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SAP102 Mediates Synaptic Clearance of NMDA Receptors

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SUMMARY

Membrane-associated guanylate kinases (MAGUKs) are the major family of scaffolding proteins at the postsynaptic density. The PSD-MAGUK subfamily, which includes PSD-95, PSD-93, SAP97, and SAP102, is well accepted to be primarily involved in the synaptic anchoring of numerous proteins, including N-methyl-D-aspartate receptors (NMDARs). Notably, the synaptic targeting of NMDARs depends on the binding of the PDZ ligand on the GluN2B subunit to MAGUK PDZ domains, as disruption of this interaction dramatically decreases NMDAR surface and synaptic expression. We recently reported a secondary interaction between SAP102 and GluN2B, in addition to the PDZ interaction. Here, we identify two critical residues on GluN2B responsible for the non-PDZ binding to SAP102. Strikingly, either mutation of these critical residues or knockdown of endogenous SAP102 can rescue the defective surface expression and synaptic localization of PDZ binding-deficient GluN2B. These data reveal an unexpected, nonscaffolding role for SAP102 in the synaptic clearance of GluN2B-containing NMDARs.

INTRODUCTION

NMDARs are ionotropic glutamate receptors that play important roles in excitatory neurotransmission, synaptic plasticity, and neuronal development (Lau and Zukin, 2007). Precise regulation of NMDAR trafficking and synaptic localization is essential for these functions. NMDARs are localized at the postsynaptic membrane, and are stabilized through interactions with membrane-associated guanylate kinases (MAGUKs)(Wenthold et al., 2003). PSD-93, PSD-95, SAP97, and SAP102 are collectively known as PSD-MAGUKs and possess three PDZ domains, a Src homology 3 (SH3) domain and an inactive guanylate kinase (GK) domain (Elias and Nicoll, 2007). The PDZ domains bind to the C termini of NMDARs, whereas the SH3 and GK domains interact with cytoskeletal proteins and intracellular signaling complexes. Although PSD-MAGUKs share a common modular structure, each family member possesses a distinct N-terminal domain. The N termini of PSD-95, PSD-93, and SAP97 contain either a pair of palmitoylated cysteines that stabilize them at synapses or an L27 domain capable of multimerization (Schlüter et al., 2006). The N terminus of SAP102, however, is not palmitoylated and does not have an L27 domain and thus has an unknown function. Recently, we found that the N terminus of SAP102 contains a GluN2B-specific NMDAR binding site (Chen et al., 2011).

Functional NMDARs are heterotetramers assembled with two GluN1 subunits and two GluN2 (GluN2A-GluN2D) and/or GluN3 (GluN3A- GluN3B) subunits. The GluN2 subunits have distinct expression patterns with GluN2A and GluN2B being the major GluN2 subunits in the forebrain. The GluN2 content of NMDARs determines their channel properties, as well as their coupling to distinct intracellular signaling cascades (Cull-Candy and Leszkiewicz, 2004). During development, GluN2B is predominantly expressed in immature neurons and the expression of GluN2A gradually increases, leading to a synaptic switch from GluN2B- to primarily GluN2A-containing NMDARs. In mature neurons, GluN2A-containing NMDARs are primarily localized at synapses, whereas GluN2B-containing receptors are still present at synapses, but also enriched at extrasynaptic sites (Li et al., 2002; Stocca and Vicini, 1998; Tovar and Westbrook, 1999). In addition, GluN2B-containing NMDARs undergo more robust endocytosis (Lavezzari et al., 2004; Roche et al., 2001) and have higher surface mobility than GluN2A-containing receptors (Groc et al., 2006). Biochemical studies have shown that GluN2A preferentially binds to PSD-95 and GluN2B preferentially binds to SAP102 (Sans et al., 2000; van Zundert et al., 2004; although, see Al-Hallaq et al., 2007), and it has been proposed that PSD-95 and SAP102 play a role in the subunit-specific regulation of receptor trafficking and localization (van Zundert et al., 2004). Interestingly, SAP102 is not palmitoylated and is highly mobile at the postsynaptic density, similar to GluN2B (Zheng et al., 2010).

GluN2A and GluN2B share an identical PDZ ligand. However, we have recently identified a secondary non-PDZ GluN2B binding site in the N-terminal domain of SAP102, which might allow for preferential binding of SAP102 and GluN2B (Chen et al., 2011). In the present study, we investigated the role of the PDZ-independent interaction of GluN2B with SAP102 in NMDAR trafficking. We identify two amino acids within the GluN2B C terminus (D1391; D1392) that are critical for binding to the SAP102 N-terminal domain.

Mutating the PDZ ligand on GluN2B profoundly reduces surface expression of NMDARs (Chung et al., 2004; Prybylowski et al., 2005) and the activity-dependent phosphorylation of GluN2B within the PDZ ligand by casein kinase 2 (CK2) drives the removal of GluN2B from the synapse (Sanz-Clemente et al., 2010). We now show that disruption of the secondary SAP102 binding site on GluN2B unexpectedly and dramatically rescues the surface and synaptic expression of PDZ binding-deficient GluN2B. Furthermore, RNAi knockdown of SAP102 also rescued the surface and synaptic expression defect of the GluN2B PDZ ligand mutant. Together, our findings reveal an unexpected role for the PDZ-independent interaction between SAP102 and GluN2B in mediating the synaptic clearance of GluN2B-containing NMDARs.

RESULTS

To identify the PDZ-independent SAP102 binding site in GluN2B, we analyzed a series of GluN2B truncations using a yeast two-hybrid assay. We found a region of the GluN2B C terminus (amino acids 1353–1441) that is required for the non-PDZ interaction (Figure 1A), but attempts to delineate this region further were limited by self-activation of the yeast two-hybrid system. We next generated GluN2A-GluN2B chimeras and found that the GluN2A (1304–1400)-GluN2B (1422–1482) chimera (Figure 1A) interacted with the SAP102 N-terminal domain (Figure 1A), suggesting a short region in GluN2B (1422–1441) is critical for the interaction. Surprisingly, however, this region alone did not interact with the N terminus of SAP102. We therefore postulated that the adjacent region of GluN2B (1353–1400) is a key molecular determinant for binding to the SAP102 N-terminal domain, whereas 1422–1441 is simply permissive but cannot interact independently. Consistently, we found that GluN2B (1–1441) and GluN2B (1–1400), but not GluN2B (1–1353), coimmunoprecipitated with SAP102 (Figures 1A and S1). Based on the chimeras, we hypothesized that the critical residues in the GluN2B (1353–1400) segment must be conserved between GluN2A and GluN2B, but that these residues only interact with the SAP102 N terminus when the GluN2B (1422–1441) region is also present. To test this possibility, we made specific amino acid substitutions within GluN2B (1353–1400), which are identical in the analogous region of GluN2A (1304–1400) (Figure 1B). We first targeted several charged residues and found that the GluN2B D1391K and D1392K mutations (Figure 1B), but not D1378K (Figure S1), disrupted the PDZ-independent binding to SAP102. Moreover, the GluN2B D1391K and D1392K (GluN2B DD-KK) double mutant further reduced the interaction (Figure 1B). We also examined the PDZ-independent interaction using a coimmunoprecipitation assay in HEK293 cells expressing GluN1, GluN2B DD-KK or GluN2B DD-KK S1480E, and SAP102. We found that GluN2B DD-KK S1480E showed a 54% reduction in SAP102 binding compared with GluN2B S1480E (Figure 1B), demonstrating that GluN2B D1391 and D1392 are involved in the interaction of GluN2B with the SAP102 N terminus.

Phosphorylation of Ser1480 within the GluN2B PDZ-binding motif by CK2 disrupts the interaction of GluN2B with PSD-95 and SAP102 and decreases surface expression of GluN2B (Chung et al., 2004). However, the role of the PDZ-independent interaction of GluN2B with SAP102 in NMDAR trafficking is not known. Therefore, we used the GluN2B DD-KK double mutant to study whether the PDZ-independent interaction with SAP102 regulates surface expression of GluN2B. We expressed GFP-GluN2B WT or GFP-GluN2B DD-KK in hippocampal neurons and visualized surface-expressed receptors with an anti-GFP antibody. Surface expression of GFP-GluN2B DD-KK was similar to that of GFP-GluN2B WT (Figure 2). We then investigated whether disruption of both the PDZ and PDZ-independent interactions could affect NMDAR trafficking. To this end, we mutated Ser1480 of GluN2B to glutamate to mimic phosphorylation of Ser1480, which disrupts the PDZ binding of GluN2B and examined the surface expression of GFP-GluN2B S1480E and a combined GFP-GluN2B DD-KK S1480E mutant. Consistent with previous reports (Chung et al., 2004), surface expression of GFP-GluN2B S1480E was dramatically reduced compared to WT (Figure 2). Strikingly, surface expression was recovered with GFP-GluN2B DD-KK S1480E and was similar to wild-type GluN2B (Figure 2), demonstrating that DD-KK mutations, which disrupt binding to the SAP102 N terminus, rescue the surface expression defect of GFP-GluN2B S1480E.

Disruption of an endocytic motif (YEKL) near the C terminus of GluN2B that binds to the clathrin adaptor protein complex AP-2 also restores the surface expression of GluN2B that lacks PDZ binding (Prybylowski et al., 2005; Sanz-Clemente et al., 2010) Thus, to determine if the surface expression rescue seen with the GluN2B DD-KK S1480E mutant (Figure 2) is due to decreased AP-2 binding, we used a yeast two-hybrid binding assay to examine the interaction of GluN2B with μ2, the medium chain of AP-2 that binds to the GluN2B YEKL motif (Lavezzari et al., 2003). However, the DD-KK mutations had no effect on GluN2B binding to μ2 (Figure S2), showing that the rescue of the surface expression of GluN2B S1480E is not mediated by the disruption of AP-2 binding.

To physiologically assess the effects of GluN2B mutations on the synaptic localization of NMDARs, we developed a genetic molecular replacement strategy in organotypic hippocampal slice cultures prepared from mice with conditional knockout alleles for both GluN2A and GluN2B ($Grin2a^{fl/fl}Grin2b^{fl/fl}$) (Granger et al., 2011). We have previously shown that neonatal injection of a Cre-expressing virus into the hippocampus of $Grin2a^{fl/fl}Grin2b^{fl/fl}$ mice completely eliminates synaptic NMDAR responses in CA1 pyramidal neurons by postnatal day 14, suggesting that GluN2A and GluN2B account for all synaptic NMDARs in these cells (Gray et al., 2011). Here, hippocampal slice cultures prepared from the $Grin2a^{fl/fl}Grin2b^{fl/fl}$ mice were biolistically transfected with Cre at DIV2-4, and paired whole-cell recordings were obtained from a Cre-expressing and a