Role of TARP interaction in S-SCAM-mediated regulation of AMPA receptors

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Abbreviations: ABP, AMPA receptor binding protein; AIP1, atrophin-1 interacting protein; AMPAR, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; EGFP, enhanced green fluorescent protein; GRIP, glutamate receptor-interacting protein; MAGUK, membrane associated guanylate kinase; MAGI-2, membrane associated guanylate kinase inverted-2; PDZ, postsynaptic density-95/disc large/zonula occludens-1; PICK-1, protein interacting with protein kinase C; S-SCAM, synaptic scaffolding molecule; TARP, transmembrane AMPAR regulatory protein

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Analyses of S-SCAM PDZ interactions based on the known examples revealed

Introduction

AMPA receptors are glutamate-gated ion channels that mediate the majority of excitatory synaptic transmission, and are crucial substrates for the expression of synaptic plasticity. For example, rapid trafficking of AMPARs into and out of excitatory synapses is responsible for long-term potentiation and long-term depression, which underlies learning and memory. Furthermore, synaptic scaling, which provides homeostatic mechanisms for the stabilization of neural network integrity, is also mediated by activity-dependent changes in the levels of synaptic AMPARs. During the dynamic trafficking of AMPARs, scaffolding proteins play a crucial role by bringing together other proteins required for the transport, insertion, anchorage/stabilization, and removal of AMPARs. PSD-95 and related proteins of the membrane-associated guanylate kinase (MAGUK) family represent such synaptic scaffolds involved in AMPAR regulation at the postsynaptic density. S-SCAM is a new and the latest member of scaffolding proteins involved in the regulation of synaptic AMPARs. In contrast to PSD-95 that regulates plasticity-involved AMPARs, S-SCAM was found to be critical for the regulation of the maintenance pool of AMPARs, which is characterized by NSF-sensitive, GluA2-containing AMPARs. Intriguingly, MAGUKs, except for SAP-97, do not bind directly to AMPARs, but indirectly through TARPs. Here we addressed the mechanism by which S-SCAM interacts with AMPARs and the contribution of TARPs in this process.

Results and Discussion

Scaffolding proteins are involved in the incorporation, anchoring, maintenance, and removal of AMPA receptors (AMPARs) at synapses, either through a direct interaction with AMPARs or via indirect association through auxiliary subunits of transmembrane AMPAR regulatory proteins (TARPs). Synaptic scaffolding molecule (S-SCAM) is a newly characterized member of the scaffolding proteins critical for the regulation and maintenance of AMPAR levels at synapses, and directly binds to TARPs through a PDZ interaction. However, the functional significance of S-SCAM–TARP interaction in the regulation of AMPARs has not been tested. Here we show that overexpression of the C-terminal peptide of TARP-γ2 fused to EGFP abolished the S-SCAM-mediated enhancement of surface GluA2 expression. Conversely, the deletion of the PDZ-5 domain of S-SCAM that binds TARPs greatly attenuated the S-SCAM-induced increase of surface GluA2 expression. In contrast, the deletion of the guanylate kinase domain of S-SCAM did not show a significant effect on the regulation of AMPARs. Together, these results suggest that S-SCAM is regulating AMPARs through TARPs.
that all six PDZ domains of S-SCAM are involved in class I PDZ interaction (Table 1), which bind to the C-terminal amino acid sequence of X-S/T-X-V/L (where X stands for any amino acids). In contrast, C-terminals of GluA2 and GluA3 possess class II PDZ interaction motifs (S-V-K-I; belongs to X-Ψ-X-Ψ consensus where Ψ represents hydrophobic amino acids). Thus, given the class specificity of PDZ-ligand interaction, it is unlikely that S-SCAM directly binds to GluA2 or GluA3. Consistent with this prediction, microarray assays showed negative interaction between the PDZ domains (PDZ-1, -4, -5) of S-SCAM and GluA2 or GluA3. S-SCAM is highly likely to interact with AMPARs indirectly through other mediator protein(s).

TARP has the class I PDZ interaction motif of T-T-A-V, and is the best candidate for this role as it has the ability to directly bind both AMPARs and S-SCAM. To address the role of TARPs in S-SCAM-mediated regulation of AMPARs, we examined the effect of preventing the S-SCAM—TARP interaction on surface AMPAR levels. To achieve this, we generated an EGFP fusion protein that has a C-terminal addition of the last 14 amino acids of TARP γ-2 (designated GFP-StgC14), whose sequence is highly conserved in all type I TARP family of proteins, especially for the critical last four amino acids (Fig. 1A). C-terminal peptides of PDZ ligands (typically 10–15 amino acids long, either as forms of synthetic peptides or fusion protein to other carrier proteins) serve as competitive inhibitors effective for preventing the PDZ-ligand interaction. This strategy has been successfully used to demonstrate the role of specific PDZ-ligand interaction, including AMPARs, PSD-95, and TARPs. Overexpression of GFP-StgC14 alone in cultured hippocampal neurons reduced surface GluA2 (sGluA2) level significantly (100 ± 6 vs 70 ± 4%, GFP Control vs GFP-StgC14, p < 0.001), indicating that GFP-StgC14 indeed prevented the function of endogenous TARPs (Fig. 1B and C). In contrast, S-SCAM overexpression increased sGluA2 level by > 1.8-fold (p < 0.001). However, co-expression of GFP-StgC14 with S-SCAM completely abolished the S-SCAM-induced increase of sGluA2 levels (183 ± 14 vs 88 ± 6%, S-SCAM vs S-SCAM + GFP-StgC14, p < 0.001; compared with GFP control, p = 0.57; Fig. 1B and C). These results strongly suggest that S-SCAM depends on the PDZ interaction with TARPs for the regulation of AMPARs.

To further corroborate the results, we tested a mutant S-SCAM that has a deletion of the last PDZ domain (designated ΔPDZ-5), which is one of the three
This may be important for S-SCAM and PSD-95 MAGUKs to serve as AMPAR-immobilizers (or "slots") at synapses. In addition, by freeing-up the cytoplasmic PDZ ligands, GluA subunits can also interact with proteins such as PICK1, GRIP/ABP, and SAP97. This allows AMPARs to make GluA subunit-specific interactions that enable complex differential trafficking that is necessary for the expression of various types of synaptic plasticity.

**Materials and Methods**

**Expression constructs.** Myc-tagged S-SCAM WT and ΔPDZ-5 (S-SCAM-8) expression constructs were gifts from Dr Y. Hata (Tokyo Medical and Dental University).23 The ΔGK construct has a deletion in amino acids sequence between 165D and 293T and was made by site-directed mutagenesis. GFP-StgC14 was constructed by inserting the following annealed oligonucleotides between the EcoRI and BamHI sites of pEGFP-C1 (Clontech): Top strand 5'-AAT TCT CAC GCC AAC ACA GCC AAC CGC CCG ACC ACC CCC GTA TGA G-3'; Bottom strand 5'-TAT CCT CAT ACG GCC GTG GTC CGG CGG TTG GCA GTG TTG GCG TCG AGA G-3'.
Neuron transfection and immunocytochemistry. Dissociated hippocampal neurons grown on top of coverslips that were pre-coated with 37.5 μg/ml poly-d-lysine (BD) and 2.5 μg/ml laminin (BD) in a standard 12-well tissue culture plate (Corning). Hippocampi were isolated from E18–19 rat embryos and dissociated with trypsin and trituration. Dissociated neurons were plated at the density of 75,000 cells per coverslip and grown in Neurobasal media (Life Technologies) supplemented with B27 (Life Technologies). Neurons were transfected at DIV 14 using Lipofectamine 2000 reagent (Life Technologies) as described previously.9 After 2 d post-transfection, neurons were fixed in 4% formaldehyde/4% sucrose/1 x PBS for 5 min at room temperature. Surface GluA2 staining was performed by incubating fixed neurons with mouse anti-GluA2 (MAB397, Millipore) diluted at 5 μg/ml in ADB (4% normal horse serum/0.1% BSA/1 x PBS). After permeabilization by incubation in cold (-20°C) methanol for 2 min, neurons were further incubated with rabbit anti-myc antibody (1:100 dilution; Cell Signaling Technology) diluted in ADB. Bound antibodies were visualized by incubating with anti-mouse Cy3 (1:500 dilution; Jackson Immunoresearch) and anti-rabbit Alexa Fluor 488 (1:250 dilution; Life Technologies). Immunofluorescence images were acquired by confocal microscopy and analyzed as described previously.9 A one-way ANOVA with a Tukey’s post hoc test was used for the statistical analyses of the data.

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