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The MUPP1–SynGAPα protein complex does not mediate activity-induced LTP☆

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At excitatory synapses of hippocampal neurons, the multi-PDZ domain scaffolding protein, MUPP1, assembles the NR2B subunit of the NMDA receptor (NMDAR), Ca^{2+}–calmodulin kinase (CamKII), and the α1 isoform of the postsynaptic density GTPase activating protein, SynGAP (SynGAPα). In order to evaluate the role of this complex in excitatory synaptic neurotransmission we specifically disrupted MUPP1–SynGAPα interactions in CA1 neurons of acute hippocampal slices using intracellular perfusion with peptides derived from SynGAPα–MUPP1 binding domains. Disruption of the interaction between MUPP1 and SynGAPα with two complementary peptides derived from SynGAP and MUPP1 mutual binding sites resulted in enhancement of excitatory postsynaptic currents (EPSCs). This potentiation did not occlude pairing-induced long-term potentiation (LTP); indeed the amplitude of postsynaptic responses of activity-potentiated synapses was further increased. Pre-potentiation of excitatory synapses with theta burst stimulations did not modify the MUPP1–SynGAPα-dependent enhancement of EPSCs. Our data suggest that MUPP1–SynGAPα complex dissociation triggers a mechanism for AMPAR enhancement that is distinct from activity-induced LTP.

Keywords: NMDA receptor; SynGAP; LTP; CaMKII; MUPP1

Introduction

NMDAR-dependent LTP of glutamatergic synapses is a model system for the understanding of memory and learning. Synaptic GTPase activating protein (SynGAP; Chen et al., 1998; Kim et al., 1998) is a potential-signal transduction intermediate between NMDAR activation and LTP. SynGAP is phosphorylated via NMDAR-dependent CaMKII, (Krapivinsky et al., 2004;Oh et al., 2004), the enzyme critical for NMDA-dependent LTP (Malinow et al., 1989;Silva et al., 1992). In the CNS, SynGAP splice variants (Li et al., 2001) encode 5 distinct SynGAP proteins. Four of these isoforms (α1, α2, β, and γ) have unique C-terminal tails; αι contains a C-terminal PDZ-binding domain and indirectly binds to CaMKII via MUPP1 (Krapivinsky et al., 2004) while β-isoforms lacking the PDZ-binding motif directly bind the non-phosphorylated form of CaMKII (Li et al., 2001). In vivo disruption of the interaction between MUPP1 and SynGAPα by peptides derived from MUPP1–SynGAPα binding domains, resulted in SynGAP dephosphorylation, inactivation of p38 MAP kinase, and an increase of the frequency and amplitude of the AMPA-receptor-mediated mEPSCs (Krapivinsky et al., 2004). The magnitudes of the mEPSCs frequency and amplitude changes resembled those observed during long lasting potentiation of mEPSCs induced by synaptic NMDAR activation in dissociated hippocampal cultures (Lu et al., 2001), called "LTP of mEPSCs". Finally, mice in which one allele of SynGAP had been deleted, exhibited decreased activity-dependent LTP (Kim et al., 2003;Komiyama et al., 2002). Taken together, the evidence suggests that SynGAPα is part of the NMDAR-mediated Ca^{2+} signal transduction cascade that controls excitatory neurotransmission.

Here we examine whether the MUPP1/SynGAPα complex is involved in NMDA-dependent long-term potentiation of EPSCs in CA1 pyramidal neurons. We find that disruption of the MUPP1/SynGAPα complex using two complementary peptides derived from SynGAP and MUPP1 mutual binding sites effectively potentiated EPSCs. This potentiation did not occlude pairing-induced LTP, but rather increased LTP amplitude. LTP of excitatory synapses induced by theta burst stimulation of Schaffer's collaterals did not occlude EPSC potentiation caused by MUPP1/SynGAPα complex dissociation. These data suggest that the MUPP1 complex effectively regulates the AMPAR EPSCs, but is not itself the signal mediating NMDAR potentiation of AMPARs during LTP.

☆ MUPP1, SynGAP and LTP.

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Results

MUPP1–SynGAPα complex disruption increases EPSC amplitudes

Previous experiments demonstrated that peptides dissociating the MUPP1/SynGAPα-complex potentiated mEPSCs in cultured hippocampal neurons (Krapivinsky et al., 2004). Exogenous molecules in the culture media, however, might influence the regulation of signaling pathways in primary neuronal cultures. Therefore, the initial experiments in this study were designed to examine whether peptide interference would modify the properties of AMPAR-mediated neurotransmission in acute hippocampal slices. For this reason, we analyzed the properties of EPSCs in CA1 neurons evoked by stimulation of the CA2 pyramidal layer. Neurons were internally perfused (via the patch pipette) with SynGAP111, a peptide encoding the last 111 amino acids of SynGAP111α. This peptide specifically disrupts native MUPP1/SynGAPα complexes (Krapivinsky et al., 2004). SynGAP33, a truncated SynGAP that neither binds MUPP1 nor disrupts the SynGAP/MUPP1 complex (Krapivinsky et al., 2004) served as the negative control for peptide specificity. Consistent with potentiation of mEPSCs observed in dissociated neuronal cultures, intraneuronal SynGAP111 peptide perfusion increased EPSC amplitudes >2-fold in slice CA1 neurons over neurons dialyzed with SynGAP33 peptide (Figs. 1A, B). Differences in EPSC amplitudes became statistically significant 6 min after the start of whole-cell recording. Steady-state levels were achieved 8–10 min after initiation of whole-cell recording.

Additional experiments using PDZ13 peptide determined if specific MUPP1/SynGAPα complex disruption, rather than SynGAP111 interaction with other proteins, potentiated EPSCs amplitudes. PDZ13 is a peptide that encodes the 13th PDZ domain of MUPP1. The only common feature of SynGAP111 and PDZ13 is their ability to dissociate the MUPP1/SynGAPα complex. A homologous peptide that did not dissociate the MUPP1/SynGAPα complex acted as the control (mutated PDZ domain; PDZm, (Krapivinsky et al., 2004), see Experimental methods). Intraneuronal perfusion with PDZ13, not PDZm, potentiated EPSCs (Figs. 1C, D). Differences in EPSC

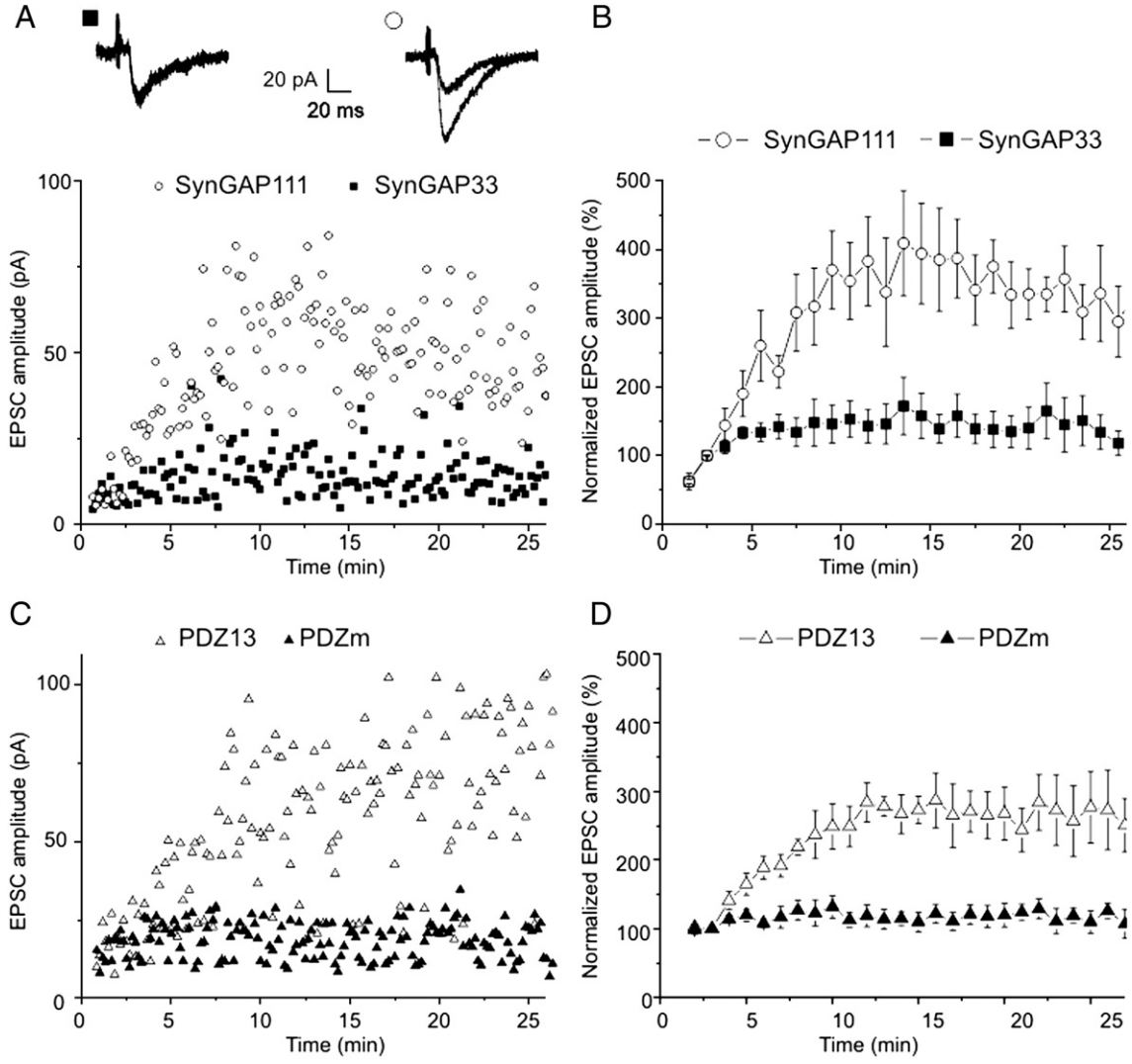

Fig. 1. Peptides SynGAP111 and PDZ13 increase EPSC amplitudes evoked in CA1 neurons of rat acute hippocampal slices. A,C. Scatterplots of single experiments in which the indicated peptides were included in the patch pipette. The inset in A shows superimposed average traces of 5 consecutive EPSCs recorded 3 and 20 min after whole-cell formation with SynGAP33 (left traces) and SynGap111 (right traces). B,D. Normalized mean values from experiments performed with the indicated peptides in the patch pipette (5 experiments under each condition).