WILEY InterScience®
DISCOVER SOMETHING GREAT

Time-Dependent Postsynaptic AMPA GluR1 Receptor Recruitment in the Cingulate Synaptic Potentiation

Hiroki Toyoda,* Long-Jun Wu,* Ming-Gao Zhao,* Hui Xu, Min Zhuo

Department of Physiology, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada M5S 1A8

Received 13 September 2006; revised 23 November 2006; accepted 12 December 2006

ABSTRACT: **The anterior cingulate cortex (ACC) is critical for brain functions including learning, memory, fear and pain. Long-term synaptic potentiation (LTP), a cellular model for learning and memory, has been reported in the ACC neurons. Unlike LTP in the hippocampus and amygdala, two key structures for memory and fear, little is known about the synaptic mechanism for the expression of LTP in the ACC. Here we use whole-cell patch clamp recordings to demonstrate that cingulate LTP requires the functional recruitment of GluR1 AMPA receptors; and such events are rapid and completed within 5–10 min after LTP induction. Our results demonstrate that the GluR1 subunit is essential for synaptic plasticity in the ACC and may play critical roles under physiological and pathological conditions.** © 2007 Wiley Periodicals, Inc. Develop Neurobiol 67: 498–509, 2007

Keywords: **long-term potentiation; anterior cingulate cortex; AMPA receptor; trafficking; PDZ domain**

INTRODUCTION

The anterior cingulate cortex (ACC) is believed to play a critical role in learning, memory, emotional fear, and persistent pain in mammalian brains (Frankland et al., 2004; Maviel et al., 2004; Wiltgen et al., 2004; Zhuo, 2004; Tang et al., 2005; Zhao et al., 2005). Glutamate is the major fast excitatory transmitter in the ACC, and postsynaptic AMPA receptors mediate majority of postsynaptic responses (Wei et al., 1999; Zhao et al., 2005). Similar to glutamatergic synapses in other central synapses (Bliss and Collingridge, 1993; Kandel, 2001; Malenka and Bear, 2004), glutamatergic synapses in the ACC can undergo long-term synaptic potentiation (Liauw et al., 2005; Zhao et al., 2005). In studies using *in vitro* brain slices of rats or mice, theta-burst stimulation or pairing training protocol produce a long lasting enhancement of synaptic responses in the ACC slices (Wei et al., 1999; Zhao et al., 2005). Similar long-term potentiation in the ACC can be found in *in vivo* anesthetized rats. Peripheral injury produces long lasting enhancement of sensory synaptic response to peripheral stimulation or local ACC stimulation (Wei et al., 1999; Wu et al., 2005b), suggesting that excitatory synaptic transmission within the ACC may under plastic potentiation after peripheral insult or injury. Parallel evidence has been obtained using the measurement of activity-dependent immediate early gene in the ACC neurons. Peripheral stimulation or fearful condition induced activation of plasticity-related immediate early genes, including *egr1*, *CREB*, and *c-fos* activation within the ACC neurons (Wei et al., 1999; Wei et al., 2002b). Therefore, these studies strongly suggest that understanding molecular mechanism for synaptic potentiation in the ACC may

*These authors contributed equally to the study.
Correspondence to: Dr. M. Zhuo (min.zhuo@utoronto.ca).
Contract grant sponsor: EJLB-CIHR Michael Smith Chair in Neurosciences and Mental Health.
Contract grant sponsor: Canada Research Chair.
Contract grant sponsor: CIHR.
© 2007 Wiley Periodicals, Inc.
Published online 1 February 2007 in Wiley InterScience (www.interscience.wiley.com).
DOI 10.1002/dneu.20380

help us to understand physiological and pathological mechanisms of brain functions.

Synaptic mechanism of LTP is one most intensely studied central mechanism. In the hippocampus, both presynaptic and postsynaptic mechanisms have been proposed for the expression of LTP, depending on the subregions of the hippocampus (Nicoll and Malenka, 1995). While LTP has been reported in other regions of the brain also, namely cerebellum, cortex, and spinal cord, it becomes clear that different synaptic signaling pathways are likely contributing to the induction and expression of LTP. The induction of ACC LTP has been investigated recently, and activation of NMDA receptor is critical for triggering the LTP (Zhao et al., 2005). Subsequent activation of calcium-stimulated adenylyl cyclases such as AC1 is thought to be a key second messenger for LTP (Liauw et al., 2005). The expression of ACC LTP, however, is less clear. Paired-pulse facilitation, a simple form of presynaptic plasticity is not altered during LTP, suggesting the expression of ACC LTP may not be presynaptic. However, the roles of postsynaptic AMPA receptors in the expression of ACC LTP have not been investigated.

In the present study, we used whole-cell patch clamp recordings to investigate the roles of AMPA receptor-interacting proteins for cingulate LTP. We decided to focus on two major subtypes of AMPA receptors: GluR1 and GluR2/3. Different peptides inhibitors were injected through the recording electrodes to selectively inhibiting GluR1 or GluR2/3 related signaling pathways. We show that the interaction between GluR1 subunit and a PDZ domain protein plays a key mechanism underlying cingulate LTP.

METHODS

Brain Slice Preparation

Coronal brain slices (300 μm) containing the ACC from 6- to 8-week-old C57BL/6 male mice were prepared as previously described (Zhao et al., 2005). Slices were transferred to a submerged recovery chamber with oxygenated (95% O_2 and 5% CO_2) artificial cerebrospinal fluid (ACSF) containing (in m*M*: 124 NaCl, 2.5 KCl, 2 $CaCl_2$, 1 $MgSO_4$, 25 $NaHCO_3$, 1 NaH_2PO_4, 10 glucose) at room temperature for at least 1 h.

Whole-Cell Patch-Clamp Recording

Experiments were performed in a recording chamber on the stage of an Axioskop 2FS microscope with infrared DIC optics for visualization of whole-cell patch clamp recording. Excitatory postsynaptic currents (EPSCs) were recorded from layer II/III neurons with an Axon 200B amplifier (Molecular Devices, CA) and the stimulations were delivered by a bipolar tungsten stimulating electrode placed in layer V of the ACC [Fig. 1(A)]. EPSCs were induced by repetitive stimulations at 0.02 Hz and neurons were voltage clamped at -70 mV. The recording pipettes (3–5 MΩ) were filled with solution containing (in m*M*) 145 K-gluconate, 5 NaCl, 1 $MgCl_2$, 0.2 EGTA, 10 HEPES, 2 Mg-ATP, and 0.1 Na_3-GTP (adjusted to pH 7.2 with KOH). The LTP induction paradigm was used within 12 min after establishing the whole-cell configuration to prevent wash out effect on LTP induction (Zhao et al., 2005). This protocol involved 80 presynaptic pulses at 2 Hz with a postsynaptic depolarization of $+30$ mV (referred to as paired training). The protocol for the induction of LTD contains 300 presynaptic pulses at 1 Hz with a postsynaptic depolarization to -45 mV (Toyoda et al., 2005). The NMDA receptor-mediated component of EPSCs was pharmacologically isolated using ACSF containing: CNQX (20 μM), glycine (1 μM) and picrotoxin (100 μM). The patch electrodes contained (in m*M*) 102 cesium gluconate, 5 TEA chloride, 3.7 NaCl, 11 BAPTA, 0.2 EGTA, 20 HEPES, 2 MgATP, 0.3 NaGTP, and 5 QX-314 chloride (adjusted to pH 7.2 with CsOH). Neurons were voltage clamped at -30 mV and NMDA receptor-mediated EPSCs were evoked at 0.05 Hz. Picrotoxin (100 μM) was always present to block $GABA_A$ receptor-mediated inhibitory currents and monitored throughout the recordings. Access resistance was 15–30 MΩ and was also monitored throughout the experiments. Data was discarded if the access resistance changed $>15\%$ during an experiment.

Postsynaptic Injection of Peptide Inhibitors

To assess the potential role of the GluR1 and GluR2/3 C-terminal PDZ binding motif in the expression of LTP, peptides were perfused into ACC neurons before or after to LTP induction. Pep1-TGL (100 μM) is peptide containing the TGL motif that corresponds to the C-terminus of the AMPA receptor GluR1 subunit. Pep2-SVKI (100 μM) is inhibiting peptide corresponding to last 10 amino acids of the C-terminus of the GluR2 subunit. Pep2-SVKI disrupts binding of GluR2 with glutamate receptor interacting protein (GRIP), AMPA receptor binding protein (ABP) and protein interacting with C kinase (PICK1). Pep2-SVKE (100 μM) is inactive control peptide analogue of pep2-SVKI. Pep2-AVKI (100 μM) is an inhibiting peptide that selectively disrupts binding of the GluR2 subunit (at the C-terminal PDZ site) to protein interacting with C kinase (PICK1). Pep2m (100 μM) is an inhibiting peptide that selectively disrupts GluR2-NSF (*N*-ethylmaleimide-sensitive fusion protein) interaction.

Intracellular Application of an Inhibitor Peptide After the Establishment of LTP

The GluR1 C-terminal peptide (Pep1-TGL) was injected through a perfusion tube directly installed in a postsynaptic patch pipette. After back-filling the tube with intracellular solutions containing Pep1-TGL, it was inserted into a patch pipette with its tip 500 μm behind the tip. After LTP induc-

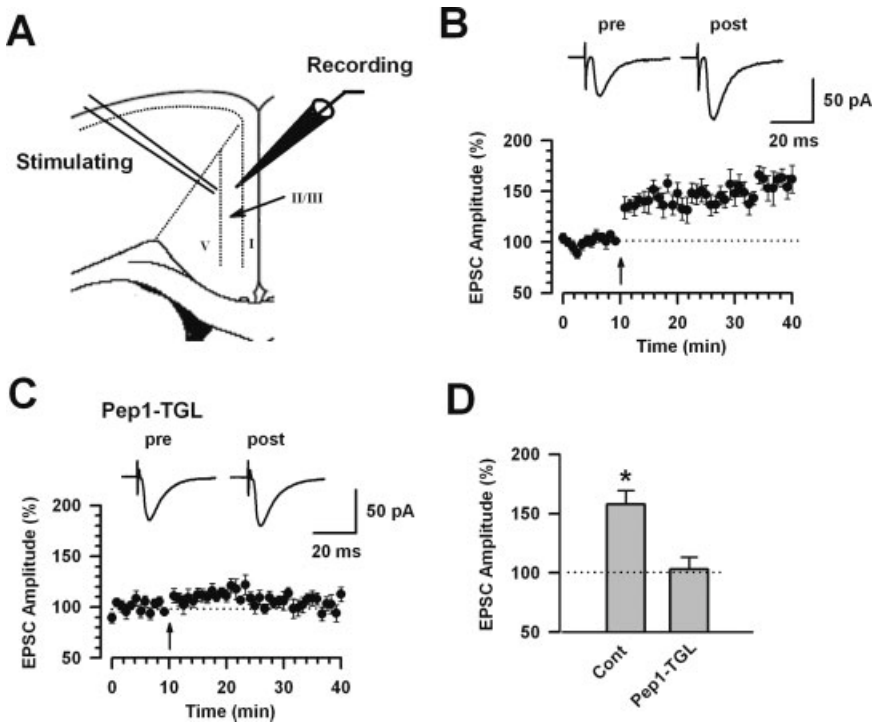

Figure 1 The C-terminal PDZ-binding domain of GluR1 subunit is required for cingulate LTP. (A) Diagram of a slice showing the placement of a whole-cell patch recording and a stimulation electrode in a cingulate slice. (B) LTP was induced in cingulate neurons ($n = 9$) by the paired training (arrow). (C) LTP was completely blocked by Pep1-TGL in the intracellular solution ($n = 9$). (B,C) The insets show averages of six EPSCs at baseline responses (pre) and 30 min (post) after the paired procedure (arrow); the dashed line indicates the mean basal synaptic responses. (D) Summary results of the effect of Pep1-TGL on cingulate LTP. * $p < 0.05$ compared to baseline.

tion, this drug was delivered into a postsynaptic patch pipette with positive pressure manually applied through a syringe (Hori et al., 1999) [Fig. 4(A)]. When Lucifer yellow (0.1%) was injected by this method, fluorescence was detected in a cingulate neuron within 1 min after injection and reached maximal intensity within 5 min.

Pharmacological Inhibitors

All chemicals and drugs for slice recordings were obtained from Sigma (St. Louis, MO), except for Pep1-TGL, Pep2-SVKE, Pep2-AVKI, Pep2-SVKI, Pep2m and QX-314, which were from Tocris Cookson (Ellisville, MO).

Data Analysis

Statistical comparisons were performed using the Student's *t*-test. Results are expressed as means ± SEM. In all cases, $p < 0.05$ was considered statistically significant.

RESULTS

We performed whole-cell patch-clamp recordings from visually identified pyramidal neurons in layer II/III of ACC slices from adult mice. Evoked EPSCs were obtained by delivering focal electrical stimulation to layer V [Fig. 1(A)]. Two sets of experiments confirmed that we were recording from cingulate pyramidal neurons. First, we identified pyramidal neurons based on the pyramidal shape of their somata by putting Lucifer yellow into the intracellular solution (Zhao et al., 2005). Second, in addition to visual identification, we confirmed that the recordings were performed from cortical pyramidal cells by injecting depolarizing currents into the neuron. The firing pattern of pyramidal neurons showed significant firing frequency adaptation.

The C-Terminal PDZ-Binding Domain of GluR1 Subunit Is Essential for Cingulate LTP

To study cingulate LTP, we used 80 presynaptic pulses at 2 Hz with postsynaptic depolarization at +30 mV (referred to as paired training) (Zhao et al., 2005). Similar to previous reports, the paired training induced a significant, long-lasting potentiation of synaptic responses in cingulate slices [157.8 ± 11.6% of baseline, $n = 9$, $p < 0.05$ compared with baseline response before the paired training; Fig. 1(B)]. The potentiation

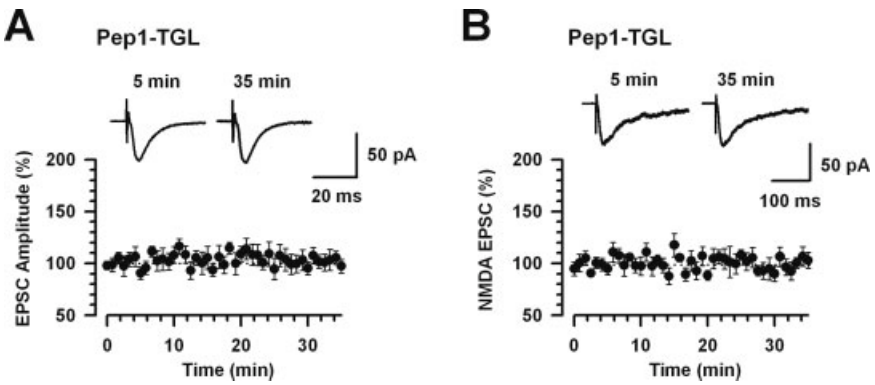

Figure 2 Pep1-TGL has no effect on basal synaptic transmission. (A) Pep1-TGL in the intracellular solution had no effect on AMPA receptor-mediated EPSCs ($n = 7$). (B) Pep1-TGL in the intracellular solution had no effect on NMDA receptor-mediated EPSCs ($n = 7$). The dashed line indicates the mean basal synaptic responses. The insets show averages of six EPSCs at the time points of 5 (pre) and 35 min (post) during the recording.

stabilized 30–40 min after LTP induction. To investigate the role of the PDZ-binding domain of the GluR1 subunit in LTP, we tested the effect of postsynaptic application of an inhibiting peptide Pep1-TGL (100 μM) (Hayashi et al., 2000) on the induction of LTP. LTP induced by paired training was completely blocked by pre-application of Pep1-TGL in the intracellular solution (102.4 ± 7.3%, $n = 9$, $p < 0.05$ compared with baseline response) [Fig. 1(C,D)]. To exclude the possible inhibition of basal synaptic transmission by the Pep1-TGL, we measured the effects of postsynaptic injection of the peptide on AMPA or NMDA receptor-mediated basal EPSCs. We found that postsynaptic injection of Pep1-TGL did not affect the AMPA receptor-mediated EPSCs ($n = 7$; see Fig. 2), suggesting that the inhibitory effect of Pep1-TGL is unlikely to be due to inhibition of basal synaptic transmission.

NMDA receptor is critical for the induction of cingulate LTP (Zhao et al., 2005). It is possible that Pep1-TGL may affect NMDA receptor mediated EPSCs and then block the induction of LTP. Thus, we measure the effects of similar postsynaptic application of Pep1-TGL on NMDA receptor mediated EPSCs isolated pharmacologically (see Methods). NMDA receptor mediated EPSCs were not significantly affected ($n = 7$; Fig. 2).

Ca^{2+} Permeable GluR2-Lacking Receptors Is Critical for the Expression of LTP

A recent study suggests that synaptic incorporation of Ca^{2+} permeable GluR2-lacking AMPA receptors is essential for LTP consolidation in hippocampus (Plant et al., 2006). Thus, we examined the role of Ca^{2+} permeable GluR2-lacking AMPA receptors in the maintenance of cingulate LTP. Philanthotoxin-433 (PhTx) is a high-affinity antagonist of Ca^{2+} permeable GluR2-lacking AMPA receptors (Washburn and Dingledine, 1996). PhTx was impotent at antagonizing NMDA receptor-mediated synaptic transmission in rat hippocampus ($IC_{50} = 144$ μM) (Albensi et al., 2000). To examine the effect of PhTx on EPSCs, PhTx (10 μM) was applied to the bath solution. EPSCs were insensitive to PhTx (last 5 min mean 95.8 ± 4.7% of first 5 min baseline response, $n = 5$, $p > 0.05$) [Fig. 3(A)]. This result suggests that Ca^{2+} permeable GluR2-lacking receptors do not significantly contribute to normal synaptic transmission in the ACC neurons.

If Ca^{2+} permeable GluR2-lacking receptors contribute to the maintenance of LTP, LTP induced by the paired training would be attenuated by bath application of PhTx. To examine this possibility, we applied PhTx (10 μM) by bath perfusion 5 min after paired training. Interestingly, although the paired training produced a significant early synaptic potentiation (138.8 ± 14.8% at 10 min post paired training) [Fig. 3(B,C)], the magnitude of synaptic potentiation after PhTx application was significantly reduced compared to the control group (mean 157.8 ± 11.6% for control neurons versus 116.5 ± 4.9% for PhTx treated, $p < 0.05$) [Fig. 3(C)]. These results suggest that the recruitment of functional GluR2-lacking AMPA receptors is critical for cingulate LTP.

The Involvement of GluR1 Subunit Is Time-Dependent

To examine the effect of Pep1-TGL on the maintenance of LTP, Pep1-TGL (100 μM) was injected

postsynaptically 5 min after paired training (Hori et al., 1999) [Fig. 4(A)]. In comparison with the application before the LTP induction, we found no significant effect of Pep1-TGL on the maintenance of LTP during the 25 min application with Pep1-TGL (157.0 ± 11.0% at 30 min post paired training, Pep1-TGL treatment, $n = 8$; $p < 0.05$ compared with baseline response before the paired training) [Fig. 4(B,C)]. These results suggest that the functional recruitment of AMPA GluR1 subunit has been completed within 5–10 min. To test if Pep1-TGL is successfully introduced into the cell, we applied Pep1-TGL immediately after whole-cell configuration by the intracellular perfusion system. We found that the induction of LTP was blocked [112.1 ± 11.8% of baseline, $n = 7$, $p > 0.05$ compared with baseline response before the paired training; Fig. 4(C,D)]. Therefore, we believe the peptide was successfully perfused into and took effect in the ACC neurons.

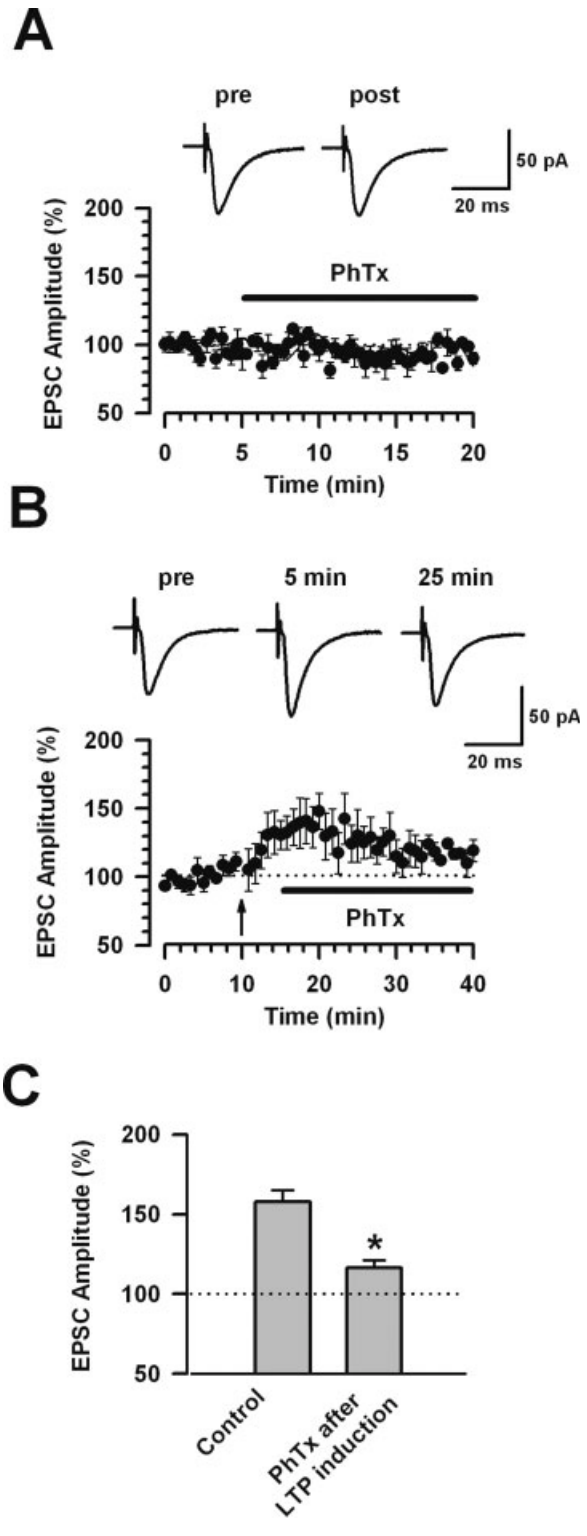

Figure 3

Cingulate LTP Does Not Require the Interaction Between GluR2 Subunits and PDZ-Domain-Containing Proteins

To examine the role of the C-terminus of GluR2 on synaptic plasticity, we used several synthetic peptides that disrupt interaction between AMPA subunits and PDZ-domain-containing proteins. The Pep2-SVKI peptide interferes with the interaction between GluR2 and GRIP. This peptide also interferes with interactions between GluR2 and other PDZ proteins such as ABP and PICK1 (Li et al., 1999; Daw et al., 2000; Kim et al., 2001). The Pep2-AVKI peptide disrupts binding of GluR2 to PICK1 (Li et al., 1999; Daw et al., 2000; Kim et al., 2001). A control peptide (Pep2-SVKE), in which the PDZ interaction motif is destroyed by substituting the last amino acid (isoleucine) with glutamate, does not interfere with GluR2 binding to GRIP. Peptides were applied through the patch recording electrode into postsynaptic neurons to examine their effect on synaptic transmission and

Figure 3 Synaptic delivery of the Ca^{2+} permeable GluR1 receptors contributes to the maintenance of LTP. (A) PhTx (10 μM; $n = 5$) had little effect on EPSCs when it was bath applied during recordings. The insets show averages of six EPSCs before and after the application of PhTx. (B) PhTx (10 μM) significantly reduced the synaptic potentiation induced by paired training ($n = 7$). PhTx was bath applied 5 min after the paired training. The insets show averages of six EPSCs at baseline responses (pre) and 5 min and 30 min after the paired training (arrow). The dashed line indicates the mean responses. (C) Summary results of the effects of PhTx on LTP induced by paired training using data in B and Figure 1B. * $p < 0.05$ compared to control LTP without PhTX.

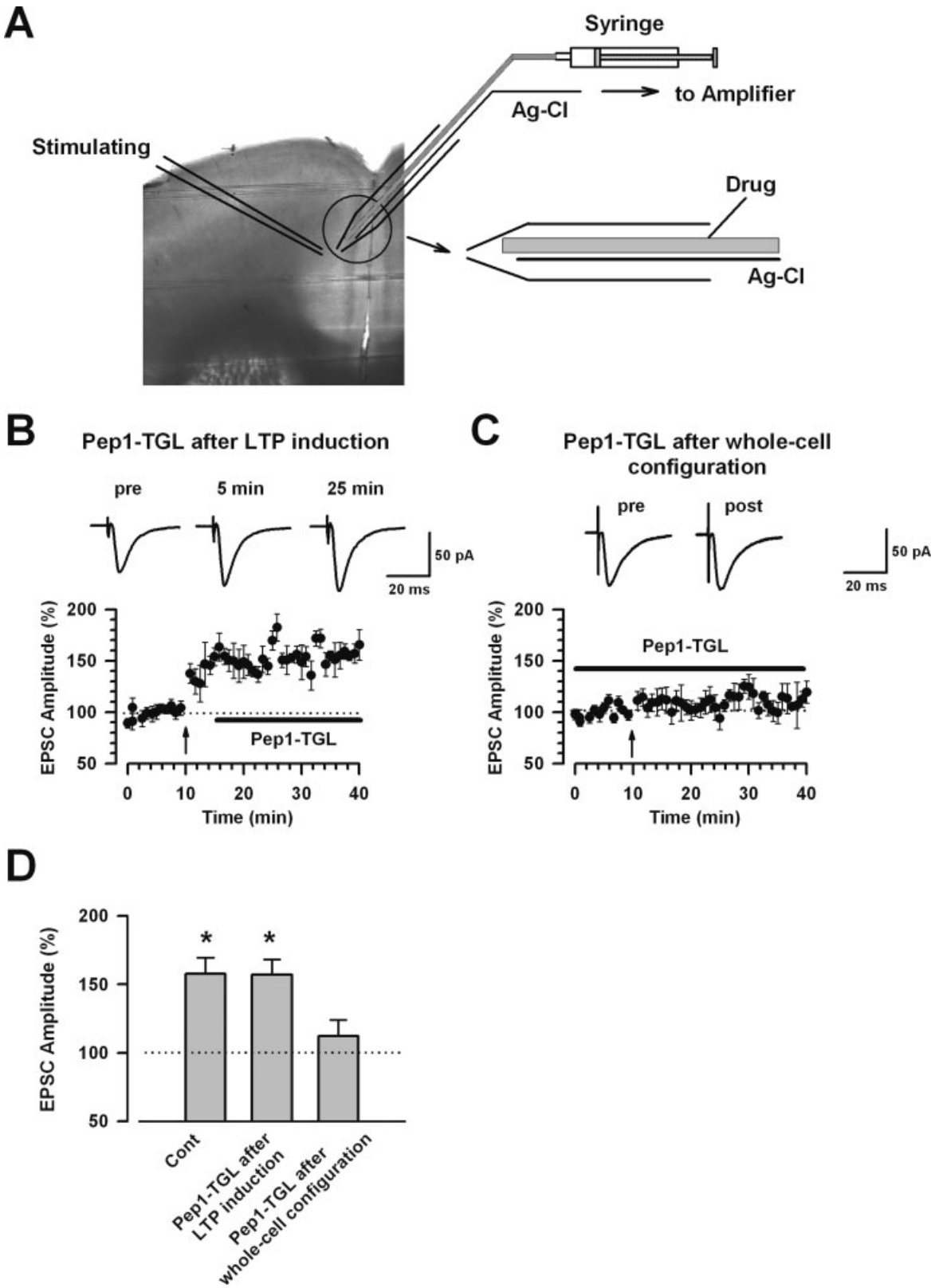

Figure 4 GluR1 subunit trafficking is completed within 10 min. (A) Diagram of a slice showing the placement of an intracellular application system and a stimulation electrode in the ACC. (B) Direct perfusion of Pep1-TGL (100 μM) 5 min after paired training had no effect on the maintenance of LTP ($n = 8$). (C) Intracellular perfusion of Pep1-TGL (100 μM) immediately after whole-cell configuration blocked the induction of LTP ($n = 7$). Traces show averages of six EPSCs at baseline responses (pre) and 5 min and 30 min after the paired training (arrow). (D) Summary results of the effects of intracellular perfusion of Pep1-TGL after LTP induction or after whole-cell configuration on LTP induced by paired training using data in B, C and Figure 1(B). * $p < 0.05$ compared to baseline.

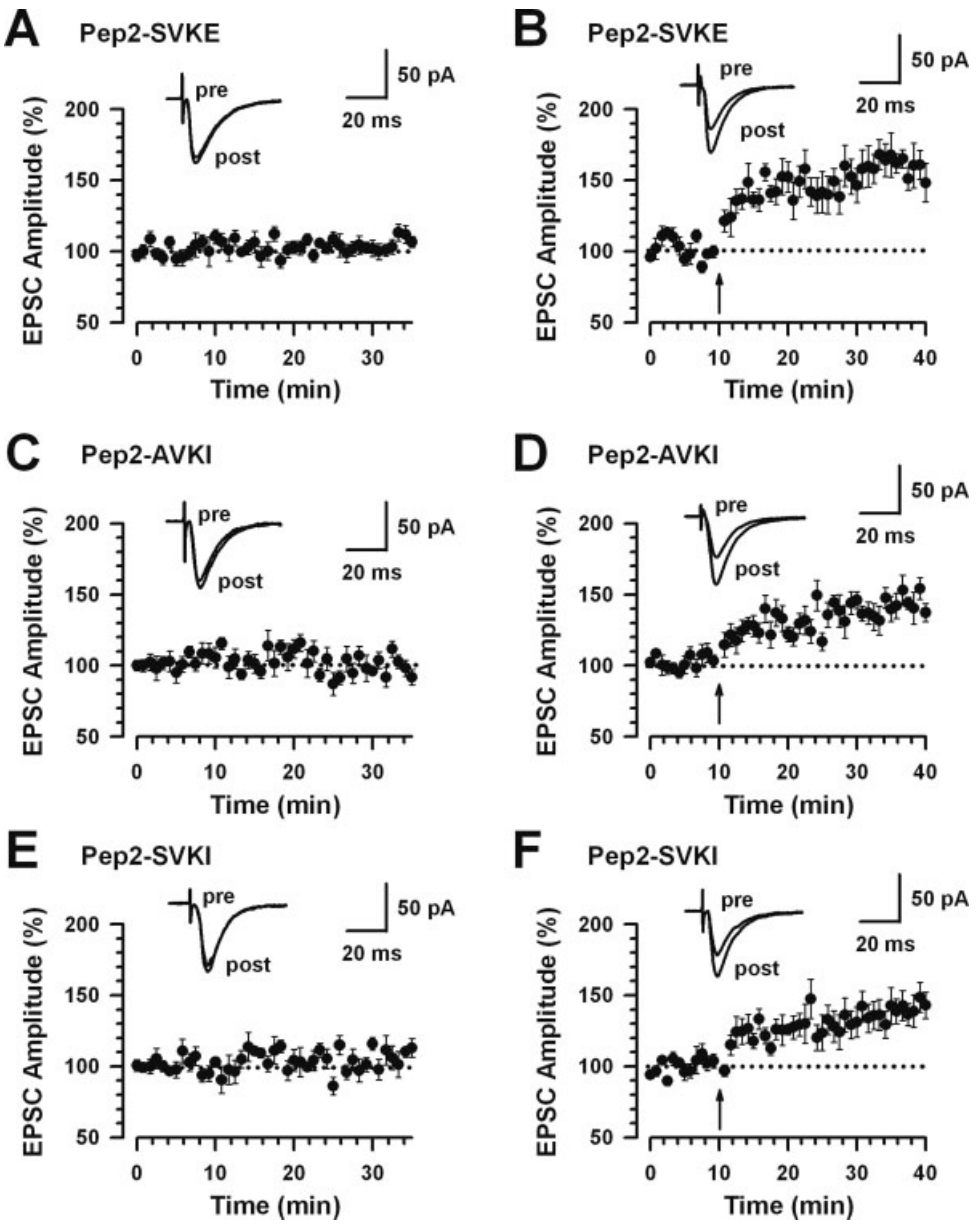

Figure 5 GluR2 C-terminal peptides have no effect on LTP. (A,C,E) Pep2-SVKE ($n = 6$), Pep2-AVKI ($n = 7$) and Pep2-SVKI ($n = 7$) did not affect baseline responses. The insets show averages of six EPSCs at the time points of 5 (pre) and 35 min (post) during the recording. The dashed line indicates the mean basal synaptic responses. (B,D,F) Pep2-SVKE (100 μM), Pep2-AVKI (100 μM) or Pep2-SVKI (100 μM) in the intracellular solution have no effect on LTP induction (Pep2-SVKE, $n = 7$: Pep2-AVKI, $n = 9$: Pep2-SVKI, $n = 8$). Traces show averages of six EPSCs at baseline responses (pre) and 30 min (post) after the paired training (arrow). The dashed line indicates the mean basal synaptic responses.

plasticity. The Pep2-SVKE (100 μM) did not affect basal synaptic responses in cingulate slices [Fig. 5(A)]. In the presence of Pep2-AVKI (100 μM) and Pep2-SVKI (100 μM), basal synaptic responses were not affected [Fig. 5(C,E)]. Thus, Pep2-AVKI and Pep2-SVKI had no effect on basal synaptic transmission.

Next, we tested whether the interactions between the C-terminus of GluR2 and PDZ-domain-containing proteins in postsynaptic neurons are critical for the induction of cingulate LTP. In the presence of Pep2-SVKE, LTP was observed and the magnitude of potentiation was comparable to controls (156.8 ±

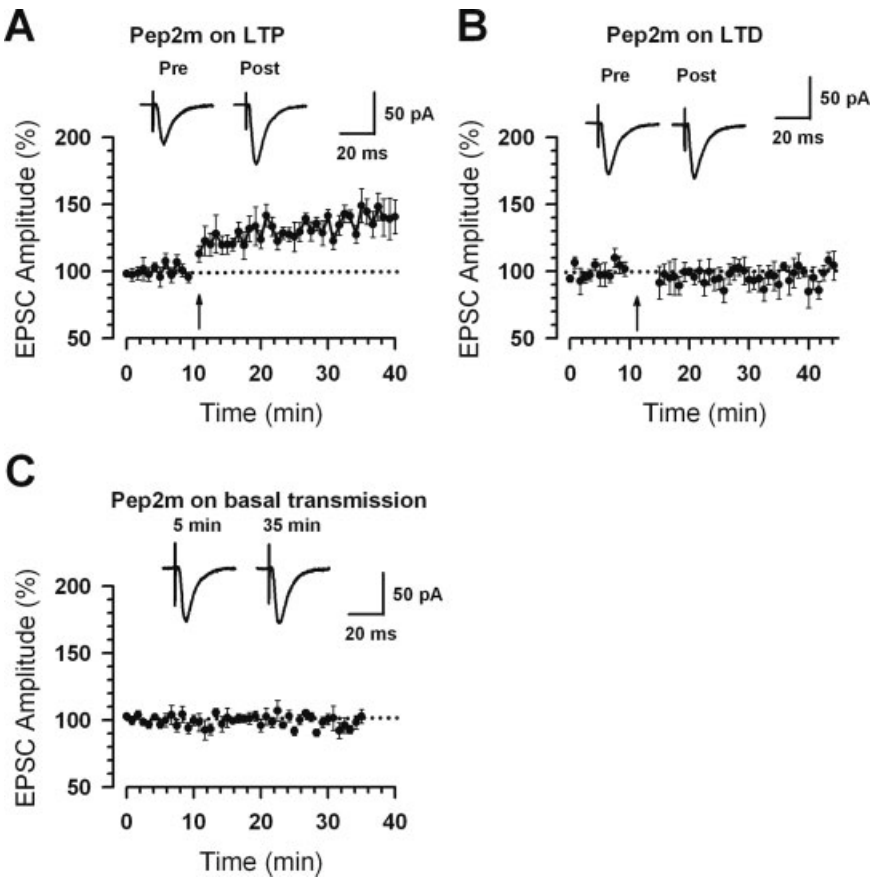

Figure 6 Peptide (Pep2m) inhibiting GluR2-NSF interaction has no effect on LTP but blocks the induction of LTD. (A) Intracellular injection of Pep2m (100 μM, $n = 7$) did not affect LTP induction. (B) Intracellular injection of Pep2m ($n = 5$) blocked LTD induced by paired training in the ACC. Traces show averages of six EPSCs at baseline responses (pre) and 30 min (post) after the paired training (arrow). The dashed line indicates the mean basal synaptic responses. (C) The peptide had no effect on the basal synaptic transmission ($n = 7$).

10.1% of baseline, $n = 7$, $p < 0.05$ compared with baseline response before the paired training) [Fig. 5(B)]. LTP was also induced in the presence of Pep2-AVKI (100 μM) or Pep2-SVKI (100 μM) [Pep2-AVKI; 143.1 $\pm$ 7.4% of baseline, $n = 9$, $p < 0.05$ compared with baseline response before the paired training; Fig. 5(D): Pep2-SVKI; 141.4 $\pm$ 9.7% of baseline, $n = 8$, $p < 0.05$ compared with baseline response before the paired training; Fig. 5(F)]. Taken together, these results suggest that interfering with GluR2/3-PDZ interactions has no effect on cingulate synaptic LTP.

NSF binds to a unique recognition site located at the C-terminus of the GluR2 subunit and regulates the membrane insertion of GluR2-containing AMPA receptors in hippocampus (Song et al., 1998; Luthi et al., 1999). We therefore tested the effect of a peptide (Pep2m) inhibiting GluR2-NSF interaction on the synaptic transmission and plasticity in the ACC. The Pep2m (100 μM) did not affect basal synaptic responses [$n = 7$, Fig. 6(C)]. Also, our results showed that the peptide had no effect on LTP [141.4 $\pm$ 11.9%, $n = 7$, $p < 0.05$ compared to baseline responses; Fig. 6(A)]. However, in the presence of Pep2m, LTD was blocked in the ACC neurons [96.2 $\pm$ 8.3%, $n = 5$, $p > 0.05$ compared to baseline responses; Fig. 6(B)].

Finally, we pooled data together and analyzed the time course of peptide effects on LTP. Synaptic responses at three different time points after the induction of LTP were examined: 5 min, 20 min and 30 min (Fig. 7). Although there is seemingly a decrease in the early phase of LTP (5 min after induction) after intracellular injection of GluR2-interacting peptides (AVKI, SVKI or Pep2m), we found that the early phase was not significantly affected in the

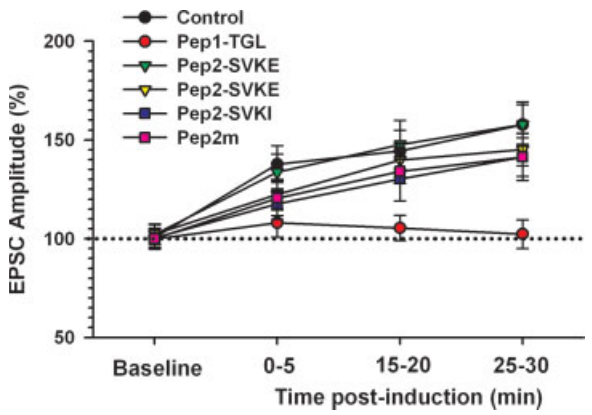

Figure 7 GluR1 C-terminal but not GluR2 C-terminal peptide inhibits cingulate LTP. Summary data for effects of GluR1 C-terminal and GluR2 C-terminal peptides on cingulate synaptic potentiation at baseline, 5 min, 20 min and 30 min after LTP induction. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com]

AVKI, SVKI, or Pep2m experiments compared with the SVKE experiment (One-way ANOVA, $p = 0.25$; Pep2-AVKI, 122.4 ± 7.8% at 5 min postpaired training, $p = 0.17$ compared with Pep2-SVKE, 135.8 ± 8.2%; Pep2-SVKI; 118.8 ± 5.4%, $p = 0.09$ compared with Pep2-SVKE; Pep2m, 120.6 ± 6.8%, $p = 0.18$ compared with Pep2-SVKE). Potentiation at 20 min and 30 min after induction of LTP were also tested, and we found that all three peptides did not affect LTP at these two time points (20 min, $p = 0.49$; 30 min, $p = 0.96$, one-way ANOVA). These results suggest that GluR2 is not involved the expression of LTP in the ACC.

DISCUSSION

In the present study, we provide strong evidence for the rapid functional recruitment of GluR2-lacking receptors, mainly GluR1 subunits, during the expression of cingulate LTP. Although similar studies have been reported in hippocampal neurons in previous studies, we believe that our results are novel for cortical neuronal functions, and indicate that hippocampal neurons and cingulate cortical neurons show similar synaptic mechanism for the expression of LTP. This is the first study, to our knowledge, to show AMPA GluR1 but not GluR2/3 receptor contributes to the expression of cingulate LTP. Considering the important physiological functions of the ACC in animals and humans (see Introduction), we are confident that this study provides a potential usual synaptic mechanism for our understanding of ACC-related cognition functions.

AMPA GluR1 Subunit and ACC LTP

A previous report suggested that the C-terminal PDZ ligand of GluR1 subunits may be a critical factor in the induction of hippocampal LTP (Hayashi et al., 2000). In contrast, a recent study using knockout mice in which the PDZ ligand-binding site of GluR1 has been deleted indicated that the GluR1 PDZ ligand is not necessary for hippocampal synaptic plasticity (Kim et al., 2005). Our results show that GluR1 subunit C-terminal peptide analog, Pep1-TGL, blocked the induction of cingulate LTP, although the reason for this difference is unclear. Thus, in the ACC, the interaction between the C-terminus of GluR1 and PDZ domain proteins is required for the induction of LTP. Synaptic delivery of the GluR1 subunit from extrasynaptic sites is the key mechanism underlying synaptic plasticity (Hayashi et al., 2000; Passafaro et al., 2001) and GluR1-PDZ interactions play a critical intermediate in this plasticity. Our pharmacological experiments show that the application of PhTx 5 min after paired training reduced to synaptic potentiation, while PhTx had no effect on basal responses. Therefore, we believe that Ca^{2+}-permeable GluR2-lacking receptors contribute to the maintenance of LTP and are necessary for subsequent LTP stabilization. Although our data did not provide direct evidence for the synaptic trafficking or insertion of GluR1 receptors at postsynaptic membrane, the present findings suggest selective contribution of AMPA subtype receptors to cingulate LTP.

Although the application of Pep1-TGL inhibited the induction of cingulate LTP, it had no effect on the basal synaptic transmission. This result is consistent with a previous report, in which the overexpression of the C-terminal domain of GluR1 had no detectable effects on basal synaptic transmission (Hayashi et al., 2000). There are at least two possible explanations for this specificity. In the basal state, the interaction between synaptic AMPA receptors and PDZ-domain-containing proteins may be stable and resistant to an interfering peptide. However, during synaptic potentiation, newly incorporated GluR1 receptors that interact with PDZ-domain-containing proteins are sensitive to this peptide. Alternatively, basal AMPA receptor responses may be mediated by receptors that do not require PDZ-domain-containing protein interactions. Do GluR1 receptors contribute to the induction of ACC LTP? We believe that GluR1 subunit alone may not be sufficient in the induction of LTP,

since cingulate LTP is completely blocked by AP-5 in our previous reports (Liauw et al., 2005; Zhao et al., 2005). In the hippocampus, GluR1 subunit is required for NMDA receptor-dependent synaptic delivery of AMPA receptors, a process thought to be responsible for adding new receptors to increase synaptic transmission during LTP (Shi et al., 1999). Future experiments are needed to demonstrate whether NMDA receptor-dependent LTP leads to synaptic delivery of GluR1 receptors in the ACC neurons.

Postsynaptic Expression of ACC LTP

In the hippocampus and cellebellum, both presynaptic and postsynaptic mechanisms are proposed to play critical roles in LTP expression. For example, the presynaptic expression of LTP is important at mossy fiber synapses, while LTP in the area of CA1 hippocampus is mainly postsynaptic (Nicoll and Malenka, 1995). At cerebellar parallel fiber (PF)-Purkinje cell synapses, cerebellar LTP induced by PF stimuli at 4-8 Hz requires an increase in glutamate release from presynaptic PF terminals (Salin et al., 1996; Storm et al., 1998). In contrast, the stimulation of PF at 1 Hz was found to induce a postsynaptic form of PF-LTP (Lev-Ram et al., 2002). What about the expression mechanisms of LTP in the ACC? Similar to the CA1 hippocampal LTP, postsynaptic mechanisms are suggested in the ACC by our current findings. We have previously shown that PPF was not altered after the induction of cingulate LTP. Furthermore, postsynaptic injection of BAPTA completely blocked the induction of LTP, indicating the importance of elevated postsynaptic Ca^{2+} concentrations (Zhao et al., 2005). However, we do not rule out the possibility of presynaptic changes in the ACC during other physiological/pathological conditions. In a recent study, we reported that an enhanced neurotransmitter release was observed in ACC synapses of mice with chronic pain (Zhao et al., 2006).

GluR2/3 Does Not Contribute to ACC LTP

GluR2/3 subunits may continually replace synaptic GluR2/3 subunits in an activity-independent manner that maintains constant synaptic transmission (Carroll et al., 2001; Malinow and Malenka, 2002; Song and Huganir, 2002; Bredt and Nicoll, 2003). Recent studies indicate that the C-terminal tails of GluR2/3 subunits can interact with PDZ-domain-containing proteins such as GRIP1, ABP, and PICK1 (Dong et al., 1997; Daw et al., 2000; Kim et al., 2001). This interaction may play a complementary role at postsynaptic membrane or inside a cell (Dong et al., 1997; Srivastava et al., 1998; Xia et al., 1999; Perez et al., 2001). In the hippocampus, postsynaptic injection of synthetic peptides (Pep2-SVKI) that disrupt the interaction between GluR2/3 and GRIP/ABP cause a change of AMPA receptor-mediated responses (Daw et al., 2000; Kim et al., 2001). Furthermore, GluR2/3-GRIP/ABP and/or GluR2/3-PICK1 interactions are critical for hippocampal and cerebellar LTD (Daw et al., 2000; Xia et al., 2000; Kim et al., 2001). Although the postsynaptic introduction of the peptide (Pep2-SVKI) in hippocampal neurons caused a change in basal synaptic transmission (Daw et al., 2000; Kim et al., 2001), it had no effect on basal synaptic transmission in the adult ACC neurons. Several possibilities, such as the difference in brain areas, the age of animals and different experimental conditions could explain the different results. We also examined the role of these peptides in synaptic potentiation in the ACC and found that the GluR2/3-PDZ interaction had no effect on cingulate LTP. In our unpublished data, we did find that the same interfering peptides inhibited cingulate LTD (unpublished data).

Physiological and Pathological Significance

The ACC is believed to play pivotal roles in learning, memory, fear and chronic pain (Frankland et al., 2004; Maviel et al., 2004; Wiltgen et al., 2004; Zhuo, 2004; Tang et al., 2005; Zhao et al., 2005). Recently, ACC has been shown to play a key role in the expression of remote fear memory (Frankland et al., 2004) and spatial memory (Maviel et al., 2004). Furthermore, ACC may play a critical role in the acquisition of fear memory (Tang et al., 2005; Zhao et al., 2005). For example, direct stimulation of the ACC has been shown to induce fear memory (Tang et al., 2005), and NR2B subunit has been demonstrated to be involved in the induction of LTP and the acquisition of contextual fear memory (Zhao et al., 2005). Future studies are necessary to reveal the role of GluR1-receptor trafficking in memory formation in the ACC, since fear conditioning drives GluR1 receptors into synapses in the amygdala, and this mechanism indicates an essential molecular process to memory formation (Rumpel et al., 2005). Recent cumulative studies suggest that the ACC plays critical roles in pain-related central plasticity (Wei et al., 2002a; Zhuo, 2004; Wu et al., 2005a). Studies using mice with genetic modification and chronic pain suggest that AC1, AC8 and NR2B receptors contribute to the behavioral allodynia (Wei et al., 2001; Wei et al., 2002a; Wu et al.,

2005a). Because little is known about the contribution of AMPA receptors in the ACC to the pathophysiology of chronic pain, it will be very important for us to understand the role of GluR1 receptors in the ACC in the formation and expression of persistent or chronic pain.

In summary, we demonstrate that the recruitment of GluR1 receptors is a major postsynaptic mechanism for cingulate LTP, and can happen on a rapid time scale. This study provides molecular basis for our understanding of the mechanism underlying activity-dependent synaptic plasticity in the ACC.

L.-J.W. is supported by postdoctoral fellowships from Canadian Institutes of Health Research and the Fragile X Research Foundation of Canada. We thank S. Ko for editing the manuscript.

REFERENCES

Albensi BC, Alasti N, Mueller AL. 2000. Long-term potentiation in the presence of NMDA receptor antagonist arylalkylamine spider toxins. J Neurosci Res 62:177–185.

Bliss TV, Collingridge GL. 1993. A synaptic model of memory: Long-term potentiation in the hippocampus. Nature 361:31–39.

Bredt DS, Nicoll RA. 2003. AMPA receptor trafficking at excitatory synapses. Neuron 40:361–379.

Carroll RC, Beattie EC, von Zastrow M, Malenka RC. 2001. Role of AMPA receptor endocytosis in synaptic plasticity. Nat Rev Neurosci 2:315–324.

Daw MI, Chittajallu R, Bortolotto ZA, Dev KK, Duprat F, Henley JM, Collingridge GL, et al. 2000. PDZ proteins interacting with C-terminal GluR2/3 are involved in a PKC-dependent regulation of AMPA receptors at hippocampal synapses. Neuron 28:873–886.

Dong H, O'Brien RJ, Fung ET, Lanahan AA, Worley PF, Huganir RL. 1997. GRIP: A synaptic PDZ domain-containing protein that interacts with AMPA receptors. Nature 386:279–284.

Frankland PW, Bontempi B, Talton LE, Kaczmarek L, Silva AJ. 2004. The involvement of the anterior cingulate cortex in remote contextual fear memory. Science 304:881–883.

Hayashi Y, Shi SH, Esteban JA, Piccini A, Poncer JC, Malinow R. 2000. Driving AMPA receptors into synapses by LTP and CaMKII: requirement for GluR1 and PDZ domain interaction. Science 287:2262–2267.

Hori T, Takai Y, Takahashi T. 1999. Presynaptic mechanism for phorbol ester-induced synaptic potentiation. J Neurosci 19:7262–7267.

Kandel ER. 2001. The molecular biology of memory storage: A dialogue between genes and synapses. Science 294:1030–1038.

Kim CH, Chung HJ, Lee HK, Huganir RL. 2001. Interaction of the AMPA receptor subunit GluR2/3 with PDZ domains regulates hippocampal long-term depression. Proc Natl Acad Sci USA 98:11725–11730.

Kim CH, Takamiya K, Petralia RS, Sattler R, Yu S, Zhou W, Kalb R, et al. 2005. Persistent hippocampal CA1 LTP in mice lacking the C-terminal PDZ ligand of GluR1. Nat Neurosci 8:985–987.

Lev-Ram V, Wong ST, Storm DR, Tsien RY. 2002. A new form of cerebellar long-term potentiation is postsynaptic and depends on nitric oxide but not cAMP. Proc Natl Acad Sci USA 99:8389–8393.

Li P, Kerchner GA, Sala C, Wei F, Huettner JE, Sheng M, Zhuo M. 1999. AMPA receptor-PDZ interactions in facilitation of spinal sensory synapses. Nat Neurosci 2:972–977.

Liauw J, Wu LJ, Zhuo M. 2005. Calcium-stimulated adenylyl cyclases required for long-term potentiation in the anterior cingulate cortex. J Neurophysiol 94:878–882.

Luthi A, Chittajallu R, Duprat F, Palmer MJ, Benke TA, Kidd FL, Henley JM, et al. 1999. Hippocampal LTD expression involves a pool of AMPARs regulated by the NSF-GluR2 interaction. Neuron 24:389–399.

Malenka RC, Bear MF. 2004. LTP and LTD: An embarrassment of riches. Neuron 44:5–21.

Malinow R, Malenka RC. 2002. AMPA receptor trafficking and synaptic plasticity. Annu Rev Neurosci 25:103–126.

Maviel T, Durkin TP, Menzaghi F, Bontempi B. 2004. Sites of neocortical reorganization critical for remote spatial memory. Science 305:96–99.

Nicoll RA, Malenka RC. 1995. Contrasting properties of two forms of long-term potentiation in the hippocampus. Nature 377:115–118.

Passafaro M, Piech V, Sheng M. 2001. Subunit-specific temporal and spatial patterns of AMPA receptor exocytosis in hippocampal neurons. Nat Neurosci 4:917–926.

Perez JL, Khatri L, Chang C, Srivastava S, Osten P, Ziff EB. 2001. PICK1 targets activated protein kinase Calpha to AMPA receptor clusters in spines of hippocampal neurons and reduces surface levels of the AMPA-type glutamate receptor subunit 2. J Neurosci 21:5417–5428.

Plant K, Pelkey KA, Bortolotto ZA, Morita D, Terashima A, McBain CJ, Collingridge GL, et al. 2006. Transient incorporation of native GluR2-lacking AMPA receptors during hippocampal long-term potentiation. Nat Neurosci 9:602–604.

Rumpel S, LeDoux J, Zador A, Malinow R. 2005. Postsynaptic receptor trafficking underlying a form of associative learning. Science 308:83–88.

Salin PA, Malenka RC, Nicoll RA. 1996. Cyclic AMP mediates a presynaptic form of LTP at cerebellar parallel fiber synapses. Neuron 16:797–803.

Shi SH, Hayashi Y, Petralia RS, Zaman SH, Wenthold RJ, Svoboda K, Malinow R. 1999. Rapid spine delivery and redistribution of AMPA receptors after synaptic NMDA receptor activation. Science 284:1811–1816.

Song I, Huganir RL. 2002. Regulation of AMPA receptors during synaptic plasticity. Trends Neurosci 25:578–588.

Song I, Kamboj S, Xia J, Dong H, Liao D, Huganir RL. 1998. Interaction of the *N*-ethylmaleimide-sensitive factor with AMPA receptors. Neuron 21:393–400.

Srivastava S, Osten P, Vilim FS, Khatri L, Inman G, States B, Daly C, et al. 1998. Novel anchorage of GluR2/3 to the postsynaptic density by the AMPA receptor-binding protein ABP. Neuron 21:581–591.

Storm DR, Hansel C, Hacker B, Parent A, Linden DJ. 1998. Impaired cerebellar long-term potentiation in type I adenylyl cyclase mutant mice. Neuron 20:1199–1210.

Tang J, Ko S, Ding HK, Qiu CS, Calejesan AA, Zhuo M. 2005. Pavlovian fear memory induced by activation in the anterior cingulate cortex. Mol Pain 1:6.

Toyoda H, Zhao MG, Zhuo M. 2005. Roles of NMDA receptor NR2A and NR2B subtypes for long-term depression in the anterior cingulate cortex. Eur J Neurosci 22:485–494.

Washburn MS, Dingledine R. 1996. Block of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors by polyamines and polyamine toxins. J Pharmacol Exp Ther 278:669–678.

Wei F, Li P, Zhuo M. 1999. Loss of synaptic depression in mammalian anterior cingulate cortex after amputation. J Neurosci 19:9346–9354.

Wei F, Qiu CS, Kim SJ, Muglia L, Maas JW, Pineda VV, Xu HM, Chen ZF, Storm DR, Muglia LJ, Zhuo M. 2002a. Genetic elimination of behavioral sensitization in mice lacking calmodulin-stimulated adenylyl cyclases. Neuron 36:713–726.

Wei F, Qiu CS, Liauw J, Robinson DA, Ho N, Chatila T, Zhuo M. 2002b. Calcium calmodulin-dependent protein kinase IV is required for fear memory. Nat Neurosci 5:573–579.

Wei F, Wang GD, Kerchner GA, Kim SJ, Xu HM, Chen ZF, Zhuo M. 2001. Genetic enhancement of inflammatory pain by forebrain NR2B overexpression. Nat Neurosci 4:164–169.

Wiltgen BJ, Brown RA, Talton LE, Silva AJ. 2004. New circuits for old memories: the role of the neocortex in consolidation. Neuron 44:101–108.

Wu LJ, Toyoda H, Zhao MG, Lee YS, Tang J, Ko SW, Jia YH, et al. 2005a. Upregulation of forebrain NMDA NR2B receptors contributes to behavioral sensitization after inflammation. J Neurosci 25:11107–11116.

Wu MF, Pang ZP, Zhuo M, Xu ZC. 2005b. Prolonged membrane potential depolarization in cingulate pyramidal cells after digit amputation in adult rats. Mol Pain 1:23.

Xia J, Chung HJ, Wihler C, Huganir RL, Linden DJ. 2000. Cerebellar long-term depression requires PKC-regulated interactions between GluR2/3 and PDZ domain-containing proteins. Neuron 28:499–510.

Xia J, Zhang X, Staudinger J, Huganir RL. 1999. Clustering of AMPA receptors by the synaptic PDZ domain-containing protein PICK1. Neuron 22:179–187.

Zhao MG, Ko SW, Wu LJ, Toyoda H, Xu H, Quan J, Li J, et al. 2006. Enhanced presynaptic neurotransmitter release in the anterior cingulate cortex of mice with chronic pain. J Neurosci 26:8923–8930.

Zhao MG, Toyoda H, Lee YS, Wu LJ, Ko SW, Zhang XH, Jia Y, et al. 2005. Roles of NMDA NR2B subtype receptor in prefrontal long-term potentiation and contextual fear memory. Neuron 47:859–872.

Zhuo M. 2004. Central plasticity in pathological pain. Novartis Found Symp 261:132–145. (discussion 145–154).