Interaction of the AMPA receptor subunit GluR2/3 with PDZ domains regulates hippocampal long-term depression

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The interaction of PDZ domain-containing proteins with the C termini of α-amino-3-hydroxy-5-methylisoxazolepropionate (AMPA) receptors has been suggested to be important in the regulation of receptor targeting to excitatory synapses. Recent studies have shown that the rapid internalization of AMPA receptors at synapses may mediate, at least in part, the expression of long-term depression (LTD). We have previously shown that phosphorylation of Ser-880 on the AMPA receptor GluR2 subunit differentially regulated the interaction of GluR2 with the PDZ domain-containing proteins GRIP1 and PICK1. Here, we show that induction of LTD in hippocampal slices increases phosphorylation of Ser-880 within the GluR2 C-terminal PDZ ligand, suggesting that the modulation of GluR2 interaction with GRIP1 and PICK1 may regulate AMPA receptor internalization during LTD. Moreover, postsynaptic intracellular perfusion of GluR2 C-terminal peptides that disrupt GluR2 interaction with PICK1 inhibit the expression of hippocampal LTD. These results suggest that the interaction of GluR2 with PICK1 may play a regulatory role in the expression of LTD in the hippocampus.

Glutamate receptors are the major excitatory neurotransmitter receptors in the central nervous system and play critical roles in synaptic plasticity, neuronal development, and neuropsychiatric disorders (1–6). Recent studies have suggested that synaptic targeting and clustering of glutamate receptors are regulated by their interaction with neuronal proteins. Specifically, the interaction of the C termini of specific *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methylisoxazolepropionate (AMPA) receptor subunits with PDZ domain-containing proteins seems to be important for the synaptic localization of the receptors (7–9). AMPA receptor GluR2/3 subunits have been shown to interact with PDZ domains within the GRIP1, GRIP2/ABP, and PICK1 proteins through their C-terminal four amino acids (−SVKI) (10–15). This interaction has been implicated in the regulation of the synaptic targeting of AMPA receptors because PICK1 coexpression with GluR2 induces clustering of GluR2 (10, 13, 14) and overexpression of the GluR2 C-terminal PDZ ligand disrupts AMPA receptor synaptic targeting (10).

Recent studies have indicated that rapid changes in synaptic efficacy such as those that occur during long-term potentiation (LTP) and long-term depression (LTD) may be caused by rapid changes in AMPA receptor function. Although some of these effects may be mediated by modulation of AMPA receptor function by phosphorylation (16–19), rapid changes in the levels of synaptic AMPA receptors may also mediate changes in synaptic efficacy (20–27). One possible mechanism for the rapid modification of the synaptic targeting of AMPA receptors is through the dynamic regulation of AMPA receptor–PDZ domain interactions. We have previously shown that phosphorylation of Ser-880 within the GluR2 C-terminal PDZ ligand differentially regulates its interaction with the PDZ domains of GRIP1 and PICK1 and modulates internalization of GluR2 subunits (28). These results suggest that the interaction of GluR2 with PDZ domain-containing proteins might rapidly regulate the surface expression of AMPA receptors during LTD. Here, we show that the modulation of GluR2 interaction with GRIP1, GRIP2/ABP, and PICK1 by Ser-880 phosphorylation may be involved in the expression of LTD in the hippocampus.

Methods

Biochemical Analysis of Hippocampal Slices. Preparation of hippocampal slices and extracellular field potential recording were performed as described (17). After the recording, the slices were quickly homogenized, and the membrane pellets were then subjected to quantitative immunoblotting with anti-GluR2–C-terminal and anti-GluR2–pS880 antibodies as described (28, 30). All data are reported as mean ± standard error. Sample size n refers to the number of pairs, and the group-paired t test was used to test the difference between the control and testing groups for the analysis of hippocampal slices (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

Electrophysiological Analysis of Hippocampal Slices. Hippocampal slices were prepared from 2- to 3-week-old male Sprague–Dawley rats. The preparation of slices and whole-cell recording was performed as described (29). All of the recordings were performed at 35°C. In the whole-cell recording of NMDA receptor-mediated excitatory postsynaptic current (EPSC), 4 mM Ca^{2+}, 1 mM Mg^{2+}, and 5 μM 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo[*f*]quinoxaline (NBQX) were in artificial cerebrospinal fluid, and cells were held at −55 to −60 mV. CA1 pyramidal neurons were visually identified by differential infrared contrast method with 40× objective of a Nikon microscope (E600FN). Cells were held at −70 mV by using an Axopatch 1D amplifier (Axon Instruments, Foster City, CA). Series and input resistance were monitored every 6 s by measuring the peak and steady-state currents in response to 2-mV, 30-ms hyperpolarizing voltage steps. Experiments with >15 MΩ series resistances were discarded. Holding current was monitored throughout the experiment. To monitor the slice stability, the field excitatory postsynaptic potential amplitude was recorded simultaneously with Dagan differential amplifier (EX1). For whole-cell LTD experiments, only slices that have no reduction of the field excitatory postsynaptic potential were chosen for analysis. Data were filtered at 2 kHz. Responses were averaged at 2-min intervals and then normalized to the average of baseline record-

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Abbreviations: AMPA, α-amino-3-hydroxy-5-methylisoxazolepropionate; NMDA, *N*-methyl-D-aspartate; LTD, long-term depression; LTP, long-term potentiation; EPSC, excitatory postsynaptic current; LFS, low-frequency stimulation; PKC, protein kinase C; AP5, 2-amino-5-phosphonopentanoic acid.

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ing. All data acquisition and analysis were done by custom software written in AXOBASIC 3.1. Mean ± SEM was used for representing average values. The data were compiled in MICROSOFT EXCEL and plotted by using MICROCAL ORIGIN. All peptides were synthesized and purified at the Howard Hughes Medical Institute biopolymer facility (The Johns Hopkins University School of Medicine). In peptide application experiments, two protease inhibitory peptides, bestatin and leupeptin (Roche Molecular Biochemicals), were added to the internal solution (100 μM each). ANOVA followed by a post hoc analysis using Dunnett's test ($\alpha = 0.05$) was used to test the statistical significance of the effects of various peptides on LTD. ANOVA was used for the peptide effect on basal synaptic transmission.

Results

LTD Induction in the Hippocampus Increases GluR2 Ser-880 Phosphorylation. We have previously shown that phorbol ester treatment of neurons significantly increased the Ser-880 phosphorylation of GluR2 and rapidly induced internalization of surface GluR2 subunits (28). Moreover, recent studies have suggested that changes in the internalization of AMPA receptors at synapses may be important for the expression of LTD (20, 24–26). We therefore examined whether GluR2 phosphorylation at Ser-880 is modulated during LTD in hippocampal slices using the phosphorylation site-specific antibody against Ser-880 of GluR2. Extracellular field potentials of pairs of hippocampal slices were recorded simultaneously in CA1 dendritic region. After collecting a stable baseline, one test slice received low-frequency stimulation (LFS, 1 Hz, 900 pulses) to induce LTD, whereas stimulation to the other control slice was turned off. After the LFS, the stimulation to the test slice was returned to the baseline frequency, and stimulation to the control slice was resumed. The LFS produced homosynaptic LTD (72 ± 5%, $n = 12$, Fig. 1*A*), whereas the synaptic strength in the control slices was stable (104 ± 3%, $n = 12$, Fig. 1*A*). The phosphorylation of GluR2 at Ser-880 in the control and LTD slices was then analyzed by quantitative immunoblot techniques. Surprisingly, LFS produced a significant increase in GluR2 phosphorylation at Ser-880 after LTD induction compared with the control slices (119 ± 4%, $n = 12$, paired t test: $P < 0.01$, Fig. 1*B*). To test whether Ser-880 phosphorylation is modulated during LTP, we induced LTP in hippocampal slices using theta burst stimulation. Slices that received theta burst stimulation produced LTP (131 ± 3%, $n = 11$, Fig. 1*C*). Interestingly, induction of LTP had no effect on Ser-880 phosphorylation (100 ± 6%, $n = 11$, paired t test, $P > 0.05$, Fig. 1*D*). These results demonstrate that the increase in Ser-880 phosphorylation of GluR2 is specific to LTD.

LTD-Induced Increase in Ser-880 Phosphorylation of GluR2 Is Not Mediated by Protein Kinase C (PKC). These results suggest that LTD induction selectively regulates a protein kinase that phosphorylates Ser-880. Although our previous results have shown that PKC activators increases Ser-880 phosphorylation in neuronal cultures and that purified PKC can phosphorylate this site *in vitro* (28), PKC has not been previously implicated in hippocampal NMDA receptor-dependent LTD (31). To investigate this further, we examined the effect of the PKC inhibitor Gö6976 (1 μM) on LTD induction and on the LTD-induced increase in Ser-880 phosphorylation. Similar to previous studies, Gö6976 had no effect on LTD induction (80 ± 2%, $n = 8$, Fig. 2*A*). Moreover, Gö6976 had no effect on the LTD-induced increase in Ser-880 phosphorylation (119 ± 9% of controls, $n = 8$, paired t test, $P < 0.05$, Fig. 2 *B* and *C*). However, under these conditions, Gö6976 specifically inhibited (79.8% of controls, $n = 4$) the basal phosphorylation of threonine 840, a recently characterized PKC site on GluR1 (H.-K.L. and R.L.H., unpublished results). These results indicate that PKC does not mediate the LTD-induced increase in Ser-880 phosphorylation.

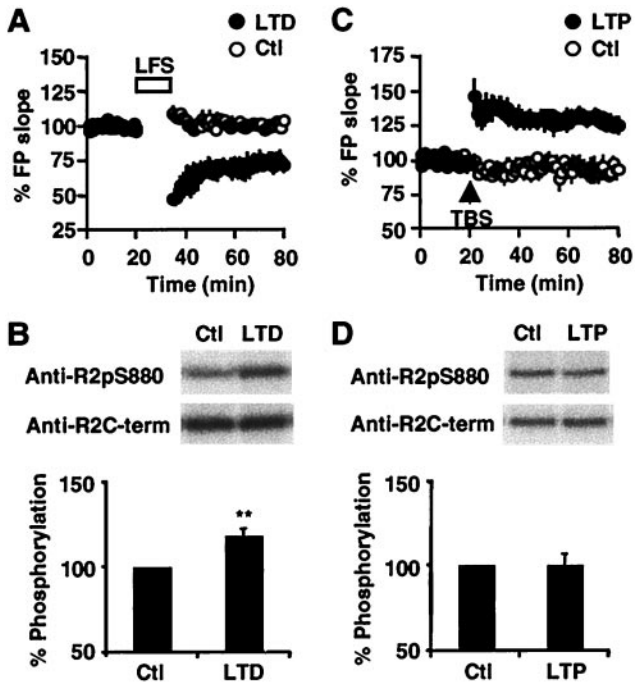

Fig. 1. LTD induction in the hippocampus increases Ser-880 phosphorylation of GluR2. (*A*) Simultaneous extracellular recording of field potentials in control slices (Ctl, ○) and LTD slices (LTD, ●). The extracellular field potential slope (FP slope) was normalized to the baseline level. (*B*) GluR2 phosphorylation at Ser-880 was increased 1 h after homosynaptic LTD. The slices from *A* were immunoblotted with anti-GluR2-pS880 and anti-GluR2 C-terminal antibodies, and the ratios of the intensity of the signal (intensity of anti-GluR2-pS880 antibody labeling/intensity of anti-GluR2 C-terminal antibody labeling) were calculated and normalized to the control slices as % phosphorylation. (*C*) Simultaneous extracellular recording of field potentials in control slices (Ctl, ○) and LTP slices (LTP, ●). (*D*) LTP induction produced no significant change in Ser-880 phosphorylation compared with the control slices. The slices from *C* were subjected to quantitative immunoblot analysis with the antibodies as described in *B*.

LTD-Induced Increase in Ser-880 Phosphorylation of GluR2 Is NMDA Receptor and Protein Phosphatase Dependent. Previous studies have demonstrated that NMDA receptor antagonists and protein phosphatase inhibitors block the induction of LTD (1, 32, 33). To examine whether these agents can also block the LTD-induced changes in Ser-880 phosphorylation, we preincubated the control and experimental slices in the NMDA receptor antagonist 2-amino-5-phosphonopentanoic acid (AP5, 100 μM), or the protein phosphatase inhibitor okadaic acid (1 μM), before LTD induction. AP5 blocked the induction of LTD (92 ± 5%, $n = 4$, t test, $P > 0.05$, Fig. 2*C*) and the LTD-induced increase in Ser-880 phosphorylation (102 ± 8% of AP5 controls, $n = 4$, paired t test, $P > 0.05$, Fig. 2*D*). Similarly, okadaic acid abolished both LTD (93 ± 4%, $n = 5$, t test, $P > 0.05$, Fig. 2*E*) and the LFS-induced increase in phosphorylation of Ser-880 (87 ± 8% of okadaic acid controls, $n = 5$, paired t test, $P > 0.05$, Fig. 2*F*). Pretreatment of the slices with AP5 had no effect on the basal level of Ser-880 phosphorylation compared with untreated slices (101 ± 6% of controls, $n = 4$, paired t test, $P > 0.05$). However, okadaic acid treatment increased the basal level of phosphorylation by 45% (145 ± 17% of controls, $n = 5$, paired t test, $P < 0.05$). These results demonstrate that LFS increases Ser-880 phosphorylation of GluR2 only under conditions that are permissive for NMDA receptor-dependent LTD induction.

Disruption of GluR2 Interaction with PDZ Domains Affects Basal Synaptic Transmission and Inhibits Hippocampal LTD. To further investigate the role of Ser-880 phosphorylation-dependent reg-

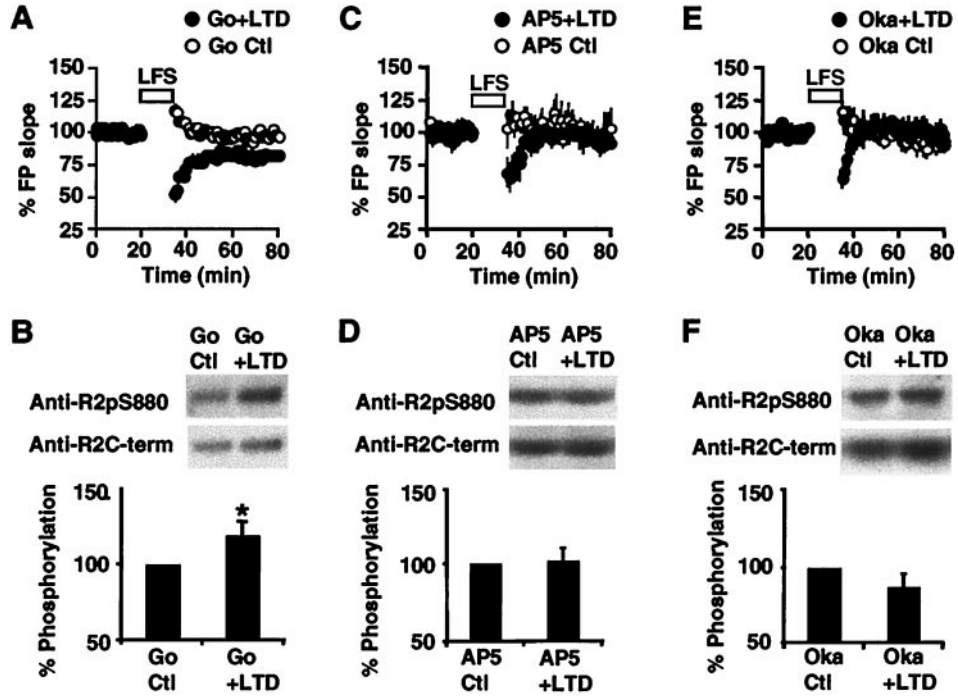

Fig. 2. The LTD-induced increase in Ser-880 phosphorylation of GluR2 is blocked by NMDA receptor antagonists and phosphatase inhibitors. (*A*) Homosynaptic LTD is not blocked by PKC inhibitor Gö6976 (Go). Both the control slices (Go ctl, ○) and experimental slices (Go + LTD, ●) were preincubated in 1 μM Gö6976 for 1 h before LFS. (*B*) Gö6976 pretreatment did not block the LTD-induced increase in Ser-880 phosphorylation. (*C*) Homosynaptic LTD is blocked by NMDA receptor antagonists, AP5. Both the control slices (AP5 ctl, ○) and experimental slices (AP5 + LTD, ●) were preincubated in 100 μM D,L-AP5 for 1 h before the LFS. (*D*) Bath application of AP5 prevented the LFS-induced increase in Ser-880 phosphorylation. (*E*) Homosynaptic LTD is blocked by a protein phosphatase 1/2A inhibitor okadaic acid (Oka). Both the control slices (Oka ctl, ○) and experimental slices (Oka + LTD, ●) were preincubated in 1 μM okadaic acid for 3 h before the LFS. (*F*) Okadaic acid pretreatment prevented the LFS-induced increase in Ser-880 phosphorylation.

ulation of GluR2 interaction with PDZ domain-containing proteins in LTD, we tested whether GluR2 C-terminal synthetic peptides that disrupt GluR2 interaction with GRIP1, GRIP2/ABP, and PICK1 would inhibit hippocampal LTD. The peptides were perfused intracellularly into hippocampal pyramidal neurons for 30 min before LTD induction using a pairing protocol in which the cell was voltage-clamped at −35 mV while giving 200 synaptic stimuli at 0.5 Hz (23, 34). The effect of peptide perfusion on the basal synaptic transmission was measured by comparing the EPSC amplitudes at 30 min just before LTD induction with the average EPSC amplitude of the initial 2-min recording. After LTD induction, the magnitude of LTD was measured by comparing the EPSC amplitude at 30 min after pairing to the average EPSC amplitude for the 6-min recording before LTD induction. In control slices without peptide perfusion, the baseline was stable for 30 min (Fig. 3 *A* and *E*, 98 ± 2%), and LTD induction reduced the EPSC amplitude by 37% (Fig. 3 *B* and *F*, 63 ± 4%). This LTD was blocked by 100 μM AP5 (n = 6, data not shown), but not by 500 μM α-methyl-4-carboxyphenylglycine, a group I/II metabotropic glutamate receptor antagonist (n = 4, data not shown), indicating that LTD induced by this pairing protocol is NMDA receptor-dependent.

In contrast, perfusion of a 15-aa peptide KKEGYNVYGIESVKI (−SVKI) corresponding to the C terminus of GluR2 induced a small increase in the basal synaptic strength, which stabilized after 10–15 min (Fig. 3 *A* and *E*, 113 ± 3%, P = 0.024, F = 5.59) and significantly inhibited LTD expression by 59% (Fig. 3 *B* and *F*, 85 ± 6%, n = 21; ANOVA: F = 8.95, $P < 0.006$) compared with the control. To test the specificity of the peptide effect, we used a control peptide KKEGYNVYGIESGKA (−SGKA) in which two amino acids, valine and isoleucine, of the PDZ binding motif (−**SVKI**) were changed to glycine and alanine, respectively (−**SGKA**). This peptide does not bind to GRIP1 and PICK1 and should not disrupt binding of the endogenous GluR2 with GRIP1, GRIP2/ABP, or PICK1 (10–13). Perfusion of −SGKA peptide had no effect on the basal synaptic strength (Fig. 3 *C* and *E*, 102 ± 2%) and LTD induction (Fig. 3 *D* and *F*, 62 ± 5%) compared with control. The field excitatory postsynaptic potential was stable and was not affected by peptide perfusion and the pairing procedure (data not shown). Moreover, the intracellular perfusion of −SVKI peptide did not affect the NMDA receptor-mediated EPSC compared with −SGKA peptide (Fig. 4*A*). Interestingly, −SVKI peptide did not block pairing-induced LTP (Fig. 4*B*). These results suggest that the interaction of the GluR2 C terminus with GRIP1, GRIP2/ABP, or PICK1 is involved in the regulation of basal synaptic transmission and the induction of NMDA receptor-dependent LTD.

GluR2 Interaction with PICK1 Is Involved in Both Constitutive and Regulated Internalization of AMPA Receptor During LTD. We have previously shown that nonphosphorylated GluR2 C-terminal peptides bind to both GRIP1 and PICK1, whereas Ser-880 phosphorylated peptides bind only to PICK1 (28). To test whether the intracellular perfusion of the GluR2 C-terminal peptide affected LTD through its interaction with GRIP1, GRIP2/ABP, or PICK1, we used a phosphorylated synthetic peptide KKEGYNVYGIES-PO_4-VKI (−(p)SVKI). Perfusion of −(p)SVKI peptide increased the basal synaptic strength by 31 ± 3% over 30 min (Fig. 5 *A* and *E*, P = 0.00002, F = 26.58) and inhibited LTD expression by 59% compared with that of −SGKA control peptide (Fig. 5 *B* and *F*, 85 ± 6%; ANOVA: F = 7.91, $P < 0.01$). We also tested the effect of a peptide KKEGYNVYGIEEVKI (−**E**VKI) in which the serine was replaced by glutamate to mimic phosphorylation. We have previously shown that −EVKI peptide binds to PICK1 but not to GRIP1 (28).

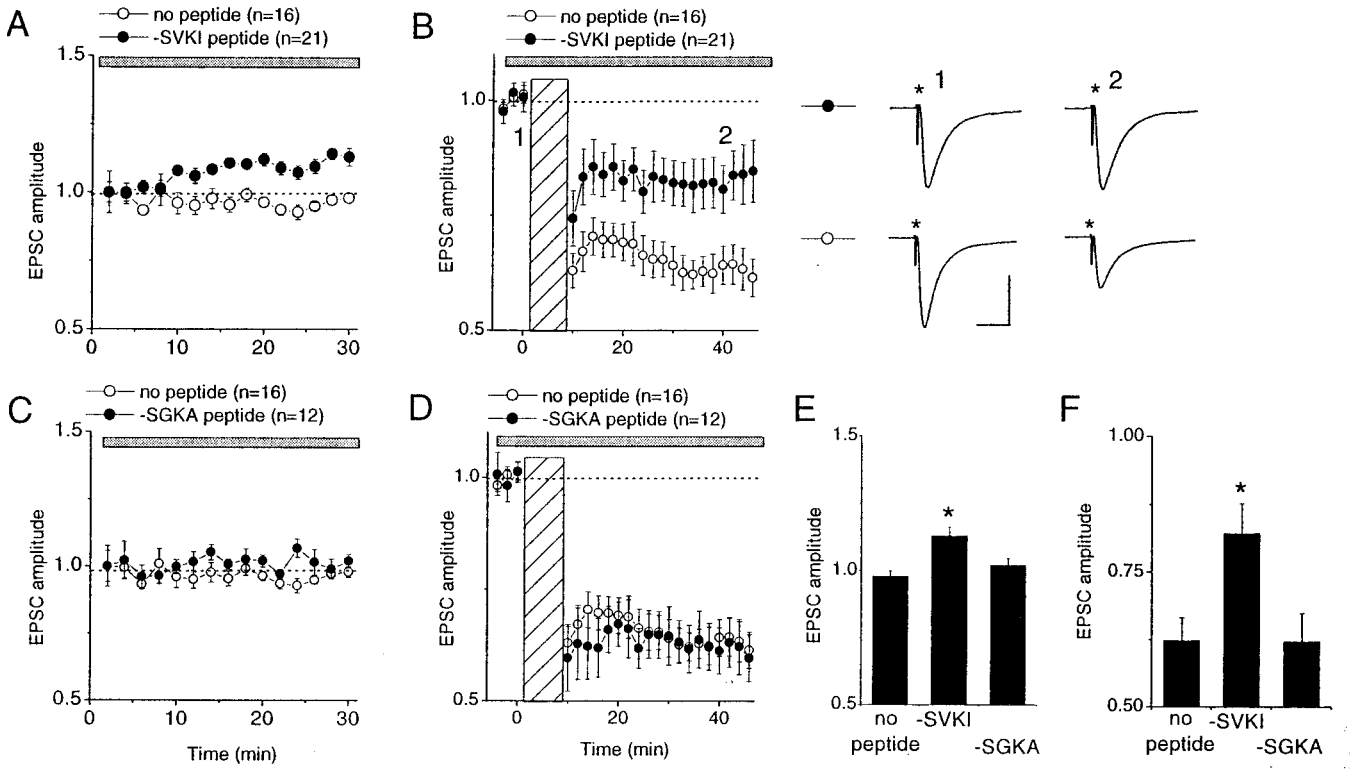

Fig. 3. Intracellular perfusion of GluR2/3 C-terminal peptides that disrupt GluR2–GRIP/PICK1 interaction affects the basal synaptic transmission and inhibits expression of LTD. (*A*) Effect of GluR2 C-terminal peptide KKEGYNVYGIESVKI (−SVKI, 200 μM, ●) on the basal synaptic transmission compared with the no peptide control (○). (*B*) After the baseline stabilized, LTD was induced by pairing in the absence or presence of −SVKI peptide (200 μM). (*Insets*) Top traces are the average of 20 EPSCs at 4 min before pairing (1) and at 30 min after pairing (2) in the presence of peptide. Bottom traces are in the absence of peptide. * indicates stimulus artifact, which is cut off. (Scale bar, 150 pA and 25 ms.) The LTD experiments in *B* were continuations of the baseline recordings in *A*. (*C*) Effect of the control peptide KKEGYNVYGIESGKA (−S GK A, 200 μM, ●) on the basal synaptic transmission. (*D*) Effect of −SGKA peptide on LTD induction compared with no peptide experiments. The LTD experiments in *D* were continuations of the baseline recordings in *C*. (*E*) The amplitude histogram of basal EPSCs at 30 min after perfusion. * indicates the significance at $\alpha = 0.05$ (ANOVA, single factor). (*F*) The amplitude histogram of LTD at 30 min after pairing. * indicates the group whose mean is significantly different from those of controls, no peptide or −SGKA peptide (Dunnett's test, $\alpha = 0.05$). Gray bar indicates the period of postsynaptic application of peptides. Hatched bar indicates the period of pairing for LTD induction.

Perfusion of −EVKI peptide also increased the basal synaptic strength (Fig. 5 *C* and *E*, 117 ± 4%, $P = 0.019$, $F = 6.5$) and inhibited LTD induction by 62% (Fig. 5 *D* and *F*, 86 ± 7%; ANOVA: $F = 6.79$, $P < 0.02$). Coperfusion of the −SVKI and −EVKI peptides increased the basal synaptic transmission by 37 ± 3% at 20 min after application but did not have an additive inhibitory effect on LTD induction (62% inhibition, $n = 9$, data not shown). These results demonstrate that synthetic peptides that block PICK1 interaction with GluR2/3 increase the basal synaptic strength and inhibit LTD expression in hippocampus.

Discussion

The present study shows that AMPA receptor internalization during the expression of hippocampal LTD may be regulated by Ser-880 phosphorylation and GluR2 interaction with PDZ domain-containing proteins. We found that phosphorylation of Ser-880 was increased after the induction of NMDA receptor-dependent LTD. Moreover, the LTD-induced increase in Ser-880 phosphorylation was blocked by pretreatment of hippocampal slices with the NMDA receptor antagonist AP5 or the protein phosphatase inhibitor okadaic acid, two agents known to specifically inhibit LTD. These results suggest that the LFS-induced increase in Ser-880 phosphorylation only occurs under conditions that are permissive for NMDA receptor-dependent LTD induction. However, okadaic acid treatment increases the basal level of Ser-880 phosphorylation, making it difficult to rule out the possibility that okadaic acid occludes LTD expression rather than blocks its induction. Although many studies have shown that LTD is associated with the activation of protein phosphatases (19, 32, 33), our results suggest that LTD induction modulates the activity of protein kinases or phosphatases that increase the phosphorylation state of GluR2 at Ser-880. However, the identity of the kinases or phosphatases is not clear. Although PKC may be one candidate kinase (28, 35, 36), our present results combined with previous studies (31, 37) suggest PKC is not involved and that another kinase may phosphorylate Ser-880 after LTD induction. Alternatively, a specific protein phosphatase that dephosphorylates Ser-880 may be inhibited during LTD. Future studies will be required to further characterize the signal transduction pathway and the relevant kinases or phosphatases involved.

Because Ser-880 phosphorylation modulates GluR2 interaction with GRIP1 and PICK1, the LTD-induced increase in Ser-880 phosphorylation suggests that receptor internalization during LTD might be regulated by the interaction of GluR2 with PDZ domain-containing proteins. Indeed, intracellular postsynaptic perfusion of GluR2 synthetic peptides that should specifically inhibit the endogenous GluR2–PICK1 interaction produced significant increases in the basal excitatory synaptic transmission and inhibited LTD expression, providing strong evidence that GluR2/3–PICK1 interaction regulates the constitutive and LTD-induced internalization of AMPA receptors. These results are similar to recent studies on cerebellar LTD, which show that both PKC phosphorylation of GluR2 and the

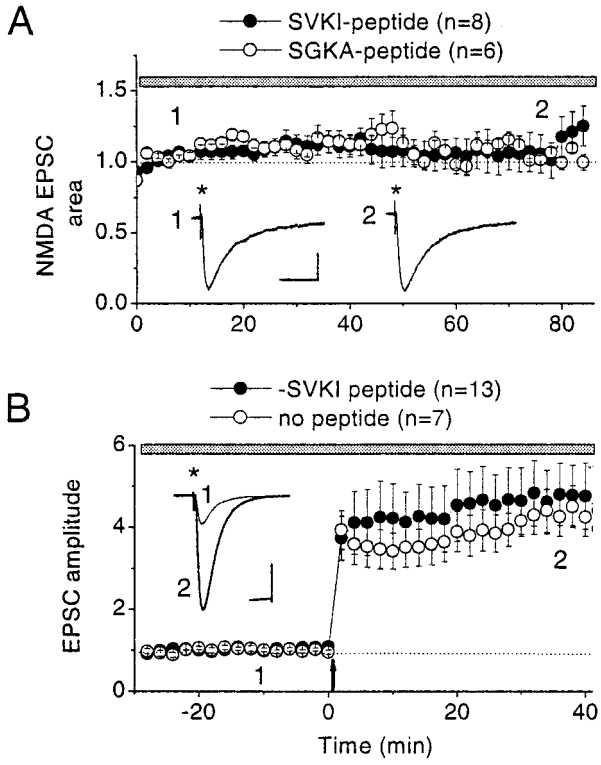

Fig. 4. Effects of GluR2 C-terminal peptide on NMDA receptor-mediated EPSC and pairing-induced LTP. (*A*) Effect of −SVKI peptide on NMDA receptor-mediated EPSC. As a control, −SGKA peptide was used. (*Insets*) The average traces of 20 traces from one example experiment measured at 8 (designated 1) and 70 min (designated 2) after whole-cell recording. The average EPSC at 30 min after whole cell (or right before pairing) was 110 ± 1% compared with the initial 2-min average EPSC. (Scale bar, 25 pA and 50 ms.) (*B*) Effect of −SVKI peptide on NMDA receptor-dependent LTP induced by pairing. Arrow indicates the time of pairing. (Scale bar, 160 pA and 15 ms.) Gray bar indicates the period of postsynaptic application of peptides. * indicates stimulus artifact, which is cut off.

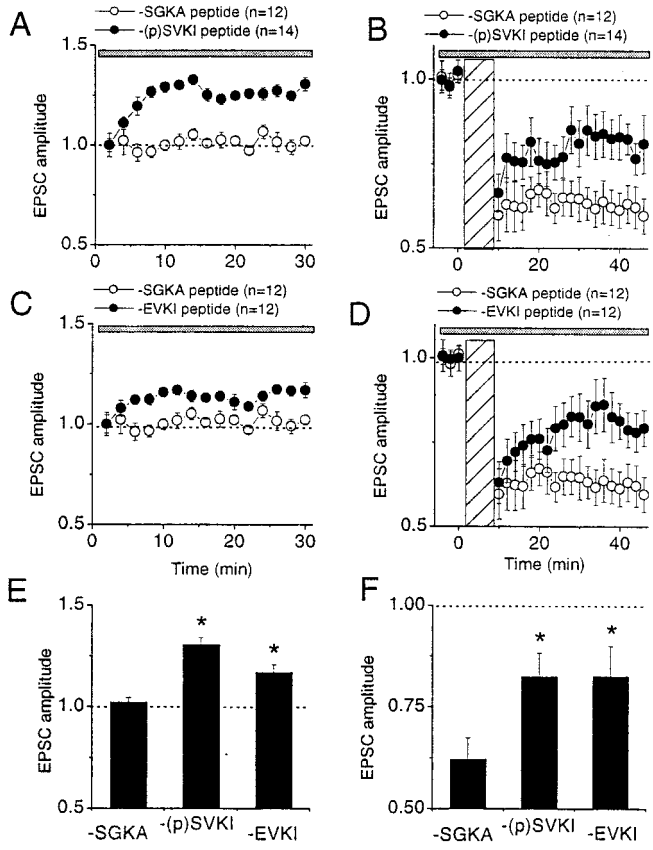

Fig. 5. Intracellular perfusion of GluR2/3 C-terminal peptides that selectively disrupt GluR2–PICK1 interaction affects the basal synaptic transmission and inhibits expression of LTD. (*A*) Effect of the phosphopeptide KKEGYNVYGIES-(PO_4)-VKI (−(p)SVKI, 200 μM, ●) on the basal synaptic transmission. (*B*) Effect of −(p)SVKI peptide on LTD induction compared with the control peptide. The LTD experiments in *B* were continuations of the baseline recordings in *A*. (*C*) Effect of KKEGYNVYGIESVKI peptide (−EVKI, 200 μM, ●) on the basal synaptic transmission. (*D*) Effect of −EVKI peptide on LTD induction compared with that of the control peptide. The LTD experiments in *D* were continuations of the baseline recordings in *C*. (*E*) The amplitude histogram of basal EPSCs at 30 min after perfusion. * indicates the significance at $\alpha = 0.05$ (ANOVA, single factor). (*F*) The amplitude histogram of LTD at 30 min after pairing. * indicates the group whose mean is significantly different from those of controls, no peptide, or −SGKA peptide (Dunnett's test, $\alpha = 0.05$). Gray bar indicates the period of postsynaptic application of peptides. Hatched bar indicates the period of pairing for LTD induction.

regulation of GluR2 interaction with GRIP1, GRIP2/ABP, and PICK1 are critical for cerebellar LTD induction (30, 36). Although perfusion of the GluR2 peptides completely blocked cerebellar LTD (30) compared with the partial block of hippocampal LTD, these results suggest that hippocampal and cerebellar LTD may share a common mechanism involving phosphorylation of GluR2 C termini and modulation of PDZ domain-mediated interaction with GRIP1, GRIP2/ABP, and PICK1. These results, taken together, suggest a model whereby the differential regulation of GluR2/3 with GRIP1, GRIP2/ABP, and PICK1 by protein phosphorylation modulates AMPA receptor internalization during LTD. Phosphorylation of GluR2 during LTD induction may disrupt the interaction of GluR2 with GRIP1, GRIP2/ABP at the synaptic plasma membrane, allowing AMPA receptors to be internalized where they may be stabilized by interaction with PICK1 (Fig. 6*A*). Alternatively, PICK1 could play a more active role in promoting the internalization of GluR2 (Fig. 6*B*).

Our model is consistent with recent studies by Osten *et al.* (38), which have shown that the interaction of the C-terminal PDZ ligand of GluR2 with GRIP1, GRIP2/ABP is important for the accumulation of GluR2 at synapses. In these experiments, Osten *et al.* showed that mutants of GluR2 that do not bind GRIP1, GRIP2/ABP, or PICK1 do not accumulate at synapses; moreover, mutants that do not bind GRIP1, GRIP2/ABP but do bind PICK1 do not accumulate at synapses. These results suggest that the interaction of GluR2 C-terminal with GRIP1, GRIP2/ABP but not PICK1 is important to stabilize the surface expression of GluR2 and are consistent with our model that GluR2 interaction with GRIP1, GRIP2/ABP is important for the stability of the surface AMPA receptors. However, recent studies by Daw *et al.* (39), analyzing the effect of perfusion of GluR2 C-terminal peptides on LTD induction, are somewhat inconsistent with our results. Similar to our findings, Daw *et al.* observed that perfusion of the −SVKI peptide increased the basal synaptic transmission and inhibited LTD in hippocampal slices. In contrast to our results, however, they found that the −EVKI peptide had no effect. These results led them to propose a model where the interaction of GluR2 with GRIP1, GRIP2/ABP stabilizes the intracellular pool of GluR2. Although it is difficult to explain the difference in the observed effects of the peptides in the two studies, several differences in the experimental procedures, such as temperature or the age of rats, may be responsible for the different results. For

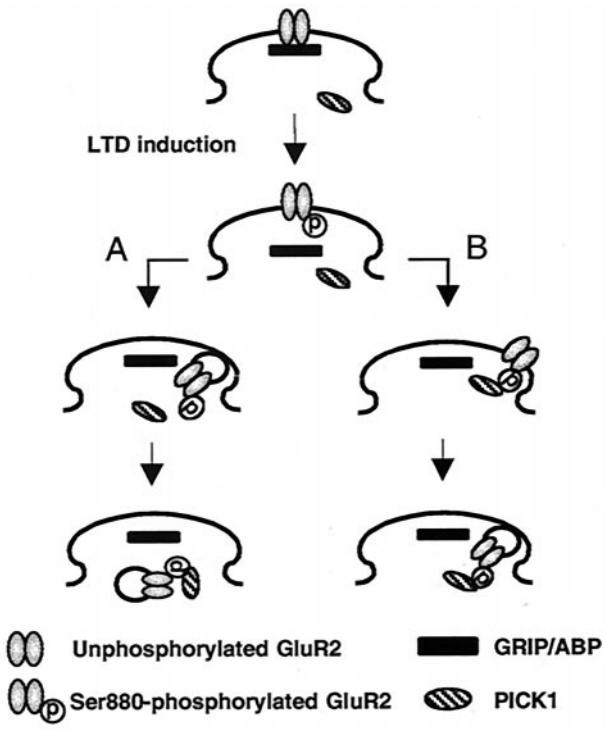

Fig. 6. Model for the regulation of AMPA receptor internalization by GluR2 C-terminal phosphorylation and GRIP1, GRIP2/ABP, and PICK1 interaction. In the basal state, AMPA receptors containing GluR2 subunits at the postsynaptic membrane are stable through their interaction with GRIP1, GRIP2/ABP. Hippocampal LTD induction increases the phosphorylation of GluR2 at Ser-880, which results in the disruption of the interaction of GluR2 with GRIP1, GRIP2/ABP, allowing AMPA receptors to be internalized. PICK1 then binds to and stabilizes the internalized receptors (*A*). Alternatively, PICK1 may play a more active role in triggering internalization (*B*).

example, Daw *et al.* (39) reported that the kinetics of the effect of the −SVKI peptide on the basal synaptic transmission depended on temperature. Our experiments were done at 35°C, whereas Daw *et al.* (39) performed their experiments at 30°C. Further studies are required to resolve the differences in the results. However, both studies indicate that the interaction of GluR2 C terminal with PDZ domain-containing proteins is important in the regulation of basal synaptic transmission and LTD.

The GluR2/3 interaction with GRIP1, GRIP2/ABP but not PICK1 has shown to be involved in the potentiation of AMPA receptors in dorsal horn sensory neurons in the spinal cord (40). Intracellular perfusion of GluR2 C-terminal peptides that disrupt GluR2 interaction with GRIP1, GRIP2/ABP in these neurons blocks the 5-hydroxytryptamine-induced potentiation of AMPA receptor responses, whereas a peptide that selectively binds to PICK1 but not GRIP1, GRIP2/ABP had no effect (40). This result is consistent with our model (Fig. 6) that the interaction of GluR2/3 with GRIP1, GRIP2/ABP stabilizes the synaptic plasma membrane AMPA receptors, whereas the GluR2/3 interaction with PICK1 stabilizes the intracellular pools of receptor. Finally, recent studies have shown that the interaction of the GluR1 subunit with PDZ domain-containing proteins may mediate the regulated insertion of AMPA receptor into synapse during hippocampal LTP (27). These studies, combined with our results, suggest that the PDZ domain-medicated interaction of AMPA receptor may play a critical role in regulating the surface expression of AMPA receptors at synapses and modulating the efficiency of excitatory synaptic transmission during many forms of synaptic plasticity.

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