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Research Report

Suppression of long-term facilitation by Rab3–effector protein interaction

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Abstract

Long-term facilitation (LTF) in *Aplysia* is achieved by the modulation of presynaptic release. However, the underlying mechanism that might be related with the regulation of synaptic vesicle release remains unknown. Since Rab3, a neuronal GTP-binding protein, is known to be a key regulator of synaptic vesicle fusion, we investigated the involvement of Rab3 in LTF. To address this issue, we examined the effect of overexpression of wild type *Aplysia* Rab3 (apRab3) and its mutant forms on LTF. Overexpression of either apRab3 Q80L, a constitutively active apRab3 mutant, or wild type apRab3 completely inhibited LTF. This inhibitory role of apRab3 appears to be mediated by an interaction with an effector molecule(s), possibly Rim. Expression of apRab3 Q80L, V54E double mutant, which do not bind effector molecules such as Rim or Rabphilin, had no effect on LTF. Furthermore, expression of apRab3 Q80L, F18L, D19E triple mutant, which has reduced binding activity with Rim but normally binds with Rabphilin, enhanced evoked basal synaptic release, and the increase in synaptic strength occluded LTF. In conclusion, our data suggest that apRab3 may act as a negative clamp of LTF through the interaction with effector protein(s), possibly Rim.

Theme: Excitable membranes and synaptic transmission
Topic: Presynaptic mechanisms

Keywords: Aplysia; apRab3; 5-HT; Long-term facilitation; Basal synaptic transmission; Microinjection

1. Introduction

The modulation of synaptic release from the presynaptic terminal is an essential mechanism of the changes in synaptic strength, which occur during learning-related synaptic plasticity [1,2,18,22]. Two important interrelated processes, which include (1) the modulation of Ca^{2+} influx into the presynaptic terminal and (2) the modulation of synaptic vesicle machinery involved in docking, priming, fusion, and exocytosis are key mechanisms for the increase in synaptic vesicle release from the presynaptic neuron [33]. Long-term and short-term synaptic facilitation (STF), which is induced by 5-HT (5-hydroxytryptamine or serotonin) treatment, is a well-established cellular basis for learning and memory in *Aplysia*. The synaptic connections between the sensory and motor neurons of *Aplysia* undergo short-term facilitation lasting <2 h and long-term facilitation lasting at least 24 h in in vitro culture in response to either a brief single or repeated pulses of 5-HT [6,9,23,27]. Whereas short-term facilitation requires the modification of pre-existing proteins, long-term facilitation requires a cascade of gene activation [4,23]. Long-term and short-term facilitation are achieved by a change in the efficacy of synaptic transmission at sensory to motor neuron synapse. The quantal analysis of spontaneously released miniature excitatory postsynaptic potential reveals that enhancement of

Abbreviations: LTF, long-term facilitation; STF, short-term facilitation; 5-HT, 5-hydroxytryptamine or serotonin; apRab3, *Aplysia* Rab3; LTP, long-term potentiation; EGFP, enhanced green fluorescent protein; EPSP, excitatory postsynaptic potential; PPF, paired-pulse facilitation; GDI, GDP dissociation inhibitor; GEP, GDP–GTP exchange protein; Rim, Rab3-interacting molecule

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synaptic strength during long-term and short-term facilitation (STF) induced by 5-HT is caused by an increase in the number of neurotransmitter released by the presynaptic sensory neuron [3,8]. These results imply that synaptic vesicle proteins may be essentially involved in 5-HT-induced long-term facilitation in *Aplysia*.

Rab3 is a member of a family of more than 30 low molecular weight GTP-binding proteins. Generally, Rab3 is attached to synaptic vesicles in the GTP-bound state and complexed with effector proteins such as Rim or Rabphilin in a GTP-bound form. Ca^{2+}-triggered membrane fusion of synaptic vesicle leads to hydrolysis of GTP-Rab3 to GDP-Rab3 following the dissociation of Rab3 from effector molecules and synaptic vesicles [32]. Rab3A plays a key role in the regulation of a late step in synaptic vesicle fusion [10]. Emerging evidence suggests that Rab3A and its effector molecules related with synaptic vesicle release are essential for presynaptically expressed long-term potentiation (LTP) in certain mammalian brain areas. In knock-out studies in mice, Rab3A is shown to be critical for presynaptically expressed LTP at hippocampal mossy fiber synapses [5,24]. In addition, PKA-mediated phosphorylation of active zone protein RIM1α (Rab3-interacting molecule), one of Rab3A effector molecules, directly induces presynaptic LTP in cerebellar parallel fiber synapses [21].

Although Rab3 acts as a key factor in synaptic transmission process and synaptic plasticity, the possible role of Rab3 during long-term facilitation in *Aplysia* sensory to motor neuron synapse has not yet been determined. Our objective in this study was to determine whether Rab3 is implicated in the enhancement of synaptic strength during 5-HT-induced LTF. We took advantage of Rab3 mutant forms to clarify the function of Rab3 in LTF. Since Rab3 on the synaptic vesicle is mostly in the GTP-bound state and only in this form, it can be complexed with its effector proteins such as Rim and Rabphilin; we first tested the effect of Rab3 Q80L mutant, which is a GTPase deficient mutant of Rab3 and so is always in the GTP-bound form, overexpression on LTF to focus on the function of Rab3–effector protein interaction in the presynaptic neuron during LTF. By electrophysiologically analyzing the effects of overexpression of various Rab3 recombinant constructs in the presynaptic neuron, we investigated the possible role of Rab3 in LTF formation.

2. Materials and methods

2.1. Cloning of apRab3

We obtained the cDNA sequence of apcRab3 (*Aplysia californica* Rab3) from the NCBI database. Based on the sequences, we cloned the full length of apkRab3 (*Aplysia kurodai* Rab3) from *A. kurodai* using nested PCR strategy. As the primary PCR with a pYESTrp2-based library in *A. kurodai*, we used two primers: *Bco*III, 5′-CGC ACT GCC AGA-3′ and H1-Rab3-Stop-A, 5′-CG GGA TCC TCA GCA GGA GCA GCC AGA-3′. With the product of the primary PCR, we did the second PCR using two primers: D3-Rab3-Start-S, 5′-CCC AAG CTT GCC ACC ACC ATG GCT TCC GCA AAC GAC-3′ and H1-Rab3-Stop-A, 5′-CG GGA TCC TCA GCA GGA GCA GCC AGA-3′. After digestion with *Hin*dIII and *Bam*HI, the second PCR product and pNEXδ were ligated. We confirmed the apkRab3 (apRab3 from now on) sequence by sequencing the ligated clone.

2.2. Plasmid constructions

2.2.1. Flag-tagged wild type apRab3

Full-length clone of apRab3 was obtained from a PCR reaction (sense primer: 5′-CCC*AAGCTT*GCCACCAC-CATG*GACTACAAGGACGACGATGACAAG*GCTTCCG-CAAACGACTCC-3′, *Hin*dIII site and Flag epitope sequences are italicized; anti-sense primer: 5′-CG*GGATCC*-TCAGCAGGAGCAGCCAGA-3′, *Bam*HI site is italicized) and subcloned into pNEXδ, a neuronal expression vector [17] using *Hin*dIII/*Bam*HI digestions.

2.2.2. Flag-tagged apRab3 mutants

To generate various apRab3 mutants, we conducted site-directed mutagenesis by recombinant PCR. We first produced flag-tagged constitutively active apRab3 (apRab3 Q80L) by recombinant PCR using the following primers: for the first fragment, sense primer: 5′-CCCAAGCTTGCCACCAC-CATGGACTACAAGGACGACGATGACAAGGCT-TCCGCAAACGACTCC-3′ and anti-sense primer: 5′-TGCGGTACCGCTCCGGC-3′ and for the second fragment, sense primer: 5′-GCCTGGAGCGGTACCGCA-3′ and anti-sense primer: 5′-CGGGATCCTCAGCAGGAGCAGCC-AGA-3′. For the construction of apRab3 Q80L, V54E mutant, we conducted recombinant PCR from apRab3 Q80L as template DNA using the following primers: for the first fragment, sense primer: 5′-CCCAAGCTTGCCACCACC-ATGGACTACAAGGACGACGATGACAAGGCT-TCCGCAAACGACTCC-3′ and anti-sense primer: 5′-GAAGTCGATGCCCTCCGT-3′ and for the second fragment, sense primer: 5′-TGCCTCCCGTAGCTGAAG-3′ and anti-sense primer: 5′-CGGGATCCTCAGCAGGAGCAGC-CAGA-3′. apRab3 Q80L, F18L, D19E mutant constructs were generated by recombinant PCR reaction from the apRab3 Q80L template DNA using the following primers: for the first fragment, sense primer: 5′-CCCA-AGCTTGCCACCACCATGGACTACAAGGACGACG-ATGACAAGGCTTCCGCAAACGACTCC-3′ and anti-sense primer: 5′-CATGTACTCGAGGTTCTG-3′ and for the second fragment, sense primer: 5′-GTCTTGCTCGAG-TACATG-3′ and anti-sense primer: 5′-CGGGATCCTCAG-CAGGAGCAGCCAGA-3′. All these recombinant PCR products were inserted into the neuronal expression vector pNEXδ using *Hin*dIII/*Bam*HI digestion.