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# Activation of Postsynaptic $\text{Ca}^{2+}$ Stores Modulates Glutamate Receptor Cycling in Hippocampal Neurons

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**Maher, Brady J., Roger L. MacKinnon II, Jihong Bai, Edwin R. Chapman, and Paul T. Kelly.** Activation of postsynaptic  $\text{Ca}^{2+}$  stores modulates glutamate receptor cycling in hippocampal neurons. *J Neurophysiol* 93: 178–188, 2005; doi:10.1152/jn.00651.2004. We show that activation of postsynaptic inositol 1,4,5-tris-phosphate receptors ( $\text{IP}_3\text{Rs}$ ) with the  $\text{IP}_3\text{R}$  agonist adenophostin A (AdA) produces large increases in AMPA receptor (AMPA) excitatory postsynaptic current (EPSC) amplitudes at hippocampal CA1 synapses. Co-perfusion of the  $\text{Ca}^{2+}$  chelator bis-(*o*-aminophenoxy)-*N,N,N',N'*-tetraacetic acid strongly inhibited AdA-enhanced increases in EPSC amplitudes. We examined the role of AMPAR insertion/anchoring in basal synaptic transmission. Perfusion of an inhibitor of synaptotagmin-soluble n-ethylmaleimide-sensitive factor attachment protein (SNAP) receptor SNARE-mediated exocytosis depressed basal EPSC amplitudes, whereas a peptide that inhibits GluR2/3 interactions with postsynaptic density-95 (PDZ) domain proteins glutamate receptor interacting protein (GRIP)/protein interacting with C-kinase-1 (PICK1) enhanced basal synaptic transmission. These results suggest that constitutive trafficking and anchoring of AMPARs help maintain basal synaptic transmission. The regulation of postsynaptic AMPAR trafficking involves synaptotagmin-SNARE-mediated vesicle exocytosis and interactions between AMPARs and the PDZ domains in GRIP/PICK1. We show that inhibitors of synaptotagmin-SNARE-mediated exocytosis, or interactions between AMPARs and GRIP/PICK1, attenuated AdA-enhanced increases in EPSC amplitudes. These results suggest that  $\text{IP}_3\text{R}$ -mediated  $\text{Ca}^{2+}$  release can enhance AMPAR EPSC amplitudes through mechanisms that involve AMPAR-PDZ interactions and/or synaptotagmin-SNARE-mediated receptor trafficking.

## INTRODUCTION

AMPA receptors (AMPARs) are primarily involved in fast excitatory synaptic transmission, and their modulation is thought to be critical for synaptic plasticity (Ehlers 2000; Heynen et al. 2000; Lin et al. 2000; Luscher et al. 1999; Shi et al. 2001). Many examples of synaptic plasticity rely on postsynaptic  $\text{Ca}^{2+}$  signaling pathways that increase or decrease synaptic transmission and are thought to result from changes in the number of postsynaptic AMPARs (Beattie et al. 2000; Linden 2001; Luthi et al. 1999; Man et al. 2000). The C-termini of AMPAR GluR2/3 subunits interact with the Discs large/zona occludens-1 (PDZ) domains of glutamate receptor interacting protein (GRIP1/2) or AMPA receptor-binding protein (ABP) (two homologous proteins referred to as GRIP/ABP), and protein interacting with C-kinase-1 (PICK1) (Chung et al. 2000; Dong et al. 1997, 1999; Sheng 1996;

Srivastava et al. 1998; Wyszynski et al. 1999; Xia et al. 1999). Interactions between PDZ domains and AMPARs are thought to regulate the anchoring, insertion and internalization of synaptic receptors (Daw et al. 2000; Kim et al. 2001; Li et al. 1999). The C-terminal amino acids SVKI of GluR2/3 bind specifically to PDZ domains, and these interactions regulate AMPAR trafficking during long-term potentiation/depression (LTP/LTD; Daw et al. 2000; Kim et al. 2001; Li et al. 1999; Shi et al. 2001). Soluble n-ethylmaleimide-sensitive factor attachment protein (SNAP) receptor SNARE complexes may also regulate the trafficking of synaptic AMPARs via vesicular exocytosis (Beattie et al. 2000; Ehlers 2000; Lin et al. 2000; Lu et al. 2001; Luscher et al. 1999; Man et al. 2000).

The exocytosis of vesicles at the cell surface involves interactions between SNARE proteins that mediate vesicle fusion with target membranes. Synaptotagmin I is a major  $\text{Ca}^{2+}$  sensor that triggers SNARE proteins to fuse vesicle lipids with target membrane lipids for secretion (Chapman 2002). Synaptotagmin I contains two regulatory C2 domains (Perin et al. 1990), and its C2A domain penetrates lipid bilayers in response to  $\text{Ca}^{2+}$  (Davis et al. 1999). C2A domains also interact with syntaxin and SNAP-25 during SNARE complex assembly (Augustine 2001). Earles et al. (2001) developed a recombinant protein with two C2A domains (C2A-C2A) that binds to SNARE complexes and rapidly inhibits exocytosis. Other results indicate that dendritic synaptotagmin I undergoes  $\text{Ca}^{2+}$ -dependent translocation, suggesting a postsynaptic role for synaptotagmin I in  $\text{Ca}^{2+}$ -regulated, SNARE-catalyzed vesicle fusion (Schwab et al. 2001).

We examined the effects of postsynaptic  $\text{Ca}^{2+}$  release on synaptic transmission in CA1 pyramidal neurons. We show that postsynaptic perfusion of adenophostin A [AdA, potent inositol 1,4,5-tris-phosphate receptor ( $\text{IP}_3\text{R}$ ) agonist] significantly increases excitatory postsynaptic current (EPSC) amplitudes, which are blocked by the  $\text{Ca}^{2+}$  chelator bis-(*o*-aminophenoxy)-*N,N,N',N'*-tetraacetic acid (BAPTA). These increases are also dependent on  $\text{Ca}^{2+}$ /calmodulin-dependent protein kinase II (CaM-KII) and/or PKC activities (J.-H. Wang, R. L. MacKinnon II, B. J. Maher and P. Kelly, unpublished data), two kinases implicated in regulating AMPAR trafficking (Daw et al. 2000; Shi et al. 2001). The studies herein examined if intracellular  $\text{Ca}^{2+}$  release plays a role in regulating AMPAR insertion and/or anchoring in postsynaptic neurons. We investigated the role of AMPAR-PDZ domain interactions in postsynaptic neurons by co-perfusing AdA with peptides

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corresponding to the C-termini of GluR2/3. These manipulations significantly attenuated increases in EPSC amplitudes observed with AdA alone, suggesting that PDZ domain interactions between GluR2/3 and GRIP/ABP/PICK1 can modulate synaptic plasticity. We also observed that co-perfusion of C2A–C2A, an inhibitor that disrupts synaptotagmin-SNARE-dependent processes, significantly attenuated AdA-enhanced increases in EPSC amplitudes. Last, we saw that postsynaptic perfusion of peptides that disrupt PDZ domain interactions, or synaptotagmin-SNARE-mediated vesicular exocytosis, altered basal synaptic transmission but in opposite directions. Perfusion of peptides that disrupt GluR2/3 binding to GRIP/ABP increased basal synaptic transmission, whereas C2A–C2A caused a decrease in transmission. These results suggest that AMPAR trafficking and/or stabilization are important for maintaining basal synaptic transmission.

## METHODS

### Brain slices and neuron selection

Transverse hippocampal slices (400  $\mu$ M) were prepared from Harlan Sprague-Dawley rats [postnatal days (PND) 14–21]. Animals were anesthetized with isoflurane and decapitated, and brains were quickly removed. Brains were dissected while immersed in ice-cold (4°C) artificial cerebral spinal fluid (ACSF) containing (in mM) 124 NaCl, 3 KCl, 1.3 NaH<sub>2</sub>PO<sub>4</sub>, 26 NaHCO<sub>3</sub>, 4 MgCl<sub>2</sub>, 0.5 CaCl<sub>2</sub>, 10 dextrose, and 10 HEPES (310–315 mOsmol), and gassed with 95% O<sub>2</sub>–5% CO<sub>2</sub> (pH 7.35). Tissue blocks containing the hippocampus and overlying cerebral cortex were sliced using a Vibratome. Slices were then transferred to oxygenated “standard” ACSF (1.3 mM MgCl<sub>2</sub>, 2.4 mM CaCl<sub>2</sub> plus other salts/HEPES at the preceding concentrations) at 25°C for 1–2 h before being transferred to a submersion chamber (300  $\mu$ l volume) for recordings. All whole cell recordings were done at 31°C at a perfusion rate of 1–2 ml/min. CA1 pyramidal neurons were selected on the basis of morphological appearance using an infrared DIC microscope ( $\times$ 40 water-immersion objective). Somas of CA1 pyramidal neurons in stratum pyramidale/oriens were triangular or olive in shape with one major apical dendrite and a few basal dendrites. All experiments using animals were conducted according to an IACUC-approved protocol (University of Kansas).

### Electrophysiology

EPSCs were recorded under whole cell patch-clamp conditions. Bipolar tungsten electrodes (12 M $\Omega$ ) were used to stimulate Schaffer collateral/commissural (S/C) axons and evoke population EPSCs in CA1 neurons at a stimulus frequency of 0.1 Hz. Patch pipettes (4–7 M $\Omega$ ) were used to perfuse modulators of intracellular signaling pathways during whole cell recordings. Standard pipette solution contained (in mM) 155 K-gluconate, 4 NaCl, 10 HEPES, 0.5 EGTA, 4 Mg-ATP, and 0.5 tris-GTP (310–315 mOsmol), and was filtered with a 0.1- $\mu$ m centrifuge filter (Millipore) before use. Where specified, pipette solutions also contained 1  $\mu$ M AdA (IP<sub>3</sub>R agonist), 100  $\mu$ M AMPAR C-terminal peptides (NVYIGIE-SVKI, -SVKE, -SVAI, or -EVKI, see RESULTS), or 20  $\mu$ M synaptotagmin I reagents (C2A–C2A, MC2A–MC2A, or C2A). Recombinant synaptotagmin I reagents were expressed and purified as described in Earles et al. (2001) and then extensively dialyzed at 4°C in dialysis cassettes (Pierce) against standard pipette solution (minus ATP and GTP). To facilitate obtaining high-resistance seals, patch pipette tips were filled with standard solution and backfilled with AMPAR C-terminal peptides or synaptotagmin reagents in standard solution. EPSCs were recorded with an Axopatch-1D amplifier in voltage-clamp mode at –75 mV. Output bandwidth was filtered at 1 kHz. Series resistances were

monitored throughout all voltage-clamp recordings by measuring responses to 5 mV (50 ms) pulses.

### Data analysis

Initial baseline values of synaptic transmission (defined as 100%) were obtained by averaging the peak amplitudes of the first five EPSCs after stable whole cell configuration was obtained and correct stimulus intensity (i.e., 75–150 pA EPSCs) was achieved. Synaptic responses are represented as means  $\pm$  SE. The peak amplitude of  $I_{in}$  was measured throughout experiments and only neurons whose amplitudes remained within 20% of initial baseline levels were considered acceptable for analysis. Data were obtained with pClamp 7.0 or 8.0 and analyzed using Clampfit. *t*-tests were used for comparisons between experimental groups from stable recordings obtained 25 min after whole cell access was achieved. Waveforms displayed in all figures are averaged from five consecutive synaptic responses selected from a representative experiment.

### Materials

AdA was purchased from Calbiochem; Dr. Morgan Sheng gratefully provided all C-terminal peptides; all other chemicals were reagent grade.

## RESULTS

### Intracellular Ca<sup>2+</sup> release modulates synaptic transmission

One pathway that increases intracellular [Ca<sup>2+</sup>] is the depletion of IP<sub>3</sub>R-mediated Ca<sup>2+</sup> stores (Supattapone et al. 1988). We have shown that perfusion of AdA, a potent IP<sub>3</sub>R agonist (Takahashi et al. 1994), into postsynaptic CA1 neurons greatly increased AMPAR-mediated EPSC amplitudes. Postsynaptic perfusion of AdA (1  $\mu$ M) dramatically increased EPSC amplitudes in a representative CA1 neuron (Fig. 1A, ●). When all neurons were averaged, AdA perfusion substantially increased EPSC amplitudes [373  $\pm$  46% at 25 min ( $n$  = 27);  $P$  < 0.0001, Fig. 1B, ●] compared with control neurons [100  $\pm$  6% at 25 min ( $n$  = 39), Fig. 1B, ■]. To examine the role of intracellular Ca<sup>2+</sup> release on increases in AMPAR-mediated EPSC amplitudes, we co-perfused AdA with the fast-acting Ca<sup>2+</sup> chelator BAPTA (5 mM). Co-perfusion of BAPTA with AdA virtually eliminated increases in EPSC amplitudes in a representative neuron (Fig. 1A, ○). When all neurons co-perfused with AdA plus BAPTA were averaged, BAPTA strongly inhibited AdA-enhanced increases in EPSC amplitudes [97  $\pm$  14% at 25 min ( $n$  = 7);  $P$  < 0.01 Fig. 1B, ○] compared with interleaved neurons perfused with AdA alone [406  $\pm$  92% at 25 min ( $n$  = 8); data not shown]. No significant differences were observed between group data for co-perfusion of BAPTA with AdA compared with control intracellular solution containing BAPTA alone [Fig. 2 ( $n$  = 7),  $P$  = 0.42]. These data suggest that activation of postsynaptic IP<sub>3</sub>Rs with AdA and subsequent intracellular Ca<sup>2+</sup> release increases AMPAR-mediated EPSCs.

### AMPA insertion and anchoring during basal synaptic transmission

The delivery of AMPARs to the postsynaptic membrane during basal transmission is believed to occur by vesicular fusion that requires specific interactions of v-SNAREs with cognate t-SNAREs (Luscher et al. 1999). Luscher et al. (1999)

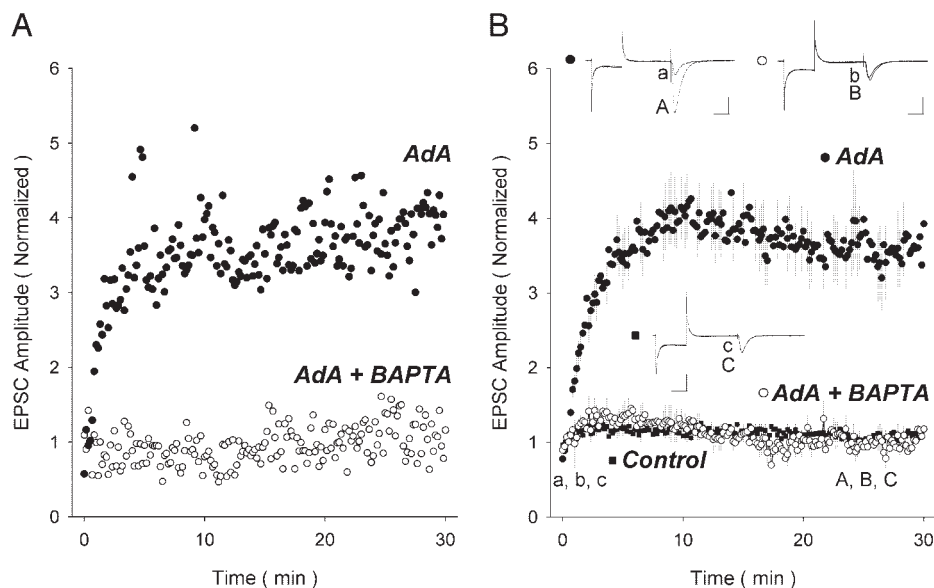


FIG. 1. Postsynaptic perfusion of adenophostin A (AdA) enhances synaptic transmission and requires postsynaptic  $Ca^{2+}$ . *A*: representative experiment for a neuron showing that co-perfusion of BAPTA, (5 mM) with AdA (1  $\mu$ M;  $\circ$ ), significantly attenuates increases in excitatory postsynaptic current (EPSC) amplitudes compared with an interleaved neuron perfused with AdA alone ( $\bullet$ ). *B*: group data showing that co-perfusion of BAPTA (5 mM) plus AdA (1  $\mu$ M;  $\circ$ ) significantly attenuates increases in EPSC amplitudes compared with neurons perfused with AdA alone ( $\bullet$ ); this panel also shows group data for control neurons ( $\blacksquare$ ). In all figures, insets show averaged waveforms of the 1st 5 EPSCs recorded during the initial minute of a representative experiment vs. averaged waveforms of 5 consecutive EPSCs recorded at 25 min. Data points represent the average EPSC amplitude  $\pm$  SE for group recordings for each experimental condition. Calibration bars are 100 pA and 25 ms for all waveforms in all figures.

used botulinum toxin type B to inhibit exocytosis in CA1 neurons. This toxin inhibits exocytosis by proteolysis of the v-SNARE synaptobrevin (VAMP) (Schiavo et al. 1992) prior to the formation in v/t-SNARE fusion complexes. We examined the involvement of postsynaptic AMPAR insertion during basal synaptic transmission by perfusing a protein reagent called C2A–C2A. C2A–C2A ( $M_r = 35$  K) is derived from synaptotagmin 1, a vesicle associated protein that interacts with SNAREs, and has tandem  $Ca^{2+}$  binding domains that regulate exocytosis (Chapman 2002). C2A–C2A binds strongly to SNAREs, displaces native synaptotagmin from preformed SNARE complexes, and rapidly inhibits exocytosis (Earles et al. 2001). Thus C2A–C2A acts at a different step and interacts with different components of the exocytotic machinery compared with type B botulinum toxin.

Postsynaptic perfusion of C2A–C2A into a representative CA1 neuron caused a gradual decrease of EPSC amplitudes relative to baseline values of 100% [ $58 \pm 4\%$  at 25 min; Fig. 3A,  $\bullet$ ]. When all neurons perfused with C2A–C2A were averaged, a consistent decrease of EPSC amplitudes was observed relative to baseline [ $40 \pm 7\%$  at 25 min ( $n = 7$ );  $P < 0.01$ , Fig. 4A,  $\circ$ ] compared with interleaved controls [ $103 \pm$

19% at 25 min ( $n = 7$ ); Fig. 4A,  $\bullet$ ]. To examine C2A–C2A's specificity, interleaved experiments using two different synaptotagmin 1 control reagents were performed. The first control reagent is a mutated version of C2A–C2A (MC2A–MC2A) that has two aspartate residues substituted for asparagines (D230N, D232N) in the  $Ca^{2+}$  binding region of each C2A domain. MC2A–MC2A has <30% of the inhibitory effect on exocytosis compared with C2A–C2A (Earles et al. 2001). The second control reagent is a single C2A domain (C2A) that still binds  $Ca^{2+}$  but has little effect on vesicular exocytosis (Earles et al. 2001). Perfusion of MC2A–MC2A did not significantly alter basal EPSC amplitudes [ $100 \pm 13\%$  at 25 min ( $n = 6$ );  $P = 0.89$ , Fig. 4B]. Likewise, postsynaptic perfusion of C2A had no significant effect on basal EPSC amplitudes relative to baseline [ $90 \pm 19\%$  at 25 min ( $n = 8$ );  $P = 0.64$ , Fig. 4C]. Our results using C2A indicate that C2A–C2A's ability to inhibit basal transmission is not simply due to its  $Ca^{2+}$  binding property. Together, these results suggest the presence of a pool of constitutively cycling AMPARs that require synaptotagmin-SNARE-mediated exocytosis to maintain stable basal synaptic transmission.

Can the anchoring of postsynaptic AMPARs help stabilize basal synaptic transmission? Peptides containing the sequence SVKI disrupt the binding of AMPARs to the PDZ domain-containing proteins GRIP1/ABP and PICK1, proteins that have been implicated in regulating the anchoring/stabilization of AMPARs during the induction of LTD (Chung et al. 2000; Daw et al. 2000; Kim et al. 2001; Li et al. 1999; Shi et al. 2001). We tested the role of AMPAR anchoring to PDZ domain-containing proteins during basal synaptic transmission by perfusing a 10 amino acid peptide containing SVKI into CA1 neurons while monitoring evoked EPSCs. Perfusion of SVKI produced small but not highly significant increases in EPSC amplitudes [ $138 \pm 25\%$  at 25 min ( $n = 12$ );  $P = 0.156$ , Fig. 5A,  $\circ$ ] compared with interleaved controls [ $104 \pm 8\%$  at 25 min ( $n = 16$ ); Fig. 5A,  $\bullet$ ]. Changing the C-terminal isoleucine of SVKI to glutamate (i.e., SVKE) completely abolishes the peptide's ability to interact with PDZ domains in GRIP/ABP/PICK1. SVKE therefore has been used as a control peptide (Daw et al. 2000; Li et al. 1999). Postsynaptic perfu-

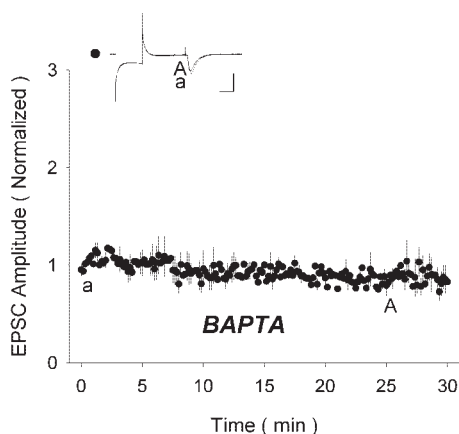


FIG. 2. Postsynaptic perfusion of BAPTA does not alter basal synaptic transmission. Group data showing that perfusion of BAPTA (5 mM) does not alter basal EPSC amplitudes.

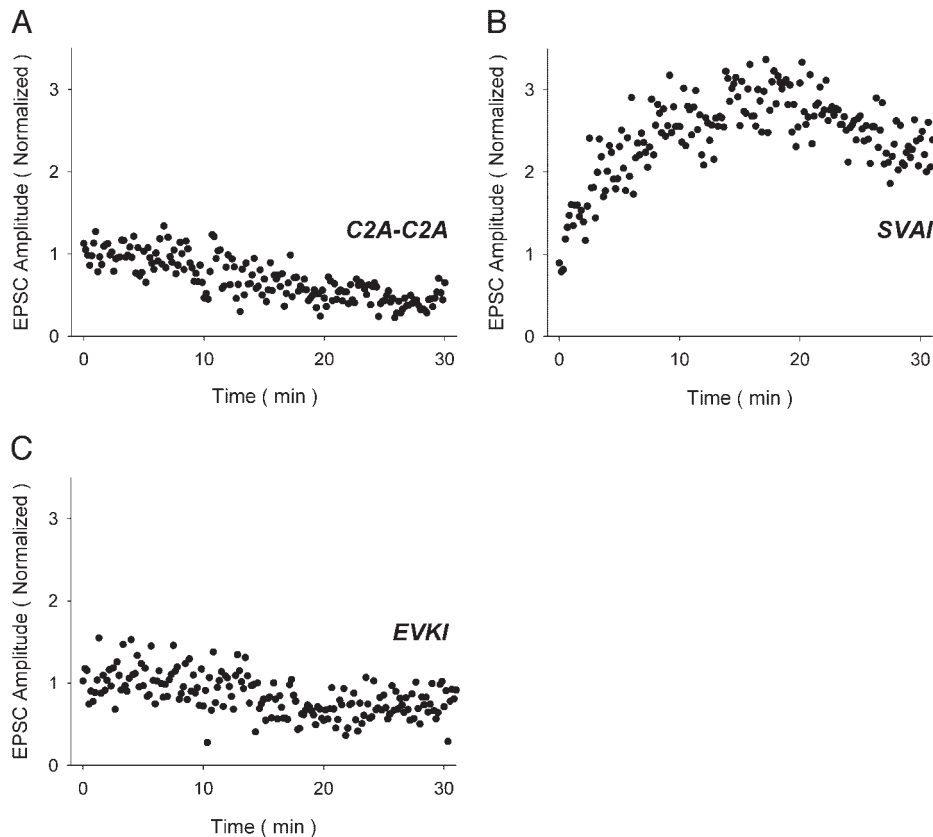


FIG. 3. Inhibiting postsynaptic SNARE-dependent exocytosis or PDZ domain interactions modulates basal synaptic transmission. *A*: representative experiment for a neuron showing that perfusion of C2A–C2A (20  $\mu$ M) depresses basal EPSC amplitudes. *B*: representative experiment for a neuron showing that perfusion of SVAI (100  $\mu$ M) enhances basal EPSC amplitudes. *C*: representative experiment for a neuron showing that perfusion of the control peptide EVKI (100  $\mu$ M) does not alter basal EPSC amplitudes.

sion of SVKE produced no significant change in basal EPSC amplitudes relative to baseline [ $103 \pm 16\%$  at 25 min ( $n = 7$ );  $P = 0.91$ , Fig. 5B].

If PKC is active under basal conditions and phosphorylates S<sup>880</sup>VKI, this would convert it to a selective PICK1-binding peptide. To test this possibility, we used a peptide with the sequence SVAI, which binds both GRIP/ABP (Li et al. 1999) and PICK1 (R. Haganir, personal communication) but cannot be phosphorylated by PKC because changing Lys<sup>882</sup> to Ala disrupts its PKC consensus phosphorylation sequence. Postsynaptic perfusion of SVAI resulted in a large increase in basal synaptic transmission in a representative individual neuron (Fig. 3B). When all neurons perfused with SVAI (Fig. 5C,  $\circ$ ) were compared with interleaved control neurons (Fig. 5C,  $\bullet$ ), SVAI significantly increased basal EPSC amplitudes [ $165 \pm 21\%$  at 25 min, ( $n = 12$ );  $P < 0.01$ ]. These results suggest that disrupting interactions between GluR2/3 and GRIP/ABP and/or PICK1 can enhance basal synaptic transmission.

The specificity of SVKI is broad because it can bind the PDZ domains of GRIP1/ABP as well as PICK1 (Daw et al. 2000; Li et al. 1999) and possibly other unknown PDZ domain-containing proteins. The phosphorylation state of GluR2/3 subunits appears to be important for PDZ domain interactions. PKC phosphorylation of Ser<sup>880</sup> within the GluR2/3 PDZ-ligand prevents GRIP/ABP interactions but does not effect PICK1 interactions (Chung et al. 2000). This phosphorylation effect can be mimicked by changing Ser<sup>880</sup> to glutamic acid (i.e., SVKI to EVKI) (Daw et al. 2000; Kim et al. 2001; Li et al. 1999). EVKI is therefore useful for determining which specific PDZ domain proteins are important for various neuronal sig-

naling pathways because it specifically disrupts PICK1 interactions. Postsynaptic perfusion of EVKI did not influence basal EPSC amplitudes in a representative neuron (Fig. 3C). Likewise, when all neurons perfused with EVKI were averaged, there was no significant change in basal EPSC amplitudes [ $109 \pm 19\%$  at 25 min ( $n = 11$ );  $P = 0.81$ , Fig. 5D,  $\circ$ ] compared with interleaved controls (Fig. 5D,  $\bullet$ ). Together, these results indicate that disrupting interactions between GluR2/3 and GRIP/ABP are primarily responsible for increasing basal synaptic transmission.

*Postsynaptic Ca<sup>2+</sup> release modulates synaptotagmin-SNARE-dependent exocytosis of AMPARs*

Fusion of receptor-containing vesicles with membranes requires the interaction of v-SNAREs with cognate t-SNAREs via Ca<sup>2+</sup>-regulated synaptotagmin-mediated exocytosis (Chapman 2002). To test the hypothesis that intracellular Ca<sup>2+</sup> release may enhance AMPAR trafficking to postsynaptic membranes, we tried to inhibit synaptotagmin-SNARE-dependent exocytosis with C2A–C2A. Co-perfusion of C2A–C2A with AdA strongly attenuated increases in EPSC amplitudes in a representative neuron [Fig. 6A,  $\circ$ ] compared with an interleaved neuron co-perfused with AdA plus the control reagent C2A (Fig. 6A,  $\bullet$ ). Comparison of all neurons co-perfused with AdA plus C2A–C2A showed significant attenuation of increases in EPSC amplitudes [ $153 \pm 53\%$  at 25 min ( $n = 7$ ); Fig. 7A,  $\circ$ ] compared with neurons co-perfused with AdA plus the control reagent C2A [ $341 \pm 67\%$  ( $n = 7$ );  $P = 0.047$ , Fig. 7A,  $\bullet$ ]. To further examine C2A–C2A's specificity, the control reagent MC2A–MC2A (a version of C2A–C2A with its Ca<sup>2+</sup>-

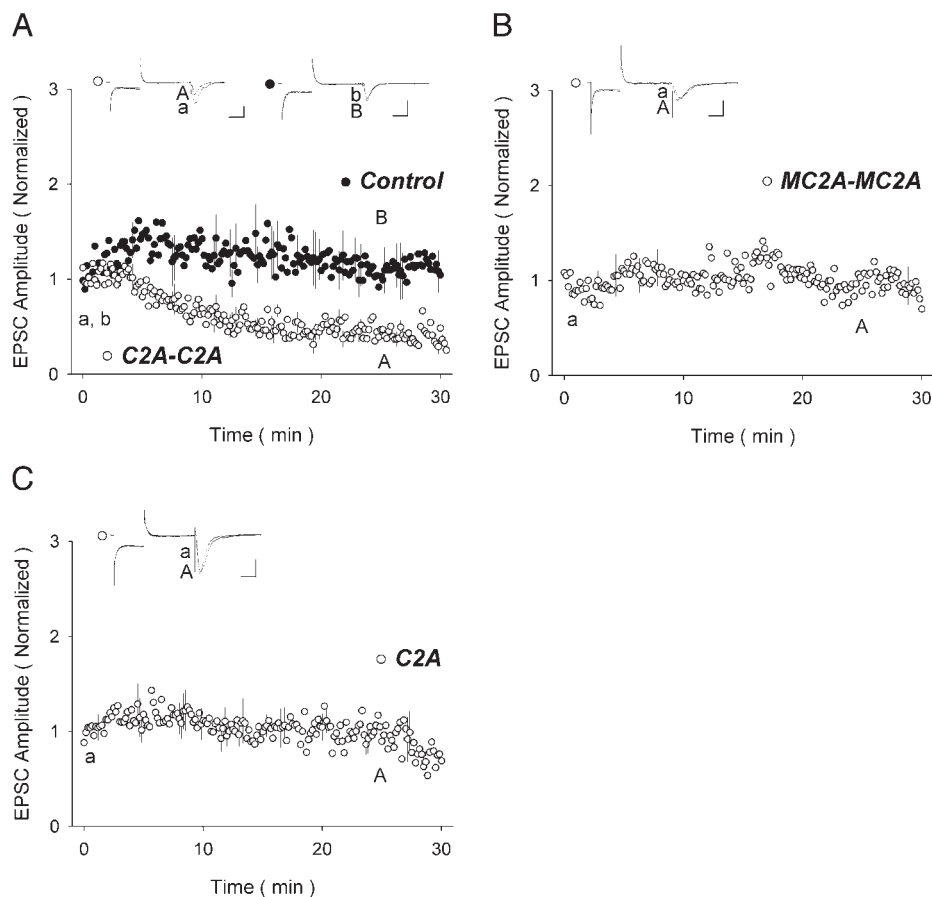


FIG. 4. Inhibiting postsynaptic SNARE-dependent exocytosis depresses basal synaptic transmission. *A*: group data showing that perfusion of C2A–C2A (20  $\mu$ M;  $\circ$ ) produces significant depression of basal EPSC amplitudes compared with interleaved controls ( $\bullet$ ). *B*: group data showing that perfusion of MC2A–MC2A (20  $\mu$ M) does not alter basal EPSC amplitudes. *C*: group data showing that perfusion of C2A (20  $\mu$ M) does not alter basal EPSC amplitudes.

binding domains mutated) was co-perfused with AdA. When all neurons co-perfused with AdA plus MC2A–MC2A were averaged, MC2A–MC2A perfusion had no significant effect on AdA-induced increases in EPSC amplitudes [ $350 \pm 85\%$  ( $n = 9$ );  $P = 0.81$ , Fig. 7*B*]. These results suggest that increases in AMPAR-mediated EPSCs resulting from AdA-enhanced intracellular  $Ca^{2+}$  release are dependent on synaptotagmin-SNARE-mediated insertion of AMPARs into postsynaptic membranes.

#### Postsynaptic $Ca^{2+}$ release modulates AMPAR anchoring

Previous results suggest that postsynaptic perfusion of AdA can convert silent synapses to active synapses (J.-H. Wang, R. L. MacKinnon II, B. J. Maher and P. Kelly, unpublished data). Thus we hypothesized that intracellular  $Ca^{2+}$  release may strengthen the anchoring of existing AMPARs and/or anchor new AMPARs in the postsynaptic membrane. We tested this hypothesis by co-perfusing AdA with the SVKI peptide that disrupts the binding of AMPARs to the PDZ domain containing proteins GRIP1/ABP and PICK1 (Daw et al. 2000; Li et al. 1999). Co-perfusion of AdA with SVKI (100  $\mu$ M) strongly attenuated increases in AMPAR-mediated EPSCs [ $167 \pm 40\%$  at 25 min, ( $n = 7$ );  $P = 0.017$ , Fig. 8*A*,  $\circ$ ] compared with interleaved neurons perfused with AdA alone [ $336 \pm 39\%$  at 25 min, ( $n = 16$ ), Fig. 8*A*,  $\bullet$ ]. Co-perfusion of AdA plus SVKE, a control peptide that doesn't disrupt PDZ domain interactions, had no significant effect on AdA-induced increases in EPSC amplitudes [ $378 \pm 79\%$  at 25 min, ( $n = 8$ );  $P = 0.59$ , Fig. 8*B*,  $\circ$ ]. These results suggest that intracellular

$Ca^{2+}$  release is a trigger for PDZ domain-dependent trafficking and/or anchoring of AMPARs in the postsynaptic membrane.

Because phosphorylation at the C-termini of GluR2/3s by PKC is important for regulating their interactions with PDZ domains, we reasoned that SVKI could inhibit AdA-induced increases in EPSC amplitudes by inhibiting PKC. In contrast to SVKI, SVAI interacts with GRIP/ABP and PICK1 but lacks a consensus PKC phosphorylation motif. SVAI should have a similar effect on AdA-enhanced increases in EPSC amplitudes unless SVKI is simply acting as a competitive substrate for PKC. Co-perfusion of AdA with SVAI (100  $\mu$ M) strongly attenuated increases in EPSC amplitudes in a representative neuron [Fig. 6*B*,  $\circ$ ] compared with an interleaved neuron perfused with just AdA [Fig. 6*B*,  $\bullet$ ]. When comparing all neurons co-perfused with AdA plus SVAI (Fig. 8*C*,  $\circ$ ) to interleaved neurons perfused with AdA alone (Fig. 8*C*,  $\bullet$ ), SVAI perfusion significantly attenuated increases in EPSC amplitude [ $119 \pm 20\%$  at 25 min,  $P < 0.001$  ( $n = 10$ )]. These results suggest that SVKI is not acting as a competitive substrate for PKC but is disrupting PDZ domain interactions between GRIP/ABP/PICK1 and AMPARs that are important for AdA-enhanced increases in EPSC amplitudes.

To examine PICK1's role in AdA's ability to increase AMPAR EPSC amplitudes, we tested a peptide with the motif EVKI that interacts with PICK1 but not GRIP/ABP. Co-perfusion of AdA plus EVKI (100  $\mu$ M) strongly attenuated increases in EPSC amplitudes in a representative neuron (Fig. 6*C*,  $\circ$ ) compared with an interleaved neuron perfused with AdA alone (Fig. 6*C*,  $\bullet$ ). Comparing all neurons co-perfused

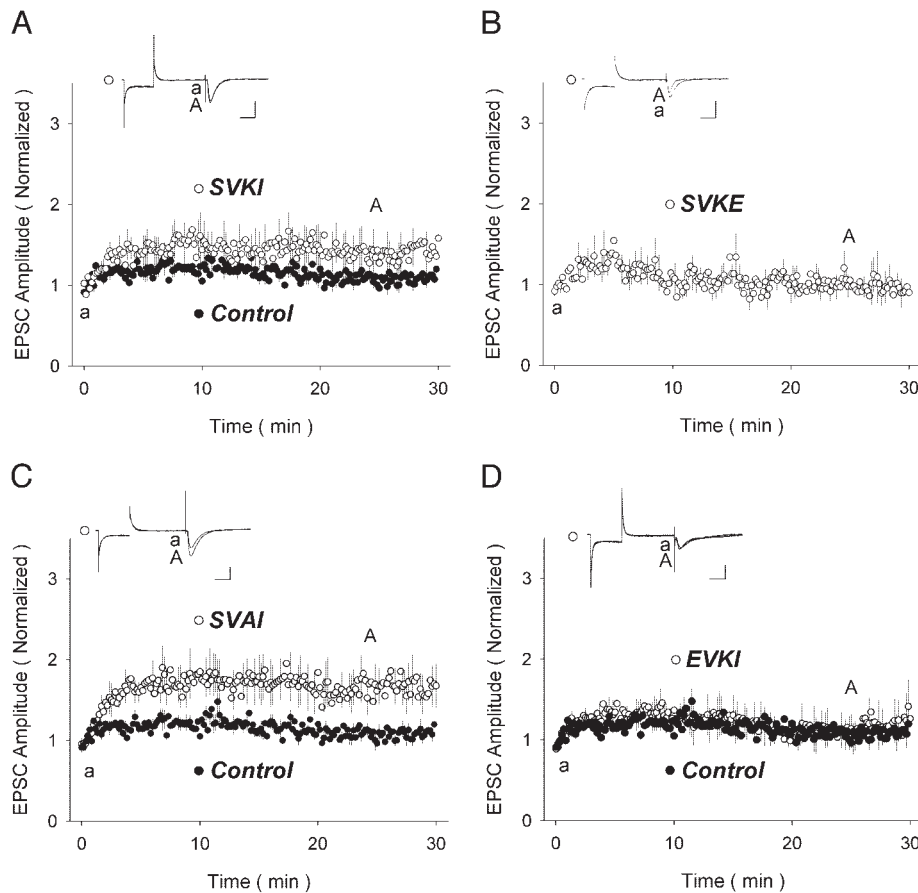


FIG. 5. Inhibiting postsynaptic PDZ domain interactions alters basal synaptic transmission. *A*: group data showing that postsynaptic perfusion of SVKI (100  $\mu$ M;  $\circ$ ) produces a small but statistically insignificant increase in basal EPSC amplitudes compared with interleaved controls ( $\bullet$ ). *B*: group data showing that the control peptide SVKE (100  $\mu$ M) does not alter basal EPSC amplitudes. *C*: group data showing that SVAI (100  $\mu$ M;  $\circ$ ) significantly enhances basal EPSC amplitudes compared with interleaved controls ( $\bullet$ ). *D*: group data showing that EVKI (100  $\mu$ M;  $\circ$ ) does not alter basal EPSC amplitudes compared with interleaved controls ( $\bullet$ ).

with AdA plus EVKI (Fig. 8*D*,  $\circ$ ) to interleaved neurons perfused with AdA alone (Fig. 8*D*,  $\bullet$ ), EVKI perfusion significantly attenuated EPSC amplitudes [ $131 \pm 16\%$  at 25 min, ( $n = 9$ );  $P < 0.001$ ]. These results suggest that interactions between AMPARs and the PDZ domain of PICK1 are important for AdA-induced increases in EPSC amplitudes.

#### DISCUSSION

Stimulating postsynaptic Ca<sup>2+</sup> release with the IP<sub>3</sub>R agonist AdA (Takahashi et al. 1994) greatly increased EPSC amplitudes in CA1 neurons (Fig. 1). This increase is blocked by a calmodulin-binding peptide, and pseudo-substrate inhibitors of PKC or CaM-KII (J.-H. Wang, R. L. MacKinnon II, B. J. Maher and P. Kelly, unpublished data). Postsynaptic perfusion of AdA also converts sub-threshold stimulation that initially failed to evoke EPSCs into supra-threshold stimulation that produced failure-free EPSCs (J.-H. Wang, R. L. MacKinnon II, B. J. Maher and P. Kelly, unpublished data). Previous studies have used similar protocols to activate silent synapses (Isaac et al. 1995; Li et al. 1999; Liao et al. 1995). One mechanism to activate silent synapses is the insertion of functional AMPARs into the postsynaptic membrane. Previous results indicate that AMPAR trafficking is important for synaptic plasticity and maintaining basal synaptic transmission (Beattie et al. 2000; Linden 2001; Luscher et al. 1999; Luthi et al. 1999; Man et al. 2000).

We observed that stimulating postsynaptic IP<sub>3</sub>R with AdA increased EPSC amplitudes and that co-perfusion of BAPTA virtually eliminated this increase (Fig. 1). These results suggest that postsynaptic Ca<sup>2+</sup> release from IP<sub>3</sub>R-regulated stores is

responsible for increasing EPSC amplitudes. It is possible that AdA could stimulate Ca<sup>2+</sup> influx through plasmalemmal stores-operated Ca<sup>2+</sup> channels (SOCs) (Minke and Cook 2002). Although it was suggested that AdA may directly stimulate SOCs (DeLisle et al. 1997), Ca<sup>2+</sup>-imaging experiments by Machaca and Hartzell (1999) clearly showed that AdA's ability to increase Ca<sup>2+</sup> in *Xenopus* oocytes depends on IP<sub>3</sub>R-mediated Ca<sup>2+</sup> release. Machaca (2003) has shown that CaM-KII activity potentiates Ca<sup>2+</sup> currents through SOCs in *Xenopus* oocytes. This finding is interesting because we have shown that AdA-stimulated increases in EPSC amplitudes is strongly blocked by inhibitors of Ca<sup>2+</sup>/calmodulin or CaM-KII (J.-H. Wang, R. L. MacKinnon II, B. J. Maher, and P. Kelly, unpublished data).

SOCs are related to the *Drosophila* transient receptor potential (TRP) protein and many have been identified in brain (Clapham 2003; Moran et al. 2004). TRP1 and TRP5 are enriched in CA1 pyramidal neurons and dendrites and appear to be primarily extrasynaptic; they form nonselective cation channels that display voltage dependence similar to NMDARs (Strubing et al. 2001). TRP1/5 heteromers are activated by G<sub>q</sub>-coupled receptors and not by depletion of intracellular Ca<sup>2+</sup> stores (Strubing et al. 2001). Philipp et al. (1998) showed that TRP5 and TRP4 are co-expressed in CA1 neurons and form Ca<sup>2+</sup> channels that are activated by depleting intracellular Ca<sup>2+</sup> stores. Baba et al. (2003) have shown that cultured hippocampal neurons contain SOC-like channels that modulate NMDA-induced Ca<sup>2+</sup> transients. They also reported that SOC inhibitors attenuated tetanus-induced increases in dendritic

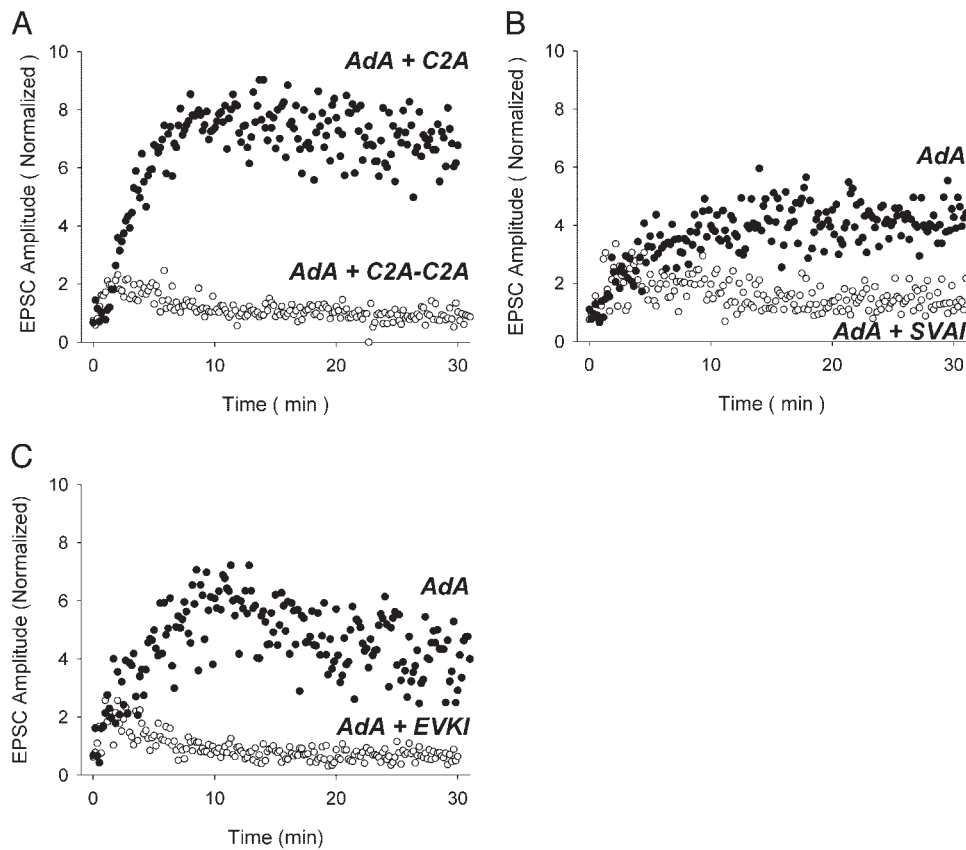


FIG. 6. SNARE-dependent exocytosis and interactions between AMPARs and PDZ domains are involved in AdA-enhanced synaptic potentiation. *A*: representative experiment for a neuron showing that co-perfusion of C2A–C2A (20  $\mu$ M) with AdA (1  $\mu$ M;  $\circ$ ) attenuates AdA-enhanced increase in EPSC amplitudes compared with a neuron co-perfused with AdA plus C2A ( $\bullet$ ). *B*: representative experiment for a neuron showing that co-perfusion of SVAI (100  $\mu$ M) with AdA (1  $\mu$ M;  $\circ$ ) attenuates the increase in EPSC amplitudes compared with an interleaved neuron perfused with AdA alone ( $\bullet$ ). *C*: representative experiment for a neuron showing that co-perfusion of EVKI (100  $\mu$ M) with AdA (1  $\mu$ M;  $\circ$ ) attenuates the increase in EPSC amplitudes compared with an interleaved neuron perfused with AdA alone ( $\bullet$ ).

$Ca^{2+}$  and LTP induction at CA1 synapses. Thus it is possible that AdA-stimulated increases in postsynaptic  $Ca^{2+}$  could stimulate additional  $Ca^{2+}$  influx via SOCs that together enhance AMPAR trafficking and synaptic transmission.

#### $Ca^{2+}$ stores in dendrites/spines

Studies at light and EM levels showed that the type-1  $IP_3R$  is a major  $IP_3R$  isoform in CA1 neurons and is predominantly enriched in postsynaptic structures including dendritic shafts and spines (Sharp et al. 1993). Ryanodine receptors are also enriched in CA1 dendrites and spines (Sharp et al. 1993) where they could contribute to  $IP_3$ -mediated  $Ca^{2+}$  release through  $Ca^{2+}$ -induced  $Ca^{2+}$  release (Friel and Tsien 1992).  $Ca^{2+}$  imaging results by Ross and co-workers (Nakamura et al.

2000) indicate that large-amplitude regenerative  $Ca^{2+}$  release occurs primarily in apical dendrites in CA1 neurons with much less release in oblique distal dendrites. These large  $Ca^{2+}$  release events in apical dendrites are elicited by high-frequency afferent stimulation in the presence of the mGluR agonist t-ACPD. Electrophysiological studies have shown that mGluR1 and mGluR5 receptors are present on CA1 pyramidal neurons where they regulate neuronal excitability and intracellular  $Ca^{2+}$  release (Mannaioni et al. 2001; Mutel et al. 2000; Rae and Irving 2004). In addition, mGluR5 knockout mice show impaired learning and reduced LTP in CA1 (Lu et al. 1997). Together, these findings indicate that mGluR signaling pathways underlying  $IP_3R$ -mediated  $Ca^{2+}$  release are present in dendrites and can regulate neuronal excitability, local  $Ca^{2+}$  release, and synaptic plasticity.

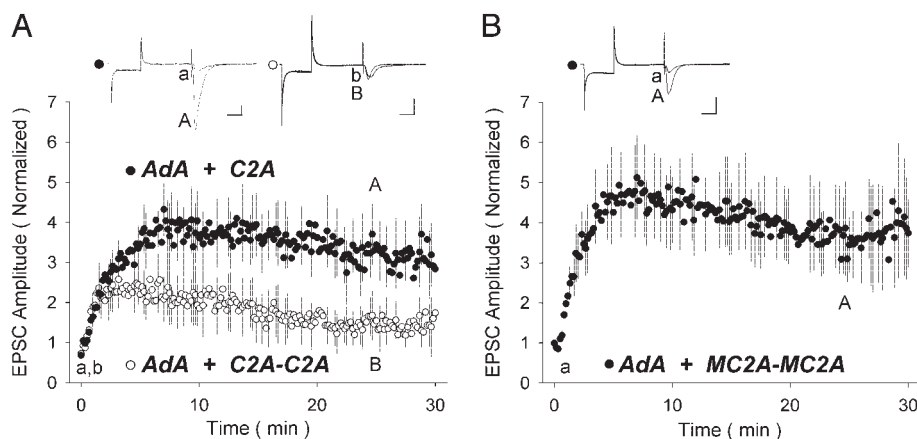


FIG. 7. SNARE-dependent exocytosis contributes to AdA-enhanced synaptic potentiation. *A*: group data showing that co-perfusion of C2A–C2A (20  $\mu$ M) with AdA (1  $\mu$ M;  $\circ$ ) attenuates increases in EPSC amplitudes compared with interleaved neurons co-perfused with AdA plus C2A ( $\bullet$ ). *B*: group data showing that co-perfusion of MC2A–MC2A (20  $\mu$ M) with AdA (1  $\mu$ M;  $\bullet$ ) does not alter AdA-enhanced increases in EPSC amplitudes.

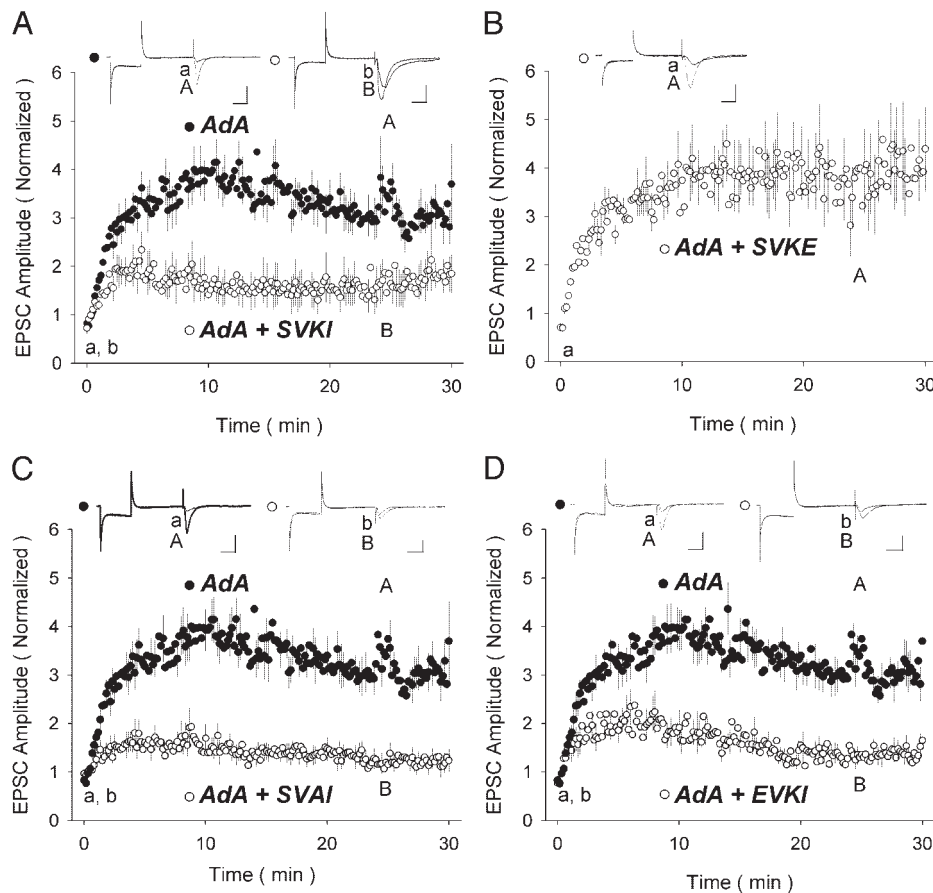


FIG. 8. Interactions between AMPARs and PDZ domains contribute to AdA-enhanced synaptic potentiation. *A*: group data showing that co-perfusion of SVKI (100  $\mu$ M) plus AdA (1  $\mu$ M;  $\circ$ ) attenuates increases in EPSC amplitudes compared with interleaved neurons perfused with AdA ( $\bullet$ ). *B*: group data showing that co-perfusion of SVKE (100  $\mu$ M) plus AdA (1  $\mu$ M) does not alter AdA-induced increases in EPSC amplitudes. *C*: group data showing that co-perfusion of SVAI (100  $\mu$ M) with AdA (1  $\mu$ M;  $\circ$ ) attenuates increases in EPSC amplitudes compared with interleaved neurons perfused with AdA ( $\bullet$ ). *D*: group data showing that co-perfusion of EVKI (100  $\mu$ M) plus AdA (1  $\mu$ M;  $\circ$ ) attenuates increases in EPSC amplitudes observed for interleaved neurons perfused with AdA ( $\bullet$ ).

*Synaptotagmin-SNARE-dependent insertion of AMPARs during intracellular Ca<sup>2+</sup> release and basal synaptic transmission*

The *N*-ethylmaleimide-sensitive factor (NSF) is a critical component of the membrane fusion machinery (Rothman 1994) and is involved in GluR2 insertion/stabilization at synapses (Braithwaite et al. 2002; Luscher et al. 1999; Nishimune et al. 1998; Song et al. 1998). NSF is required for the recycling of SNARE complexes (Chapman 2002) and is believed to be important for the insertion of synaptic AMPARs (Luscher et al. 1999). Synaptotagmin 1, a critical component of SNARE-dependent exocytosis, has been visualized in dendrites where it could modulate postsynaptic functions (Schwab et al. 2001). We examined the role of SNARE proteins in AdA-enhanced increases in synaptic transmission by co-perfusing C2A–C2A, a synaptotagmin-1-derived inhibitor of SNARE-mediated membrane fusion. C2A–C2A binds strongly to SNAREs, displaces native synaptotagmin 1 from SNARE complexes, and rapidly inhibits exocytosis (Earles et al. 2001). C2A–C2A strongly attenuated AdA-enhanced EPSC amplitudes (Fig. 7A), suggesting that intracellular Ca<sup>2+</sup> release increases AMPAR insertion into postsynaptic membranes via synaptotagmin-SNARE-mediated exocytosis. Two control reagents (C2A and MC2A–MC2A) failed to inhibit AdA-enhanced increases in EPSC amplitudes (Fig. 7, A and B). Our results provide the first evidence suggesting a postsynaptic function for synaptotagmin 1–SNARE interactions in AMPAR trafficking.

Proteins that regulate exocytosis/endocytosis are thought to modulate basal synaptic transmission (Luscher et al. 1999;

Luthi et al. 1999; Song et al. 1998). The balance between exocytosis and endocytosis may be regulated by AMPARs that constitutively cycle using SNARE-dependent processes to deliver receptors to synapses. C2A–C2A decreased basal EPSC amplitudes (Fig. 4A), suggesting that C2A–C2A inhibits the delivery of AMPARs to synapses, leaving endocytosis unchecked and thus decreases the number of synaptic AMPARs.

Postsynaptic perfusion of C2A–C2A depressed basal transmission (Fig. 4A), whereas C2A–C2A attenuated AdA-enhanced increases in EPSC amplitudes (Fig. 7A). These results suggest that C2A–C2A may not directly inhibit AdA's action in increasing EPSC amplitudes but simply depresses transmission by the same mechanism that it depresses basal synaptic transmission. This seems unlikely because all known examples of vesicular exocytosis require SNARE-dependent mechanisms (McNew et al. 2000) and therefore should be inhibited by C2A–C2A. Therefore we believe that both basal and AdA-enhanced synaptic transmission require SNARE-dependent exocytosis of AMPARs.<sup>1</sup>

*AMPA interactions with PDZ domains during intracellular Ca<sup>2+</sup> release and basal synaptic transmission*

PDZ domains are important protein–protein interacting motifs involved in regulating the trafficking/anchoring of AMPARs (Dong et al. 1997) and NMDARs (Kornau et al. 1995). The SVKI sequence in GluR2/3 preferentially binds class II PDZ domains in GRIP/ABP and PICK1. Because the two major types of heteromeric AMPARs in CA1 neurons are GluR1/2 and GluR2/3 (Wenthold et al. 1996), the anchoring/trafficking

of CA1 AMPARs could be modulated by interactions between GluR2 and GluR3 and the PDZ domains in GRIP/ABP and PICK1. Postsynaptic perfusion of peptides containing the PDZ-binding sequence SVKI in GluR2/3 can alter synaptic transmission and plasticity (Daw et al. 2000; Kim et al. 2001; Li et al. 1999; Matsuda et al. 1999). The overexpression of GRIP1 in neurons reduces the number of cell-surface AMPARs (Dong et al. 1997), suggesting that GRIP1 regulates AMPAR trafficking. GRIP/ABP has been localized near the postsynaptic density (PSD) and in membrane vesicles in dendritic shafts (Dong et al. 1999; Wyszynski et al. 1999). The palmitoylation of ABP is thought to target ABP to spine plasma membranes where it anchors GluR2s, whereas nonpalmitoylated ABP localizes GluR2 to intracellular storage sites (DeSouza et al. 2002). Thus GRIP/ABP is believed to anchor GluR2/3 in both synaptic and dendritic compartments. PICK1 is also found in spines and dendrites (Xia et al. 1999), and phorbol esters induce PICK1, GluR2 and PKC to become preferentially co-localized in spines (Chung et al. 2000; Perez et al. 2001).

We tested if PDZ domains were involved in AdA-enhanced increases in EPSC amplitudes. Co-perfusion of SVKI, which disrupts interactions between GluR2/3 and GRIP/ABP/PICK1, strongly attenuated AdA-enhanced increases in EPSC amplitudes (Fig. 8A), suggesting that interactions between GluR2/3 and class II PDZ domains of GRIP/ABP/PICK1 play a role in AdA-enhanced increases in EPSC amplitudes. In addition, SVAI which disrupts GluR2/3 binding to GRIP/ABP and PICK1 and does not contain a PKC phosphorylation motif, strongly attenuated AdA's ability to increase EPSC amplitudes (Fig. 8C). This suggests that SVKI acts by disrupting interactions between GluR2/3 and GRIP/ABP/PICK1 rather than acting as a competitive PKC inhibitor.

Are both GRIP/ABP and PICK1 involved in AdA-enhanced increases in EPSC amplitudes? Changing the serine in SVKI to glutamic acid (i.e., EVKI) mimics PKC phosphorylation. The resulting EVKI peptide selectively interacts with PICK1 but not GRIP/ABP. EVKI strongly attenuated AdA-enhanced increases in EPSC amplitudes (Fig. 8D), suggesting that interactions between GluR2/3 and PICK1 are mainly responsible for AdA's enhancement of synaptic transmission.<sup>1</sup>

#### *Role of PICK1 and PKC in AdA-induced increases in synaptic transmission*

AdA-enhanced increases in EPSC amplitudes were significantly attenuated by SVKI (Fig. 8A). SVKI inhibits interactions between GluR2/3 and GRIP/ABP/PICK1 and could inhibit PKC by acting as a competitive substrate. In addition, we have shown that inhibition of postsynaptic PKC activity strongly attenuated AdA-induced increases in synaptic transmission (J.-H. Wang, R. L. MacKinnon II, B. J. Maher and P. Kelly, unpublished data). Because the PICK1 selective peptide EVKI was effective in attenuating AdA-enhanced increases in EPSC amplitudes (Fig. 8D), it appears that signaling between PKC, PICK1, and GluR2/3 may be critical in this type of synaptic plasticity. Although the exact role of PKC is unclear,

we suggest that AdA-enhanced  $Ca^{2+}$  release stimulates PKC phosphorylation of Ser<sup>880</sup>, which releases GluR2/3 tethered to intracellular storage sites by GRIP/ABP. This facilitates GluR2/3's binding to PICK1, promoting receptor trafficking to the postsynaptic membrane and increasing synaptic transmission. A model sharing some of these properties<sup>1</sup> has been proposed by DeSouza et al. (2002) and Daw et al. (2000).

The mechanism proposed in the preceding text shares some similarities with recent results describing the effects of overexpression of PICK1 in CA1 neurons. PICK1 overexpression significantly increased AMPAR EPSC amplitudes, decreasing synaptic/cell-surface GluR2s without changing surface GluR1 levels or the total number of surface AMPARs (Terashima et al. 2004). AMPARs lacking GluR2 display greater single-channel conductance than those that contain GluR2 (Swanson et al. 1997), and this may be the basis for increased synaptic transmission during PICK1 overexpression. PICK1-mediated increases in synaptic transmission observed by Terashima et al. were blocked by inhibitors of PKC or CaM-KII. This dependence on PKC and CaM-KII activities is similar to our results on AdA-stimulated increases in synaptic transmission (J.-H. Wang, R. L. MacKinnon II, B. J. Maher and P. Kelly, unpublished data). Terashima et al. (2004) also observed that viral-mediated expression of SVKI/EVKI peptides in CA1 neurons decreased AMPAR EPSC amplitudes, which is different from the actions of these peptides on basal synaptic transmission that we (Fig. 5) and others have observed (Daw et al. 2000; Kim et al. 2001). The differences between our results and those of Terashima et al. (2004) may be due to experimental conditions. We have studied short-term effects (30 min) of SVKI/EVKI peptides and IP<sub>3</sub>R agonists during whole cell perfusion in acute hippocampal slices whereas they examined viral-mediated expression of PICK1 and SVKI/EVKI peptides over longer times (20–48 h) using organotypic slices or cultured neurons. Despite these differing results, it is possible that AdA perfusion activates PICK1 via PKC and CaM-KII signaling pathways, decreases the proportion of GluR2s in synaptic AMPARs and thereby enhances EPSC amplitudes.

Although there are several targets of IP<sub>3</sub>R-mediated  $Ca^{2+}$  release that could modulate synaptic transmission, two noteworthy candidates are calpain and the clathrin-mediated endocytotic complex. The  $Ca^{2+}$ -activated protease calpain is present in dendrites (Perlmutter et al. 1990) and calpain inhibitors attenuate LTP (Denny et al. 1990; Oliver et al. 1989; Vanderklisch et al. 1996). NMDAR stimulation activates calpain in dendritic spines (Vanderklisch et al. 2000). Calpain degrades GRIP *in vitro* and in hippocampal slices stimulated with *N*-methyl-D-aspartate (NMDA) show enhanced GRIP proteolysis (Lu et al. 2001). AdA-stimulated  $Ca^{2+}$  release could activate postsynaptic calpain which degrades GRIP, disrupts its binding to GluR2/3 and thereby modulates AMPAR trafficking.

Cell-surface receptors undergo endocytosis by the action of a protein complex containing clathrin, clathrin adapters, dynamin, amphiphysin, and synaptojanin (Slepnev and De Camilli 2000). Lai et al. (1999) reported that calcineurin (CaN), a  $Ca^{2+}$ /calmodulin-dependent phosphatase, is physically associated with dynamin, and this association is  $Ca^{2+}$ -dependent. They showed that dynamin physically links CaN to the endocytic

<sup>1</sup> The Supplementary Material for this article (a figure) illustrates the roles of synaptotagmin-SNARE-mediated exocytosis, GRIP/ABP, and PICK1 in AMPAR cycling during basal (A) and AdA-enhanced synaptic transmission (B). This figure is available online at <http://jn.physiology.org/cgi/content/full/00651.2004/DC1>.

complex and most importantly, that inhibition of CaN in vivo attenuates transferrin endocytosis. These results suggest that the CaN-dynamin complex is a Ca<sup>2+</sup> sensor for vesicular endocytosis. Consistent with this role for CaN, we have shown that inhibiting postsynaptic CaN activity increased basal synaptic transmission (Wang and Kelly 1997). Beattie et al. (2000) have shown that activation of NMDA receptors triggers AMPAR endocytosis by a mechanism that involves Ca<sup>2+</sup> influx and CaN activity. The dephosphorylation of dynamin by CaN is an important step in the functional assembly of the endocytosis complex (Slepnev et al. 1998). It is possible that Ca<sup>2+</sup>-mediated endocytosis may be activated following AdA-stimulated intracellular Ca<sup>2+</sup> release; however, our results indicate that the balance between AMPAR insertion versus internalization greatly favors insertion. Clearly, more information is needed to understand how these complicated but opposing pathways regulate AMPAR trafficking.

What role do PDZ domains play in regulating basal synaptic transmission? We observed that SVAI significantly increased basal transmission (Fig. 5C), while SVKI perfusion produced small but not significant increases in basal EPSC amplitudes (Fig. 5A). One explanation for the different actions of SVAI versus SVKI may be that SVKI is phosphorylated by PKC under basal conditions and becomes a selective PICK1 inhibitor (Kim et al. 2001) and therefore doesn't modulate basal synaptic transmission. Consistent with this notion, we observed that EVKI (a selective PICK1 inhibitor) did not alter basal synaptic transmission (Fig. 5D); this is in agreement with Daw et al. (2000); however, see Kim et al. (2001). Because SVAI significantly increased basal synaptic transmission and isn't phosphorylated by PKC, we believe SVAI is acting primarily by inhibiting interactions between GluR2/3 and GRIP/ABP. Two potential mechanisms could account for SVAI's ability to increase basal transmission. First, SVAI may disrupt GluR2/3s that are anchored to GRIP/ABP at intracellular storage sites and allow these AMPARs to traffic to the postsynaptic membrane. Second, SVAI may inhibit the intracellular storage of GluR2/3s that are being constitutively internalized and thus allow GluR2/3s to return directly to the postsynaptic membrane. Both of these potential mechanisms could increase the number of synaptic AMPARs being inserted versus the number being constitutively internalized and therefore contribute to SVAI's ability to increase basal EPSC amplitudes.<sup>1</sup>

Our findings suggest that stimulating postsynaptic Ca<sup>2+</sup> release with AdA increases synaptic transmission by enhancing the insertion and anchoring of AMPARs. AdA-enhanced postsynaptic Ca<sup>2+</sup> release increases AMPAR function, possibly via synaptotagmin-SNARE-dependent exocytosis that controls receptor insertion and interactions with PICK1 PDZ domains that mediate receptor anchoring. In addition, the regulation of basal synaptic transmission involves synaptotagmin-SNARE-dependent exocytosis and PDZ domain interactions of GRIP/ABP that regulate the constitutive cycling of AMPARs.

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