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Trafficking of α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic Acid Receptor (AMPA) Receptor Subunit GluA2 from the Endoplasmic Reticulum Is Stimulated by a Complex Containing Ca^{2+}/Calmodulin-activated Kinase II (CaMKII) and PICK1 Protein and by Release of Ca^{2+} from Internal Stores*

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Wei Lu[1,2], **Latika Khatri**[1], **and Edward B. Ziff**[3]

From the Department of Biochemistry and Molecular Pharmacology, New York University School of Medicine, New York, New York 10016

Background: ER exit is a rate-limiting step for GluA2 synaptic delivery, which regulates AMPAR trafficking and synaptic calcium dynamics.
Results: ER calcium release, CaMKII activity and PICK1 are important for GluA2 ER exit.
Conclusion: ER-mediated intracellular calcium dynamics regulate GluA2 trafficking out of the ER.
Significance: Novel Ca^{2+}-dependent signaling pathways underlie the regulation of GluA2 trafficking from the ER.

The GluA2 subunit of the AMPA receptor (AMPAR) dominantly blocks AMPAR Ca^{2+} permeability, and its trafficking to the synapse regulates AMPAR-dependent synapse Ca^{2+} permeability. Here we show that GluA2 trafficking from the endoplasmic reticulum (ER) to the plasma membrane of cultured hippocampal neurons requires Ca^{2+} release from internal stores, the activity of Ca^{2+}/calmodulin activated kinase II (CaMKII), and GluA2 interaction with the PDZ protein, PICK1. We show that upon Ca^{2+} release from the ER via the IP3 and ryanodine receptors, CaMKII that is activated enters a complex that contains PICK1, dependent upon the PICK1 BAR (Bin-amphiphysin-Rvs) domain, and that interacts with the GluA2 C-terminal domain and stimulates GluA2 ER exit and surface trafficking. This study reveals a novel mechanism of regulation of trafficking of GluA2-containing receptors to the surface under the control of intracellular Ca^{2+} dynamics and CaMKII activity.

Membrane proteins are synthesized in the ER,[4] and the vast majority of them are eventually delivered to the plasma membrane to mediate cellular function. It has been recognized that regulated exit out of the ER is a key step for membrane protein forward trafficking. However, the mechanisms underlying this step for many membrane proteins remain largely elusive.

AMPA receptors are transmembrane protein complexes, mediating rapid central nervous system excitatory synaptic transmission, and their regulated synaptic trafficking is thought to underlie synapse long term potentiation and long-term depression, respectively (1–3). Trafficking depends on assembly of subunits GluA1–4 into tetrameric channels (4), which takes place within the endoplasmic reticulum and which for GluA2 is controlled by the pore apex hairpin residue, arginine 607, which restricts incorporation of multiple GluA2s and also imposes Ca^{2+} impermeability (5–9). In hippocampal neurons, GluA2 may reside for long periods (hours) in the ER, and its exit from the ER is rate-limiting for surface trafficking (5–7).

In the steady state, most AMPA receptors contain GluA2, and GluA1/2 is the most abundant heteromer type in hippocampal pyramidal neurons, with smaller proportions of GluA2/3 heteromers and GluA1 homomers (10, 11). GluA2 genetic deletion reduces synaptic transmission (10, 12, 13), and receptors that lack GluA2 are Ca^{2+}-permeable and rectifying, whereas receptors that contain GluA2 are non-rectifying and Ca^{2+}-impermeable (4, 8, 9). Also, GluA2 inclusion diminishes single channel conductance (4). Various transmembrane proteins and postsynaptic density proteins anchor GluA2-containing AMPA receptors at synapses, including the PDZ scaffolds, GRIP, and ABP (2, 3, 14). Among these, GRIP/ABP is released from GluA2 by PKC phosphorylation of the GluA2 C-terminal

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[1] Both authors contributed equally to this work.
[2] Present address: Synapse and Neural Circuit Research Unit, NINDS, National Institutes of Health, Bethesda, MD 20892.
[3] To whom correspondence should be addressed: Dept. of Biochemistry and Molecular Pharmacology, New York University School of Medicine, 550 First Ave, New York, NY. Tel.: 212-263-5774; Fax: 212-263-8214; E-mail: edward.ziff@nyumc.org.
[4] The abbreviations used are: ER, endoplasmic reticulum; CaMKII, Ca^{2+}/calmodulin-activated kinase II; AMPAR, AMPA receptor; PDZ, postsynaptic density (PSD)-95/Discs large/zO-1; BAR, Bin-amphiphysin-Rvs; PICK1, protein interacting with C-kinase 1; GRIP, glutamate receptor interacting protein; ABP, AMPA receptor-binding protein; KN93, *N*-[2-[[[3-(4-chlorophenyl)-2-propenyl]methylamino]methyl]phenyl]-*N*-(2-hydroxyethyl)-4-methoxybenzenesulphonamide; KN92, 2-[*N*-(4′-methoxybenzenesulfonyl)]amino-*N*-(4′-chlorophenyl)-2-propenyl-*N*-methylbenzylamine phosphate; Endo H, endoglycosidase H; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; BAPTA, 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid; IP3, inositol trisphosphate; DIV, days *in vitro*; TARP, transmembrane AMPA receptor regulatory protein; RT, room temperature; D-APV, D-(2*R*)-amino-5-phosphonovaleric acid; 2-APB, 2-aminoethoxydiphenyl borate; ANOVA, analysis of variance.

domain, whereupon GluA2 binds the PICK1 protein (2, 3, 15, 16).

Synaptic GluA2-lacking AMPA receptors may be replaced by ones containing GluA2. Such replacement has been reported during the early phase of long term potentiation (Refs. 17 and 18, but see Ref. 19), in cerebellar stellate neurons (20–23), and at synapses of dopaminergic ventral tegmental area neurons of cocaine-sensitized rats (24), in the latter case involving the activation of group I metabotropic glutamate receptors. Where characterized in ventral tegmental area dopamine neurons, GluA2 insertion depends upon synaptic activity and thus differs from the reported GluA2 synapse trafficking mechanism in hippocampal neurons, which is activity-independent (25, 26).

Here we have investigated the mechanism of GluA2 surface trafficking. We found that release of Ca^{2+} from the ER via the IP3 and ryanodine receptors activates CaMKII, which enters a complex that contains PICK1. Association of this complex with GluA2 stimulates GluA2 exit from the ER leading to trafficking to the plasma membrane. We suggest that this pathway acts as a feedback mechanism to limit Ca^{2+} influx through Ca^{2+}-permeable AMPARs.

EXPERIMENTAL PROCEDURES

Expression Vectors—Plasmids and viral vectors expressing PICK1, GluA1, and GluA2 have been described before (27–29). Expression plasmids of CaMKIIα and the CaMKIIα (H282R) mutant were a gift from Professor Thomas R. Soderling at the Oregon Health Sciences University. Expression plasmids of GFP-CaMKIIα mutants were a gift from Professor Tobias Meyer at Stanford University. Expression plasmids of CaMKIIα and its mutants were created by PCR amplification and inserted into the pEGFP-N2 vector. Sindbis viral vectors of GFP-CaMKIIα mutants were created by PCR amplification and inserted into the pSinRep5 viral expression vector. The Sindbis viral vector expressing a truncation mutant of CaMKIIα encoding the catalytic domain was a gift from Professor Roberto Malinow of the University of California, San Diego.

Antibodies—The following antibodies were used: polyclonal and monoclonal anti-Myc, polyclonal and monoclonal anti-HA antibodies, monoclonal anti-tubulin, goat polyclonal anti-GRIP, and polyclonal anti-PICK1 N18 antibodies (Santa Cruz); polyclonal and monoclonal anti-FLAG antibodies, polyclonal anti-CaMKIIα antibody (Sigma); polyclonal anti-PICK1 antibody and monoclonal anti-pCaMKIIα antibody (Affinity BioReagents); monoclonal anti-CaMKII α antibody (Roche Applied Science); monoclonal anti-GluA2 and polyclonal anti-GluA2/3 (Chemicon) and polyclonal anti-GluA1 (Oncogene) antibodies; rabbit anti-GFP antibody.

HeLa Cell Culture, Transfection, and Immunocytochemistry—HeLa cells culture and immunostaining were performed as described before (36). Briefly, HeLa cells were seeded on glass coverslips and maintained in Dulbecco's minimum Eagle's medium (DMEM) culture medium, 10% fetal bovine serum, and antibiotics under 37 °C and 5% CO_2 in a cell culture incubator. After overnight incubation, cells were ~30% confluent, and plasmids were introduced into cells using Effectene reagent (Qiagen) following the instructions of the manufacturer. 15–18 h after transfection, cells were washed with PBS and fixed with 4% paraformaldehyde, 4% sucrose for 15 min at room temperature (RT). After washing with PBS three times, cells were permeabilized with 0.2% Triton X-100 for 10 min at RT. Cells were then washed with PBS and blocked with 10% BSA for 1 h at RT. Primary antibody diluted in 3% BSA was incubated with cells for 1 h at RT. Cells were then washed 3 times with PBS for 5 min and incubated with secondary antibody conjugated with the appropriate fluorophore diluted in 3% BSA for 1 h at RT. After washing 3 times with PBS for 5 min, coverslips with cells were mounted with mounting oil, Citifluor (Ted Pella), and stored at 4 °C until examination under the confocal microscope.

293T Cell Culture, Transfection, and Cell Lysate Preparation—293T cells were seeded onto 6-cm Petri dishes and maintained in DMEM culture medium under 37 °C plus 5% CO_2 in a cell culture incubator. After overnight incubation, plasmids were introduced into cells using Effectene reagent (Qiagen) following the instructions of the manufacturer. 36–48 h after transfection, cells were washed once with PBS, and 0.5 ml 1% Triton X-100 buffer (150 mM NaCl, 2 mM EDTA, 50 mM Tris-HCl, pH 7.4, and 1% Triton X-100) plus protease inhibitor mixture tablet (Roche Applied Science) was added to each dish. Cells were collected into 1.5-ml Eppendorf tubes with Cell Lifter and agitated for 30 min to 1 h at 4 °C. Lysates were then clarified by microcentrifugation at 4 °C for 15 min at maximal speed. Supernatants were immediately used for experiments or stored in −80 °C until needed.

Virus Production and Neuronal Infection—Sindbis viruses encoding proteins of interest were produced based on instructions from the manufacturer (Invitrogen). Briefly, viral vectors encoding the proteins of interest were digested with the restriction enzyme, Not1, to linearize vectors, which were transcribed for 2 h at 37 °C using *in vitro* transcription/RNA capping kits (Ambion). RNAs were then electroporated into BHK cells (6×10^6 cells/electroporation) along with RNAs transcribed from the DH-26S helper plasmid (Invitrogen), which contains genes necessary for pseudovirion production. Electroporated BHK cells were then plated onto a 10-cm Petri dish in α-minimum Eagle's medium (Invitrogen) and incubated at 37 °C and 5% CO_2 for about 30–40 h to allow virus production. The growth medium containing the viruses was subsequently collected, aliquoted, and frozen at −80 °C until needed.

Neurons seeded on coverslips were infected at days *in vitro* (DIV) 17–21, and neurons seeded on 6-cm Petri dishes were infected at DIV 10–14. For infections, viral stocks were thawed and diluted (generally 1:25) in 500 μl of conditioned Neurobasal medium (for neurons plated on coverslips) or in 1 ml of conditioned Neurobasal medium (for neurons plated on 6 cm Petri dishes) that was then placed over neurons with occasional rocking for 1 h. Neurons were then supplemented with additional conditioned Neurobasal medium for about 15–18 h until experimental manipulation.

Primary Hippocampal Neuron Culture—The day before dissection, coverslips or 6-cm Petri dishes were coated with poly-L-lysine in boric acid buffer at 37 °C overnight. Before dissec-

tion, coverslips or dishes were washed twice with PBS and stored in the incubator ready for plating neurons.

Primary hippocampal neuron cultures were obtained from E18–19 SD rat embryos. Pregnant rats were anesthetized with CO_2, and embryos were then removed. All dissection work was carried out in ice-cold PHG buffer (1× PBS, 10 mM HEPES, and 0.6% glucose, pH 7.35). After decapitation of the embryos, hippocampi were isolated under a dissection microscope in the sterile hood. Hippocampi were collectively trypsinized in 1× trypsin for 15 min at 37 °C, washed 3 times in dissection buffer, and then resuspended in 5 ml of plating medium (minimal essential medium, 10% horse serum, 0.45% glucose, 1 mM pyruvate, 1× penicillin/streptomycin) warmed to 37 °C. Hippocampi were triturated with a 5 ml of sterile pipette until the cell suspension appeared homogeneous, and cells were then counted with a hemocytometer. Cells were plated at a density of 120,000 per coverslip or 1,000,000 per 6-cm Petri dish in plating medium. 2–4 h after plating, all media were removed and replaced with Neurobasal medium supplemented with B27 supplement (Invitrogen), glutamine (500 μM), and antibiotics. Every 4 days, half of the volume of medium remaining on the cells was removed and replaced with fresh Neurobasal medium. Anti-glia growth drug was usually added into growth media after 8 DIV.

Pharmacological Manipulation of Hippocampal Cultures—To study the role of protein kinases and intracellular calcium dynamics in GluA2 surface expression and GluA2 ER exit, antagonists that inhibit protein kinases, ion channels, or receptors indicated in corresponding experiments were added to growth medium immediately after virus infection and maintained until immunostaining or metabolic labeling.

Autocamtide-3 pseudosubstrate, myristoylated (CaMKII inhibitory peptide) (Myr-KKALHRQEAVDAL-OH) (42) and scramble control peptide were purchased from Quality Controlled Biochemicals (Hopkinton, MA). The peptides were dissolved in water and added to neuronal cultures at a final concentration of 50 μm 2 h after Sindbis virus infection. The cells were incubated with the peptides for an additional 16 h before experiments.

Neuronal Immunocytochemistry—For fixed immunostaining, 15–18 h after infection, neurons were washed once with PBS and fixed with 4% paraformaldehyde, 4% sucrose for 10 min at RT. Neurons were then washed 3 times with PBS and permeabilized with 0.2% Triton X-100 for 8 min at RT. After permeabilization, neurons were washed once with PBS and blocked with 10% BSA for 1 h at RT. Primary antibodies were then diluted in 3% BSA and applied to neurons on coverslips for 1 h at RT. Neurons were then washed 3 times with PBS and incubated with secondary antibodies diluted in 3% BSA for 1 h at RT. Neurons were then mounted with mounting oil, Citifluor (Ted Pella), and stored at 4 °C until examination under the confocal microscope.

For recombinant receptor live staining, neurons were stained live with anti-Myc 9E10 (4 μg/ml) or anti-HA (4 μg/ml) monoclonal antibodies for 15 min at 37 °C to detect surface tagged receptors. After live labeling, neurons were washed once with PBS and fixed with 4% paraformaldehyde, 4% sucrose for 10 min at RT. Neurons were then washed 3 times with PBS and blocked with 10% BSA for 1 h at RT. Secondary antibody diluted in 3% BSA was applied to neurons for 1 h at RT. Neurons were then washed once with PBS and permeabilized with 0.2% Triton X-100 for 8 min at RT. After permeabilization, neurons were blocked again with 10% BSA for 1 h at RT, and primary anti-Myc polyclonal antibody (A14, 0.5 μg/ml) or polyclonal anti-HA antibody (0.5 μg/ml) diluted in 3% BSA was incubated with neurons to detect total tagged receptors for 1 h at RT. Neurons were then washed 3 times with PBS and incubated with secondary antibody diluted in 3% BSA for 1 h at RT. Coverslips with neurons were then washed, mounted, and stored as described above.

Image Analysis and Quantitation—Immunofluorescence images were acquired on a Nikon PCM 2000 confocal microscope or a Zeiss Axovert 200 fluorescence microscope under 60× objective and, for the PCM2000, analyzed with Simple32 Imaging software (C-IMAGING Systems). All images were acquired at the same setting for one experiment. To analyze the effect of drugs treatments on Myc-GluA2 or HA-GluA1 trafficking, neuronal areas were defined as the extent of total Myc or HA staining, and total fluorescence was measured in this area. The ratio of surface Myc or HA signal to cell area was then calculated. However these ratios do not indicate actual receptor distribution but were used for relative differences across different treatment conditions. Each experimental manipulation was performed at least three times. *Error bars* are S.E., and *t* tests were carried out to determine the significance between two groups. Statistical differences for multiple groups were determined by one-way ANOVA.

Immunoprecipitation and Immunoblotting—For immunoprecipitation assays in 293T cells, appropriate amounts of 293T cell lysates were incubated with 1 μg of M2 monoclonal anti-FLAG antibody to immunoprecipitate FLAG-tagged species for 2 h at 4 °C. The immunocomplexes were precipitated with Protein G beads for 2 h at 4 °C. Immunoprecipitates were washed with lysis buffer, eluted with SDS-PAGE sample buffer, and heated at 100 °C for 5 min. The samples were then run on SDS-PAGE gels for electrophoresis. After electrophoresis, the protein samples in the SDS-PAGE gel were transferred overnight to nitrocellulose or PVDF membrane for Western blotting with the indicated antibodies.

Metabolic Labeling and Endo H Assay—DIV 10–14 neurons were washed once with warmed DMEM lacking Met/Cys (containing 0.5 mM glutamine and 1× B27; Invitrogen). Neurons were then Met-depleted in this medium for 20 min and pulsed with 160 μCi/ml of Expre^{35}S^{35}S (1175 Ci/mmol; PerkinElmer Life Sciences) for 15 min. Neurons were then washed with warmed Neurobasal medium and chased in 50:50 of conditioned Neurobasal medium:fresh Neurobasal medium (+600 μM cold methionine) for 5 h. Neurons were then washed 3 times with ice-cold PBS and lysed with Neuronal lysis buffer (400 μl/dish, 150 mM NaCl, 20 mM Hepes, 2 mM EDTA, 1% Triton, 0.1% SDS, pH 7.4, and 1× fresh protease inhibitor mixture tablets) for 1 h at 4 °C. Lysates were cleared for 20 min at 16,000 × *g* and used for immunoprecipitations. Lysates were incubated with 1.5 μg of Myc polyclonal antibody or 1.5 μg of GluA2 monoclonal antibody from Neuromab for 1 h at 4 °C. Twenty-

five microliters of Protein A or G-agarose beads (Santa Cruz) were then added for 50 min. Immunoprecipitates were washed three times with lysis buffer.

Immunoprecipitates were first denatured with 100 °C heating for 5 min in 0.5% SDS solution. When immunoprecipitates were cooled to RT, Endo H (Roche Applied Science) digestion was carried out. Enzyme reactions on immunoprecipitates were in 50 mM sodium citrate, pH 5.9, with 15 milliunits of enzyme per reaction. Digestions were at 30 °C for 16–19 h. Samples were separated on 6% SDS-PAGE gel, fixed with 45% methanol and 5% acetic acid buffer for 20 min, and amplified with amplification solution (Amersham Biosciences) for 30 min. Gels were then dried and autoradiographed.

RESULTS

CaMKII Is Required for GluA2 Trafficking to the Surface in Hippocampal Cultures—To test the roles of protein kinases in GluA2 surface trafficking, we measured the effects of various protein kinase inhibitors on GluA2 levels in the plasma membrane of cultured embryonic hippocampal neurons (17–21 DIV). We expressed Myc-GluA2 (Myc epitope tag fusion to GluA2 N terminus) from a Sindbis virus vector and treated the cultures with different kinase inhibitors. At 15–18 h after infection, surface and total Myc were measured by live and permeabilized cell immunofluorescent staining, respectively, and the ratio of surface Myc-GluA2 to total Myc-GluA2 was calculated. Neither a PKC inhibitor (bisindolylmaleimide (2 μM) nor a PKA inhibitor (KT5720; 2μM) affected GluA2 plasma membrane levels. In contrast, a CaMKII inhibitor, KN93 (10μM), significantly reduced the levels of surface GluA2 (Fig. 1*A*). As a control, the inactive analog KN92 did not significantly inhibit surface levels of GluA2 (Fig. 1*B*). This suggests a role for CaMKII in establishing the level of GluA2 in the neuronal plasma membrane.

To corroborate the role of CaMKII in GluA2 trafficking, we employed a different approach to inhibit neuronal CaMKII activity. We found that trafficking of MycGluA2 to the neuronal surface was sensitive to a peptide (myristoylated autocamtide-3 pseudosubstrate, 50 μM) that inhibits CaMKII activity (42), but not to a control peptide (Fig. *1C*). Taken together these data demonstrate an important role of CaMKII in the regulation of GluA2 trafficking.

We previously showed that a large fraction of intracellular GluA2 resides in the endoplasmic (ER), where its glycosylation is sensitive to Endo H (7). We employed an Endo H assay to determine whether CaMKII is required for export of GluA2 from the ER, an early step in surface trafficking. CaMKII inhibition by KN93 significantly suppressed maturation of Myc-GluA2 as assayed by pulse-chase analysis of Endo H sensitivity. In contrast, KN92 did not suppress (Fig. 1*D*). Thus, CaMKII activity was specifically required for GluA2 exit from the ER.

Ca^{2+} Release from Internal Stores Stimulates GluA2 Surface Trafficking—Because CaMKII is a Ca^{2+}/calmodulin-activated kinase, we next examined the roles of extracellular and intracellular Ca^{2+} in GluA2 surface expression. We found that blockage of Ca^{2+} influx through plasma membrane Ca^{2+} channels, including L-type voltage-gated Ca^{2+} channels (nimodipine 10 μM, data not shown) and ionotropic glutamate receptors (100 μM D-APV, *N*-methyl-D-aspartate receptor antagonist; CNQX 100 μM, AMPAR antagonist), did not impair GluA2 surface expression, nor did blockage of voltage-gated sodium channels with tetrodotoxin (2 μM) (Fig. 2*A*). This suggested that in cultured hippocampal neurons, GluA2 trafficking to the surface is not dependent on Ca^{2+} influx through channels in the plasma membrane or on neuronal activity. In agreement, a reagent (BAPTA, 2 mM) that chelates extracellular Ca^{2+} also failed to reduce GluA2 surface expression (Fig. 2*A*). Thus, extracellular Ca^{2+} influx is not required to maintain surface levels of GluA2.

Because Ca^{2+} release from the ER via IP3 and ryanodine receptors contribute to intracellular Ca^{2+} dynamics (30, 31), we examined the role of this release in GluA2 surface expression. Interestingly, simultaneous inhibition of the IP3 receptor by 2-APB (50 μM) and the ryanodine receptor by dantrolene (25 μM), but not inhibition of either receptor alone, significantly reduced surface GluA2 but did not inhibit GluA1, a control (Fig. 2, *A* and *B*). This suggests that release of Ca^{2+} from internal stores through either IP3 or ryanodine receptors is sufficient for the maintenance of GluA2 surface levels.

Regulation of Trafficking of Endogenous GluA2 by CaMKII Activity and Ca^{2+} Release from Internal Stores—Previous studies have shown that maturation of GluA2 at the ER is a rate-limiting step for surface expression of the receptor (7). To test the role of CaMKII activity and Ca^{2+} release from internal stores in native GluA2 maturation and forward trafficking, we treated neuronal cultures with KN93 or 2-APB and dantrolene and assayed GluA2 maturation through an Endo H assay. As shown in Fig. 2*C*, blockade of CaMKII activity or inhibition of Ca^{2+} release from internal stores suppressed maturation of endogenous GluA2 at the ER.

PICK1-dependent GluA2 Trafficking to Neuronal Surface—We have previously shown that the exit of GluA2 from the ER depends on the PICK1 protein (7), a Ca^{2+}-responsive, PDZ protein that specifically binds the GluA2 C-terminal domain and mobilizes trafficking of GluA2 (28, 32–35). To study the role of PICK1 in CaMKII-dependent GluA2 trafficking to the neuronal surface, we took advantage of well-characterized GluA2 mutants (GluA2-AVKI and GluA2-SVKE) (27). Although GluA2-SVKE cannot bind to either PICK1 or GRIP/ABP, GluA2-AVKI selectively loses its ability to bind to GRIP/ABP without disrupting the interaction with PICK1 (27). We determined which of these MycGluA2 mutants trafficked by a CaMKII-dependent and ryanodine or IP3 receptor-dependent mechanism by comparing trafficking in inhibitor-treated cultures with trafficking in control cultures lacking drugs. As shown in Fig. 3, KN93 or dantrolene/2-APB inhibits trafficking of GluA2 and GluA2-AVKI to the neuronal surface. In contrast, neither KN93 nor dantrolene/2-APB has an effect on GluA2-SVKE delivery to the plasma membrane. These data suggest that the ability of CaMKII activity or internal calcium release to stimulate GluA2 trafficking depends on an interaction of PICK1 with GluA2.

PICK1 Associates with CaMKII in Heterologous Cells—We next investigated how Ca^{2+} release from internal stores,

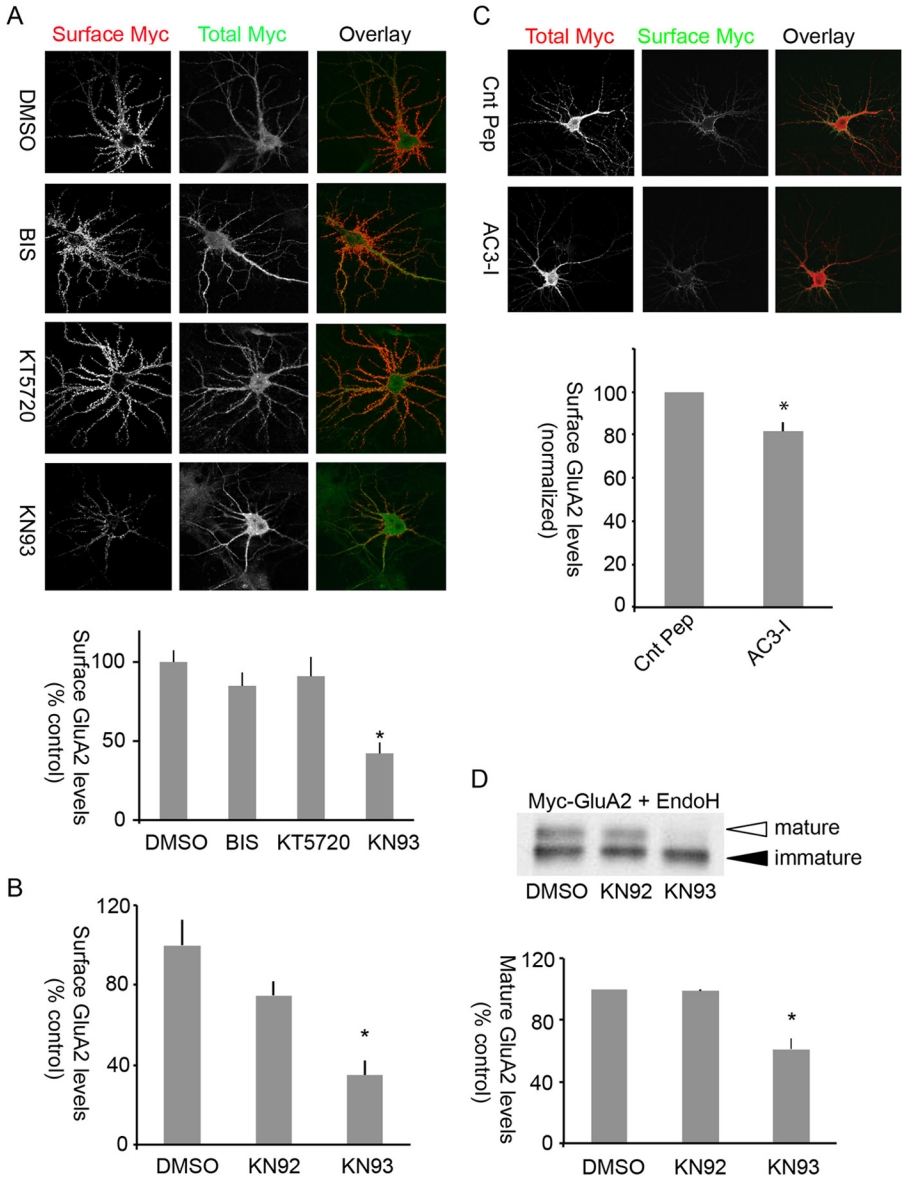

FIGURE 1. **CaMKII activity is required for surface expression of GluA2 in cultured hippocampal neurons.** *A* and *B*, requirement of CaMKII activation for surface delivery of Myc-GluA2 in neurons. Cultured hippocampal neurons (17–21 DIV) were infected with Sindbis virus expressing Myc-GluA2. PKC inhibitor, bisindolylmaleimide (*BIS*, 2 μM), or PKA inhibitor, KT5720 (2 μM) or CaMKII inhibitor, KN93 (10 μM), KN92 (10 μM) or the vehicle, DMSO, were added to cultured media immediately after infection. Surface and total Myc-labeled receptors were quantitated by immunocytochemistry as described under "Experimental Procedures." The *bar graph* in *A* shows the mean ± S.E.; $n = 29$ for DMSO; $n = 23$ for bisindolylmaleimide; $n = 24$ for KT5720; $n = 30$ for KN93. The *bar graph* in *B* shows the mean ± S.E.; $n = 37$ for DMSO, KN93, or KN92. Significance between individual drug treatment and DMSO in *A* and *B* was determined with two-tailed *t* test; *, $p < 0.001$. *, $p < 0.05$ with one-way ANOVA; *, $p < 0.05$ post hoc Fisher's test. *C*, cultured hippocampal neurons (18–21 DIV) were infected with Sindbis virus expressing Myc-GluA2 (R) in the presence of control peptide (*Cnt Pep*) or the CaMKII inhibitor, autocamtide (*AC3-I*). Surface and total Myc-labeled receptor was quantitated by immunocytochemistry as described under "Experimental Procedures." The *bar graph* shows the mean ± S.E.; $n = 51$ for control peptide and $n = 50$ for autocamtide-3. Significance was determined with two-tailed *t* test. *, $p < 0.05$. *D*, CaMKII activity is involved in GluA2 exit from the ER. Cultured hippocampal neurons (~12 DIV) were infected with Sindbis virus expressing Myc-GluA2. 15 h after infection, neurons were pulsed with [^{35}S]Met-Cys for 20 min and chased for 5 h. CaMKII inhibitor, KN93 (10 μM), or the inactive analog, KN 92 (10 μM), or the vehicle, DMSO, was added to the medium throughout the pulse-chase experiment. Myc-GluA2 was immunoprecipitated with an anti-Myc antibody, Endo H-digested, and analyzed on 6% SDS-PAGE followed by fluorography. Maturely glycosylated Myc-GluA2 is denoted with an *empty arrowhead*, and immaturely glycosylated Myc-GluA2 with a *filled arrowhead*. The *bar graph in the bottom* shows the quantitation ($n = 4$ for DMSO and KN93 and $n = 3$ for KN92. *, $p < 0.05$ with one-way ANOVA. *, $p < 0.05$ with post hoc Fisher's test.).

CaMKII, and PICK1 could cooperate to control GluA2 trafficking. Based on the precedent that PKCα regulates GluA2 through a PICK1-PKCα complex (28, 36–38), we hypothesized that CaMKII might act through a complex containing PICK1 and CaMKII to stimulate GluA2 trafficking. To determine whether PICK1 enters such a complex, we expressed wild type PICK1 or PICK1 truncation mutants with CaMKIIα in 293T cells. Taking into account that activation of PKCα is required to form the PICK1-PKCα complex, we assayed whether the CaMKIIα mutant, CaMKIIα H282R, would coimmunoprecipitate with PICK1 after 293 cell expression (Fig. 4). The H282R mutation releases the kinase regulatory domain from its catalytic domain, as does binding of Ca^{2+}-calmodulin to CaMKII, constitutively acti-

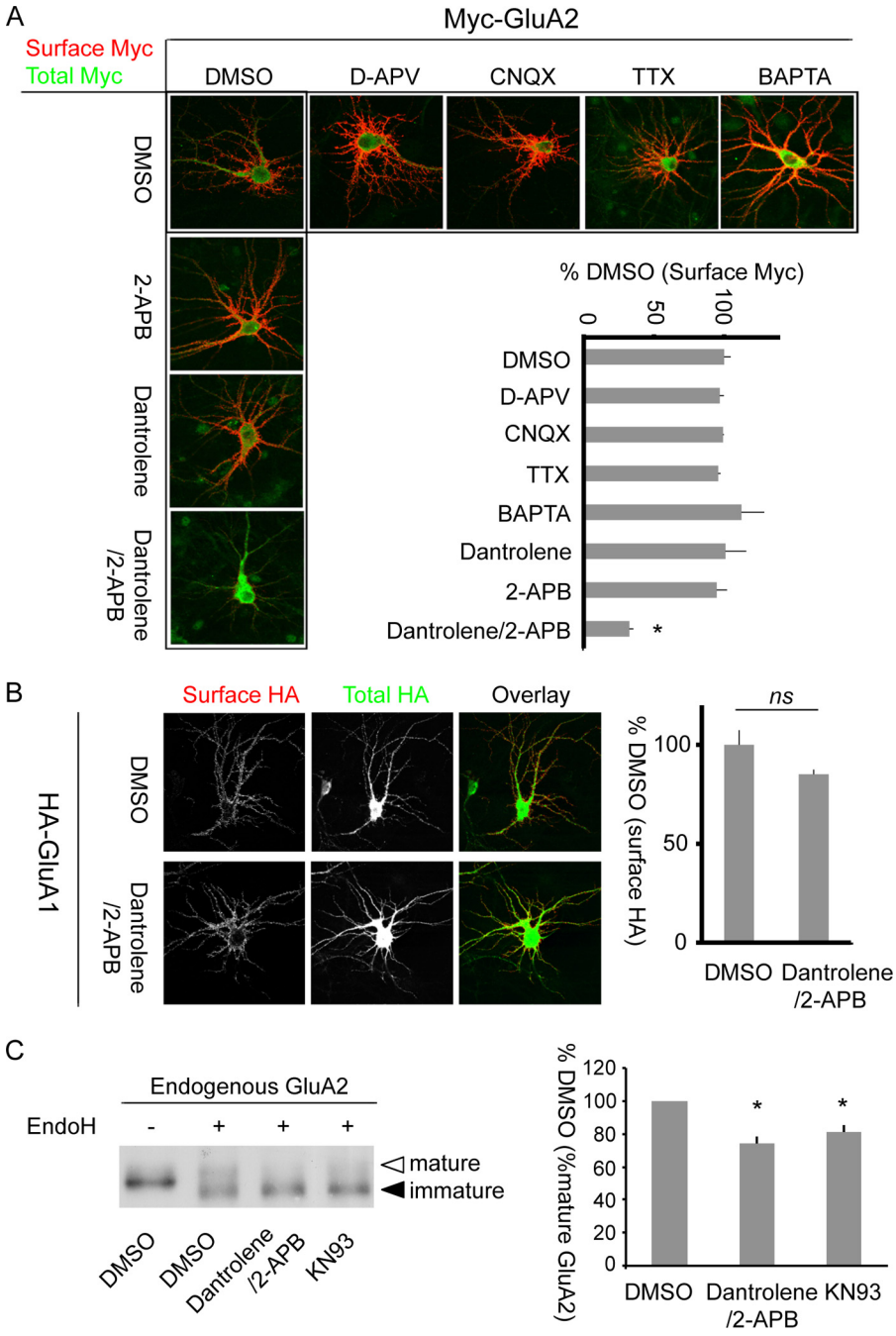

FIGURE 2. **Calcium release from internal stores is required for surface delivery of GluA2.** *A*, cultured hippocampal neurons (18–21 DIV) were infected with Sindbis virus expressing Myc-GluA2. Pharmacological reagents D-APV (100 μM), CNQX (100 μM), Triton X-100 (*TTX*; 2 μM), BAPTA (2 mM), 2-APB (50 μM), and dantrolene (1 μM) or the vehicle, DMSO, were added to the culture medium immediately after infection. Surface and total Myc-labeled receptor was quantitated by immunocytochemistry as described under "Experimental Procedures." The *bar graph* shows the mean ± S.E.; $n = 57$ for DMSO, $n = 29$ for D-APV, $n = 12$ for CNQX, $n = 18$ for Triton X-100, $n = 12$ for BAPTA, $n = 18$ for 2-APB, $n = 18$ for dantrolene, and $n = 18$ for dantrolene/2-APB. Significance was determined with two-tailed *t* test. *$p < 0.001$. *B*, neurons were infected with Sindbis virus expressing HA-GluA1. Pharmacological treatment, immunostaining for surface and internal HA-GluA1, and quantitation of normalized surface levels were performed as described under "Experimental Procedures." The *bar graph* shows the mean ± S.E.; $n = 26$ for DMSO and $n = 30$ for dantrolene/2-APB. Significance was determined with two-tailed *t* test. $p > 0.05$. *ns*, not significant. *C*, neurons were labeled with [^{35}S]Met and [^{35}S]Cys for 90 min and chased for 18 h in the presence of KN93 or dantrolene/2-APB or DMSO (control) as noted, and endogenous GluA2 was immunoprecipitated from whole cell extracts, treated with Endo H, and Endo H sensitivity was analyzed by polyacrylamide gel electrophoresis and autoradiography, all as described under "Experimental Procedures." *, $p < 0.05$ with one-way ANOVA; *, $p < 0.05$ with post hoc Fisher's test.

vating the kinase (39). CaMKIIα H282R coimmunoprecipitated with the WT PICK1 protein and the PICK1 mutant Δ121, which lacks the PICK1 PDZ domain. It also coimmunoprecipitated with a PICK1 mutant that contains the PICK1 coiled-coil domain (CC mutant), whose dimerization forms a BAR domain (33), and it also bound to PICK1 mutant 379Δ, which lacks sequences C-terminal to the coiled-coil domain. However, it did not coimmunoprecipitate with mutant 135Δ, which contains the PICK1 PDZ domain but lacks sequences C-terminal to it, including the coiled-coil

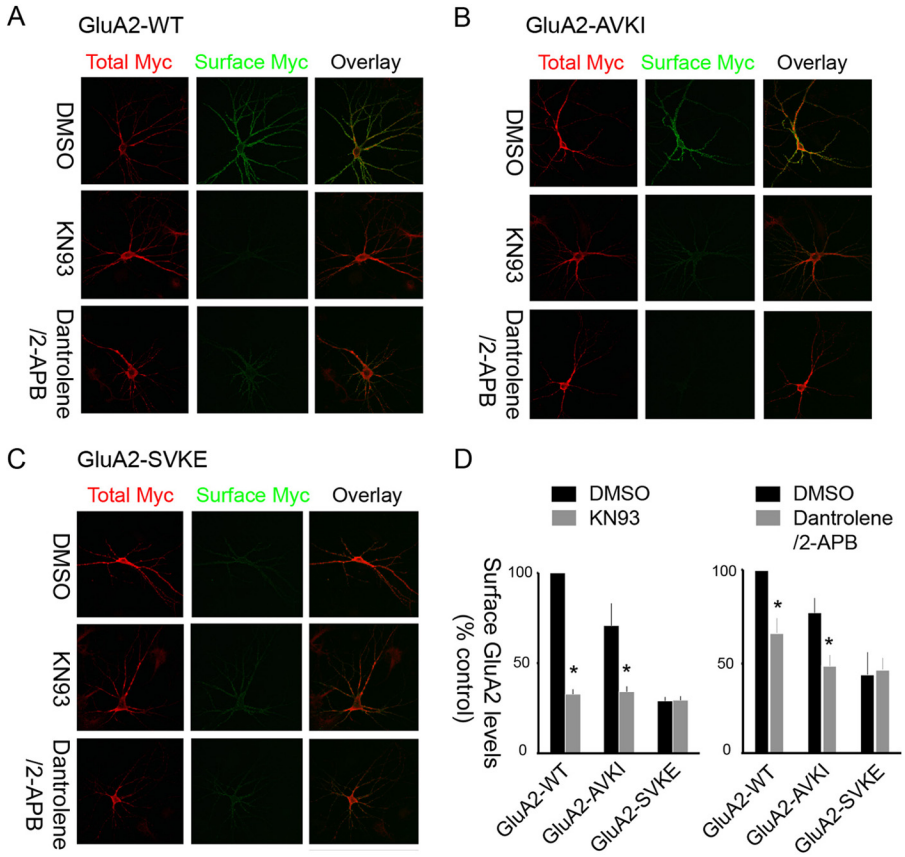

FIGURE 3. **GluA2 C-tail interaction with PICK1 is required for plasma membrane trafficking of GluA2 dependent on ER Ca^{2+} and CaMKII.** Cultured hippocampal neurons (18–21 DIV) were infected with Sindbis virus expressing Myc-GluA2 or its mutants. Pharmacological reagents or the vehicle, DMSO, were added to the culture medium immediately after infection. Surface and total Myc-labeled receptor was quantitated by immunocytochemistry as described under "Experimental Procedures." The subunit species were Myc-GluA2 Wt (*A*), Myc GluA2-AVKI (binds to PICK1 but not ABP or GRIP) (*B*), Myc GluA2 SVKE (binds to neither PICK1 or GRIP or ABP (*C*). *D*, *bar graph* shows the mean ± S.E.; $n = 52$ (from four experiments) for all conditions. Significance was determined with two-tailed *t* test. $p < 0.005$ with one-way ANOVA; *, $p < 0.05$ with post-hoc Fisher's test.

domain. Thus PICK1 coimmunoprecipitation with CaMKIIα requires the coiled-coil region, which can form a BAR domain, rather than the PDZ domain, which is the binding site for PKCα. These results indicate that activated CaMKIIα can enter a complex containing PICK1, dependent upon the PICK1 BAR domain, although it did not determine if the interaction was direct or indirect.

To analyze further the roles of CaMKII domains in the interaction of CaMKIIα with PICK1 and to determine the effects of PICK1 interaction on CaMKIIα subcellular localization, we expressed wild type CaMKIIα or its truncation mutants in HeLa cells together with Δ121, which contains the BAR domain and the site required for CaMKIIα interaction (Fig. 5, *A* and *B*). Δ121 by itself formed intracellular clusters, and CaMKIIα was diffuse on its own (Fig. 5*A*). A CaMKIIα mutant containing only the catalytic domain colocalized with Δ121 (Fig. 5*B*), but constructs containing the catalytic domain plus the regulatory domain or the catalytic, regulatory, and association domains (*i.e.* WT) did not colocalize and neither did the isolated CaMKII association domain or a mutant lacking the catalytic domain (Fig. 5*B*). In agreement with the coimmunoprecipitation studies, this indicated that the site on CaMKII necessary for entering a complex with PICK1 was the CaMKII catalytic domain. Because inclusion of the regulatory domain with the catalytic domain blocked colocalization, it appeared that only CaMKII with the unmasked form of the catalytic domain could enter into a complex with PICK1. The fact that the unmasked catalytic domain, which colocalizes with PICK1, corresponds functionally *in vivo* to the activated kinase, is consistent with the finding made through coimmunoprecipitation that PICK1 enters into a complex with the activated CaMKIIα (Fig. 4) via the catalytic domain of CaMKIIα (Fig. 5*C*).

The PICK1 BAR Domain Colocalizes with Activated Forms of CaMKII—CaMKII H282R colocalized with coexpressed Δ121 in clusters surrounding the nucleus (Fig. 5*D*), but on its own, H282R was diffusely distributed, further supporting that activated CaMKIIα binds directly or indirectly to the BAR domain and suggesting that Δ121 could recruit CaMKIIα to its subcellular location as does PICK1 for PKCα (28).

Binding of Ca^{2+}-calmodulin to CaMKIIα stimulates CaMKIIα Thr-286 autophosphorylation, which releases the regulatory domain from the catalytic domain and stably activates the kinase (40, 41). To evaluate the role of CaMKII autophosphorylation in kinase interaction with the PICK1 BAR domain, we coexpressed Δ121 with wild type CaMKIIα in HeLa cells (Fig. 5*E*). Although immunofluorescence revealed that the bulk of the CaMKII population was diffuse and did not colocalize with Δ121, Thr-286 autophosphory-

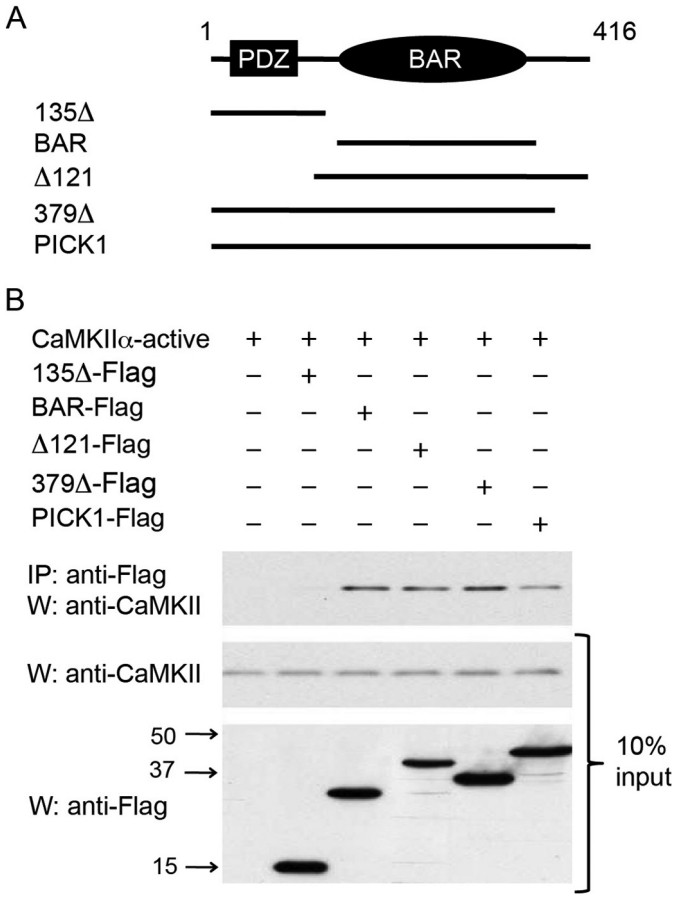

FIGURE 4. **PICK1 forms a complex with a constitutively active CaMKIIα mutant in 293T cells.** *A*, schematic drawings of PICK1 and PICK1 mutants tagged with FLAG in the C termini. *B*, CaMKIIα (H282R) was expressed alone or together with FLAG tagged PICK1 and PICK1 mutants in 293T cells. FLAG-tagged species in cell lysates were immunoprecipitated with an anti-FLAG antibody, and immunoprecipitates (*IP*) were subjected to Western blotting (*W*) assay with an anti-CaMKIIα antibody. 10% input used for immunoprecipitation was also probed with the indicated antibodies to confirm protein expression.

lated CaMKIIα, detected with a phospho-specific antibody extensively colocalized with Δ121 (Fig. 5*E*). This demonstrated that CaMKIIα that had been physiologically activated by autophosphorylation, but not unphosphorylated CaMKIIα, selectively colocalized with Δ121 and suggests that interaction via the PICK1 BAR domain is selective for the physiologically activated kinase. In addition, full-length PICK1 colocalized with the CaMKIIα catalytic domain in cells expressing both constructs (Fig. 5*F*). Furthermore, in hippocampal cultures the expressed catalytic domain of CaMKIIα was diffuse throughout neuronal processes (Fig. 6*A*). Upon coexpression with Δ121, the expressed catalytic domain of CaMKIIα extensively colocalized with Δ121 in intracellular clusters (Fig. 6*B*). Finally Δ121 colocalized with pCaMKIIα in neurons expressing both Δ121 and CaMKIIα-GFP but not in neurons expressing both Δ121 and $CaMKII\alpha_{T286A}$-GFP (Fig. 6, *C* and *D*). Taken together, these results demonstrated that the CaMKIIα catalytic domain colocalized with the PICK1 BAR domain in both heterologous cells and in neurons.

Differential Regulation of GluA2 (R) and GluA2 (Q) Trafficking by CaMKII and Ca^{2+} Release from Internal Stores—Finally we sought to determine the role of the GluA2 pore loop apex residue, Arg-607, which imposes a barrier on assembly of channels with high GluA2 content that is not observed for GluA1, whose pore apex residue is Gln. Indeed, the hippocampal neuron ER contains a pool of dimeric and monomeric GluA2, whereas GluA1 is almost exclusively tetrameric and in the plasma membrane (6, 7). Changing the GluA2 pore apex from Arg to Gln overcomes the assembly barrier leading to rapid ER exit (6). To determine the role of the pore apex residue in the Ca^{2+}-dependent export of GluA2, we compared the sensitivities of GluA2 (R) and GluA2 (Q) to CaMKII or to ER Ca^{2+} release inhibitors. Significantly, trafficking of MycGluA2 (R), but not MycGluA2 (Q), was sensitive to KN93, and MycGluA2 (Q) trafficking exceeded the trafficking level of MycGluA2 (R) both in the presence and absence of KN93 (Fig. 7*A*). In addition, although MycGluA2 (R) is inhibited by the treatment with 2-APB and dantrolene, MycGluA2 (Q) is not sensitive to such treatment (Fig. 7*B*). These data suggested that the pore apex residue mutation to Gln, a change that promotes ER assembly of GluA2, may overcome the ER trafficking dependence on ER Ca^{2+} flux and CaMKII activity. Finally, to test if ER Ca^{2+} release from stores can contribute to CaMKII activation, we treated cultured neurons with DMSO or 2-APB/dantrolene. Five hours after treatment we performed a Western blot assay to measure total CaMKIIα and pCaMKIIα. As shown in Fig. 7*C*, 2-APB/dantrolene treatment significantly reduced the levels of pCaMKIIα, suggesting that Ca^{2+} release from ER stores contributes to CaMKII activation.

DISCUSSION

The AMPAR GluA2 subunit determines receptor biophysical properties, including single channel current, rectification, and Ca^{2+} conductance (4). Therefore, trafficking of GluA2-containing receptors to synapses plays a key role in shaping excitatory synaptic transmission. Trafficking of AMPARs to the synapse begins at the ER, where the AMPAR subunits are synthesized and assembled into a functional ion channel (43). In the present work we report a pathway for controlling trafficking of GluA2 from the endoplasmic reticulum to the plasma membrane. Ca^{2+} released from ER stores via the IP3 and ryanodine receptors activates CaMKII, which directly or indirectly associates with the BAR domain of PICK1 to form a complex that can bind to the C-terminal domain of GluA2. CaMKII activity then promotes the release of GluA2 from the ER whereupon the receptor traffics to the cell surface.

Previous studies from our laboratory have shown that RNA editing of the GluA2 pore, which changes the pore apex residue Gln-607 to Arg, resulted in a majority of intracellular GluA2 residing in the ER (6, 7). This raises a critical question for mechanisms underlying GluA2 trafficking: how is GluA2 trafficking out of the ER regulated? Here we show that GluA2 forward trafficking from the ER is stimulated by CaMKII activity. Pharmacological inhibition of CaMKII activity by either KN93 or a CaMKII inhibitory peptide prevented GluA2 trafficking out of the ER and to the plasma membrane. This suggests that CaMKII functions in the early secretory pathway of GluA2 trafficking. Surprisingly, inhibition of PKC activity failed to prevent GluA2 trafficking to the surface. PKC has been shown to phosphorylate GluA2 and regulates the interaction of GluA2 with

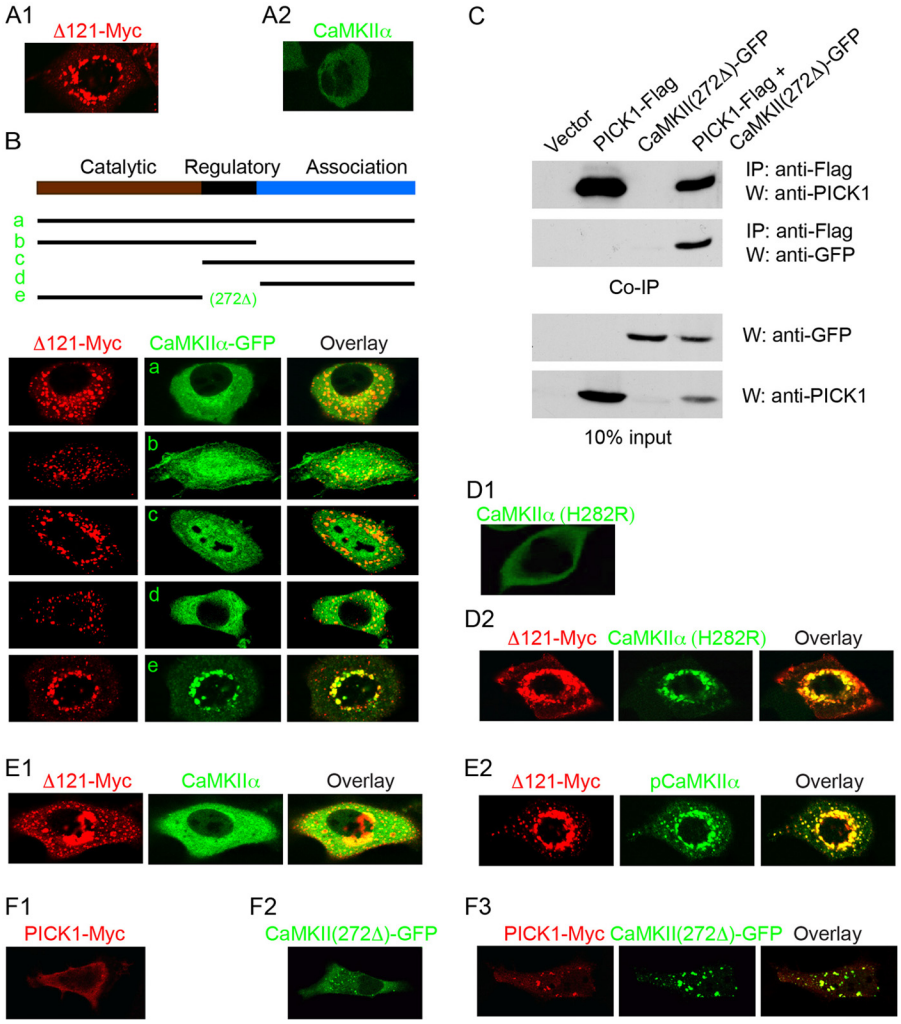

FIGURE 5. **Catalytic domain of CaMKII mediated the interaction with the PICK1 BAR domain.** *A*, distribution of CaMKIIα and PICK1 BAR domain in HeLa cells. *A1*, when expressed on its own, Δ121-Myc formed perinuclear clusters in HeLa cells. *A2*, CaMKIIα was diffuse in the cytoplasm when expressed on its own. *B*, the catalytic domain of CaMKIIα mediated the co-localization with the PICK1 BAR domain. Schematic drawings showed CaMKIIα and its deletion mutants (*a*–*e*) that are fused to GFP on GFP N terminus. Full-length CaMKIIα-GFP or its mutants were transfected together with Δ121-Myc into HeLa cells, and subcellular localizations of both proteins were then examined. Full-length CaMKIIα (*a*) did not extensively co-localize with Δ121-Myc. The mutant that lacks the association domain (*b*) did not co-localize with Δ121-Myc. In addition, the mutants that lack the catalytic domain (*c* and *d*) did not co-localize Δ121-Myc. In contrast, the mutant that only contains the catalytic domain (*e* or 272Δ-GFP) co-localized with Δ121-Myc. *C*, 272Δ-GFP was co-immunoprecipitated with PICK1 in 293T cells. 272Δ-GFP and PICK1-FLAG were expressed alone or together in 293T cells. FLAG-tagged species in cell lysates were immunoprecipitated with an anti-FLAG antibody, and immunoprecipitates (*IP*) were subjected to Western blotting (*W*) assay with an anti-GFP antibody. 10% input used for immunoprecipitation was also probed with indicated antibodies to confirm protein expression. *D*, co-localization of CaMKIIα (H282R) with Δ121-Myc (H282R) in HeLa cells. Expressed on its own, CaMKIIα (H282R) was diffuse in the cytoplasm (*D1*). When co-expressed with Δ121-Myc in HeLa cells, CaMKIIα (H282R) entered into perinuclear clusters where it co-localized with Δ121-Myc (*D2*). *E*, autophosphorylated CaMKIIα at Thr-286 co-localized with Δ121-Myc in HeLa cells. In cells co-transfected with CaMKIIα and Δ121-Myc, CaMKIIα appeared not to extensively co-localize with Δ121-Myc (*E1*). In contrast, an antibody specifically recognizing CaMKIIα phosphorylated at Thr-286 (*pCaMKIIα*) showed that pCaMKIIα co-localized with Δ121-Myc in perinuclear clusters (*E2*). *F*, PICK1-Myc and the CaMKIIα catalytic domain were diffuse in HeLa cells on their own (*F1* and *F2*). When coexpressed, they were colocalized in intracellular clusters (*F3*).

PDZ domain proteins (*e.g.* PICK1 and ABP/GRIP) (28, 37, 38). This suggests that PKC may primarily be involved in trafficking of GluA2 that has already exited the ER and arrived at the plasma membrane, whereas an earlier step in GluA2 trafficking, movement out of the ER, depends on CaMKII activity.

A role of CaMKII in GluA2 trafficking suggests the involvement of Ca^{2+} signaling in GluA2 forward transport. In hippocampus, CaMKII is highly expressed in pyramidal neurons (40, 41). Studies have shown that a substantial fraction of CaMKII is found at excitatory synapses where it may be strategically located to transduce signals generated by Ca^{2+} influx through *N*-methyl-D-aspartate receptors to the regulation of intracellular pathways involved in synaptic plasticity (40, 41). However, in the current work, pharmacological manipulations preventing Ca^{2+} influx from extracellular space failed to suppress GluA2 forward trafficking, suggesting that Ca^{2+} influx through Ca^{2+}-permeable channels at the plasma membrane is unlikely to be necessary for GluA2 trafficking to the surface in hippocampal neurons. This is reminiscent of previous work that showed that blockade of neuronal activity did not prevent GluA2 trafficking to synapses (25). This is also a striking difference from the mechanism underlying trafficking of GluA1-con-

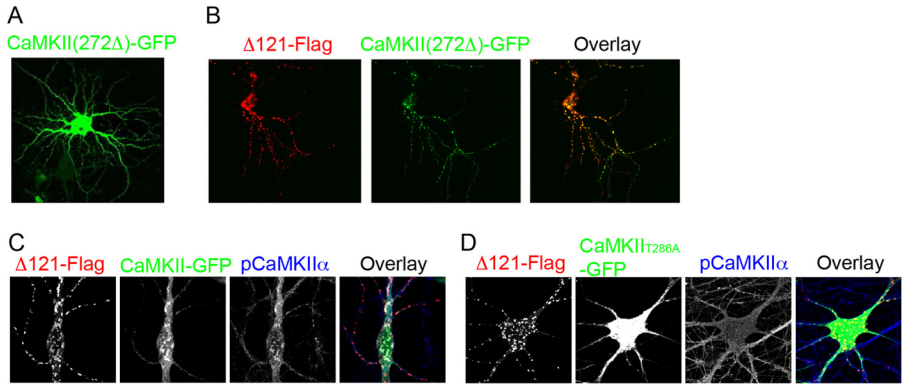

FIGURE 6. **Colocalization of CaMKIIα with PICK1 BAR domain in neurons.** *A*, diffuse distribution of 272Δ-GFP in cultured hippocampal neurons. *B*, when co-expressed, 272Δ-GFP co-localized in clusters with Δ121-Myc in cultured hippocampal neurons. *C* and *D*, when Δ121-FLAG and CaMKIIα-GFP were co-expressed in neurons, Δ121-FLAG co-localized with pCaMKIIα (*C*). In contrast, when Δ121-FLAG and CaMKIIα_{T286A}-GFP were co-expressed in neurons, there was no co-localization of Δ121 with pCaMKIIα (*D*).

taining receptors. Trafficking of GluA1-containing receptors to synapses has been shown to depend on CaMKII activity and Ca^{2+} influx through the synaptic *N*-methyl-D-aspartate receptor (25, 44). Thus, our data suggest that although trafficking of both GluA1 and GluA2 to the surface requires activation of CaMKII, the Ca^{2+} source responsible for CaMKII activation is different for the two receptor subunits. Indeed, we found that Ca^{2+} release from the internal stores, mediated by both IP3 receptors and ryanodine receptors, is crucial for GluA2 but not GluA1 forward trafficking. We further showed that Ca^{2+} release from internal stores acts through CaMKII to regulate GluA2 trafficking. Taken together, these data suggest that Ca^{2+} release from internal stores activates CaMKII, which in turn promotes GluA2 trafficking out of the ER and to the surface.

AMPAR subunits synthesized in the ER are first assembled into dimers, which subsequently undergo tetramerization to form tetrameric channels (45). Our data, however, do not distinguish whether CaMKII activity acts upon fully assembled tetrameric receptors or subunit dimers or monomers or all of these. Nevertheless, our data indicate an important role for CaMKII activity in the maturation of AMPAR GluA2 subunit, potentially through stimulating the assembly of channels with a high proportion of GluA2. Although GluA2 (R) subunits assemble freely with GluA1, channels do not readily form with high GluA2 (R) content, which leads to an accumulation of GluA2 (R) monomers and dimers in the ER (6, 7). The barrier to assembly is relieved in the case of GluA2 (Q) (6, 7). In the current work we found that inhibition of CaMKII did not impede trafficking of GluA2 (Q) to the plasma membrane, which suggests that the step in ER exit that is stimulated by CaMKII is not required for GluA2 (Q). Although this suggests that CaMKII overcomes a barrier imposed by the pore apex residue Arg, the specific role of CaMKII phosphorylation remains to be determined. In a preliminary experiment, we failed to detect a direct phosphorylation of GluA2 by CaMKII (data not shown). Therefore, it is likely that CaMKII may regulate GluA2 trafficking through phosphorylating other factors, for example TARPs (transmembrane AMPA receptor regulatory proteins), which have been shown to be involved in AMPAR trafficking out of the ER (46). Indeed, TARPs have recently been shown to be a phosphorylation substrate of CaMKII (47). Significantly, TARPs have also been proposed to contribute to AMPA receptor assembly in the ER (23, 48). In this scenario, we envision that calcium release from ER activates CaMKII, facilitating direct or indirect interaction of CaMKII with PICK1, which in turn brings complexes containing the PICK1 and CaMKII to GluA2 residing in the ER through the PICK1-GluA2 interaction. Activated CaMKII in the vicinity of GluA2 may stimulate GluA2 trafficking out of ER through TARP-dependent mechanisms.

It is worth noting that blockade of CaMKII activity and ER calcium release has a larger effect on virally expressed GluA2 than endogenous GluA2 (Figs. 1 and 2). Virally expressed GluA2 largely forms GluA2 homomers. In contrast, in hippocampal neurons native GluA2 mainly forms heteromers with GluA1 (10, 11). As many previous studies have shown that GluA1 trafficking out of ER and to the surface is different from GluA2 (2, 3, 16) and our data in this manuscript also showed that GluA1 trafficking is not dependent on ER Ca^{2+} release, it is, therefore, plausible that native GluA1/2 heteromers are less sensitive to KN93 and 2APB/Dan treatment as compared with overexpressed GluA2 homomers.

The finding that GluA2 exit out of the ER is under the regulation of ER calcium release suggests an unappreciated mechanism governing AMPAR maturation. Although the mechanism regulating internal calcium release that underlies GluA2 trafficking remains to be determined, it is possible that metabotropic glutamate receptors, which upon activation can mobilize intracellular calcium release from internal stores via IP3 receptors, may play a role in this. Indeed, a recent study shows that activation of metabotropic glutamate receptor 1 induces long term depression at excitatory glutamatergic synapses on dopamine neurons of the ventral tegmental area by removing synaptic GluA1-containing calcium-permeable receptors, which do not contain the GluA2 subunit, and by replacing them with GluA2-containing receptors (24). This suggests that metabotropic glutamate receptors function in promoting GluA2 trafficking to synapses in ventral tegmental area dopamine neurons.

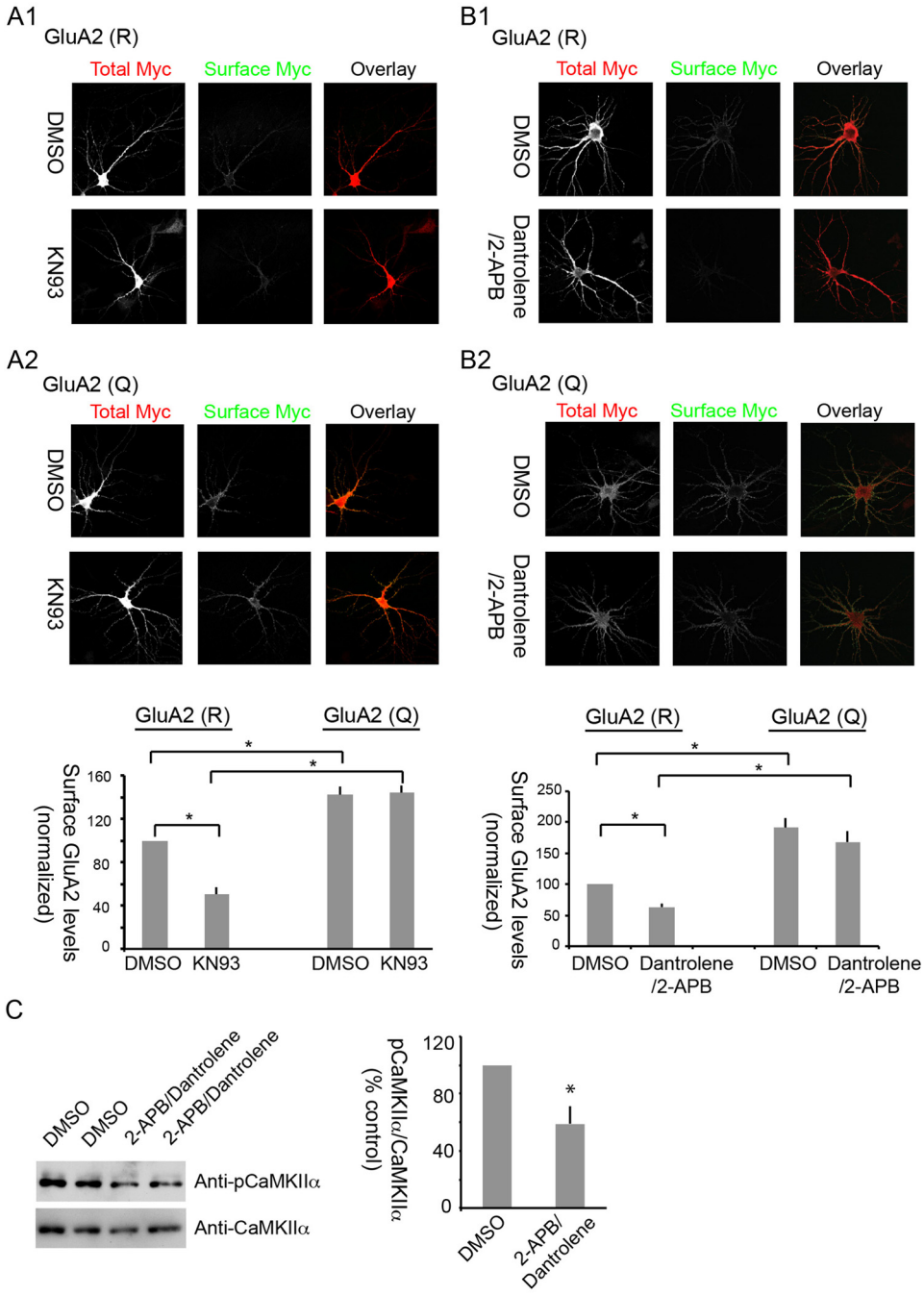

FIGURE 7. **Surface trafficking of GluA2 pore apex residue mutant R607Q displays reduced dependence on CaMKII and ER calcium.** Cultured hippocampal neurons (18–21 DIV) were infected with Sindbis virus expressing Myc-GluA2 (*R*) or Myc-GluA2 (*Q*) in the presence of DMSO (control) or the KN93 (*A1*, *A2*) or 2-APB/dantrolene (*B1*, *B2*). Surface and total Myc-labeled receptor was quantitated by immunocytochemistry as described under "Experimental Procedures." The *bar graph* shows the mean ± S.E.; $n = 32$ for all conditions in *A*; $n = 29$ for DMSO and $n = 30$ for dantrolene/2-APB in *B*. *, $p < 0.05$ with one-way ANOVA. *, $p < 0.05$ with post hoc Fisher's test. *, $p < 0.05$. *C*, 2-APB/dantrolene treatment reduced the levels of neuronal pCaMKIIα. Significance was determined with two-tailed *t* test. *, $p < 0.05$.

Regulation of GluA2 trafficking by intracellular Ca^{2+} dynamics may have important implications in protecting neurons from ischemic insults. Substantial evidence shows that trafficking of GluA2-containing receptors to synapses in the hippocampus is impaired after ischemic insults, which may prime neuronal death after transient ischemia (49). Significantly, metabotropic glutamate receptors, which contribute to cognitive aging, stimulate the formation of IP3 (50), which activates the IP3 receptor, shown here to contribute to GluA2 trafficking. Therefore, promoting the delivery of GluA2-containing receptors to the surface through the mechanism discovered here may be utilized to reduce prevent neuronal death upon ischemia.

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