinal LTP in the same manner, suggesting that persistent activation of the kinase is necessary for the early phase (1–3 h) of the spinal LTP.

To our knowledge, the role of CaMKII in the maintenance of late-phase (>3 h) LTP has not been studied. The method of recording C-fiber–evoked field potentials in spinal dorsal horn of anesthetized rats makes it possible to observe the effect of CaMKII inhibitors on LTP for a longer time. Our results showed that KN-93 or AIP, applied at 3 h after LTP induction, never affected established LTP of C-fiber–evoked potentials, indicating that CaMKII may not be relevant to the late-phase maintenance of the spinal LTP, no matter whether CaMKII was still activated or not. The results may also exclude the possibility that the drugs at the dosages used in this study may have toxic effects on spinal neurons. Late-phase LTP seemed to be supported by other factors rather than CaMKII. There have been a lot of publications showing that the late-phase but not early-phase maintenance of LTP is translation-dependent, which relies on new protein synthesis and elevated translation (Bliss and Collingridge 1993; Frey et al. 1988). Our recent work has shown that inhibition of de novo protein synthesis by spinal application of cycloheximide or anisomycin before tetanic stimulation selectively inhibits the late-phase LTP of C-fiber–evoked field potentials but does not affect the induction and early-phase maintenance of spinal LTP (Hu et al. 2003).

**PKA is required for the induction and maintenance of spinal LTP**

Our results demonstrated that a competitive PKA inhibitor Rp-CPT-cAMPS, when applied prior to tetanic stimulation, completely blocked the induction of LTP of C-fiber–evoked field potentials in the spinal dorsal horn. When applied 15 min but not 30 min after LTP induction, Rp-CPT-cAMPS reversed the spinal LTP. The results suggest that activation of PKA may be involved in the induction and early-phase (<30 min) maintenance but not the late phase of spinal LTP.

In the hippocampus, it has been shown that the level of cAMP increases immediately after the initiation of LTP and returns to control levels over the next 10–20 min (Chetkovich and Sweatt 1993; Chetkovich et al. 1991), and PKA activity is only transiently increased (2–10 min) after LTP induction (Roberson and Sweatt 1996), indicating that PKA may be relevant to induction and early-phase maintenance of LTP. Consistent with this, some studies have shown that early LTP is almost completely blocked by PKA inhibitor Rp-cAMPS (Blitzer et al. 1995) and by postsynaptic intracellular perfusion of the peptide PKA inhibitor PKI (6–22) amide (Otmakhova et al. 2000) or strongly suppressed by pretreatment with the PKA inhibitor H89. In the spinal dorsal horn, central sensitization produced by capsaicin injection is blocked by the PKA inhibitor H89 (Lin et al. 2002), which is in agreement with our findings. However, there is also evidence demonstrating that, in the hippocampus, late-phase but not early LTP depends on PKA (Frey et al. 1993; Huang and Kandel 1994; Winder et al. 1998).

In the hippocampus, PKA may facilitate LTP through regulation of CaMKII activity by phosphorylation of inhibitor-1 (I-1). The activated I-1 inhibits protein phosphatase 1, which leads to dephosphorylation of CaMKII. The final effect of PKA is to maintain the level of activated CaMKII (Blitzer et al. 1998; Carroll et al. 1998; Fukunaga and Miyamoto 2000). Recently, it has been reported that PKA directly controls the synaptic incorporation of AMPA receptors by phosphorylating subunits GluR4 and GluR1 of the receptor (Esteban et al. 2003). If PKA functions with the same mechanisms in the spinal dorsal horn, blockage of PKA at 30 min after LTP induction should depress the spinal LTP, as CaMKII inhibitors do. In contrast, our results showed that the PKA inhibitor Rp-CPT-cAMPS depressed LTP only within 15 min after LTP induction. Thus the mechanism of PKA participating in the maintenance of the spinal LTP remains to be elucidated.

**PKC is involved in the induction and maintenance of spinal LTP**

Many studies have shown that PKC is involved in the induction of NMDA receptor-dependent LTP at CA1 synapses in the hippocampus. Activation of PKC by phorbol esters potentiates synaptic transmission, which resembles LTP in hippocampal slices (Linden et al. 1986, 1987; Malenka et al. 1986), and direct injection of PKC into the postsynaptic hippocampal pyramidal cells mimics LTP (Hu et al. 1987). Moreover, PKC inhibitor prevents the induction of LTP (Denny et al. 1990; Ling et al. 2002; Malinow et al. 1989). Thus activation of PKC is necessary and sufficient for LTP induction.

The role of PKC in the maintenance of hippocampal LTP is still unclear, because an early study showed that postsynaptic application of PKC inhibitor (PKC19–31) after 30-min LTP induction does not affect established LTP (Malinow et al. 1989), but a recent work indicated that chelerythrine reversed
established LTP when applied 1 h after LTP induction (Ling et al. 2002).

PKC is also abundant in the dorsal horn of the spinal cord and has been suggested to be important in sensory signal processing, including pain (Igwe and Chronwall 2001; Saito and Shirai 2002; Wen et al. 2003; Willis 2001). Activation of PKC in the spinal cord produces mechanical allodynia and thermal hyperalgesia (Pamecek et al. 1999), and inhibition of PKC prevents central sensitization of spinohalamic tract neurons produced by intradermal injection of capsaicin (Lin et al. 1996). Our results further demonstrated that spinal application of PKC inhibitor, chelerythrine, or G6 6983 blocked the induction of LTP of C-fiber-evoked field potentials in the spinal dorsal horn and reversed spinal LTP when applied at 15 min but not 30 min after LTP induction, suggesting activation of PKC is involved in the induction and early phase but not the late phase of spinal LTP.

Both PKC and CaMKII phosphorylate the AMPA receptor, thereby altering its sensitivity to glutamate (Barria et al. 1997; Giese et al. 1998; Mammen et al. 1997; Roche et al. 1996). PKC and CaMKII share a same phosphorylation site (serine 831) on the GluR1 subunit of AMPA receptors (Barria et al. 1997). In addition, PKC also potentiates the NMDA receptor function by phosphorylation of the receptors (MacDonald et al. 2001). PKC potentiates NMDA currents in the neurons isolated from the trigeminal caudalis nucleus by increase in channel open time and partial relief of the magnesium block (Chen and Huang 1992). Potentiation of both NMDA and AMPA receptors may contribute to the expression of LTP of C-fiber-evoked field potentials in the spinal dorsal horn.

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