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The regulation of glutamate receptor trafficking and function by TARPs and other transmembrane auxiliary subunits

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Abstract

At excitatory synapses in the brain, glutamate released from nerve terminals binds to glutamate receptors to mediate signaling between neurons. Glutamate receptors expressed in heterologous cells show ion channel activity. Recently, native glutamate receptors were shown to contain auxiliary subunits that modulate the trafficking and/or channel properties. The AMPA receptor (AMPA) can contain TARP and CNIHs as the auxiliary subunits, whereas kainate receptor (KAR) can contain the Neto auxiliary subunit. Each of these auxiliary subunits uniquely modulates the glutamate receptors, and determines properties of native glutamate receptors. A thorough elucidation of the properties of native glutamate receptor complexes is indispensable for the understanding of the molecular machinery that regulates glutamate receptors and excitatory synaptic transmission in the brain.

Introduction

Neurons connect with each other physically and functionally to form neuronal circuits. Neurons communicate mainly at synapses via chemical neurotransmission. The principal excitatory neurotransmitter in the vertebrate brain is glutamate. Glutamate released from presynaptic terminals binds to postsynaptic ionotropic glutamate receptors (iGluRs) to depolarize postsynaptic membranes. The spatial and temporal summation of postsynaptic depolarizations then may generate action potentials in the postsynaptic neuron that transmit information to subsequent neurons in the neuronal circuit. The strength and pattern of synaptic transmission are crucial factors for the proper function of neuronal connections. Synaptic strength is mediated by changes in the concentration of glutamate in the synaptic cleft, as well as by the number and channel properties of glutamate receptors. Thus, it is important to understand the physiological mechanisms that govern glutamate receptor abundance and functional properties at synapses.

Glutamate receptors and auxiliary subunits

Ionotropic glutamate receptors (iGluRs) are classified, using pharmacology, into three types: the α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-, the N-methyl-D-

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aspartate (NMDA)-, and the kainate (KA)-type [1-3]. When expressed in heterologous cells, the iGluRs show glutamate-dependent ion channel activity, and their expression alone is sufficient to form a channel pore. However, native iGluRs also contain auxiliary subunits, which modulate the trafficking and/or the channel properties of the receptors. Several molecules have been proposed to be auxiliary subunits of iGluRs; these include the Transmembrane AMPA receptor Regulatory proteins (TARPs), the Cornichon-like proteins (CNIHs), and the Neuropilin and Tolloid like proteins (Netos) [4-6]. The current review summarizes recent progress, with emphasis on papers published in the past two years, in understanding the regulation of iGluRs by the TARP, CNIH2/3, and Neto auxiliary subunits.

TARPs and CNIH2/3 regulate AMPAR trafficking and function

At synapses, the number and channel properties of AMPARs must be controlled in basal transmission and regulated upon neuronal activity. The AMPAR auxiliary subunits, TARPs and CNIH2/3, are important for the regulation of AMPAR activity at synapses. CNIH2 and CNIH3, but not CNIH1 or CNIH4, have been shown to bind to AMPARs [7]. Based upon characteristic features in their modulation of channel properties and/or trafficking of AMPARs, the six TARP isoforms are classified as type 1a (γ -2/stargazin and γ -3), type 1b (γ -4 and γ -8), and type 2 (γ -5 and γ -7) TARPs [8,9].

TARPs regulate AMPA receptor numbers at synapses

TARPs stabilize AMPARs at synapses via direct interactions with the post-synaptic density (PSD)-enriched PSD-95 and other MAGUKs (Figure 1) [4-6,10-12]. TARPs can be co-purified with AMPA receptors from the brain [13-15], and the c-terminal PDZ binding motifs of the TARPs directly interact with the PDZ domains of PSD-95 or other MAGUKs [16,17]. This interaction was shown to be necessary for synaptic AMPAR function by measuring AMPAR-mediated excitatory post-synaptic currents (EPSCs) following PDZ-domain mutation [18], and by acute disruption of the interaction between TARPs and PSD-95 using biomimetic divalent ligands [19].

The six isoforms of TARPs display distinct distributions in the brain [8,20]. A loss or reduction in the AMPAR-mediated EPSCs is observed in cerebellar granule cells and stellate cells from stargazer mice carrying a disrupted γ -2/stargazin gene, in cerebellar Golgi cells from $\text{C}\text{N}\text{I}\text{H}\text{2}/\gamma$ -3 double knockout mice (KO), in hippocampal pyramidal cells from γ -8 KO, and in Purkinje cells from γ -2/ γ -7 double KO mice [21-25]. AMPA-evoked whole cell currents in the hippocampal neurons from γ -8 KOs were reduced to 10% of wild-type levels [22]. Importantly, severe reductions in AMPAR expression are also observed in the hippocampus of γ -8 KO mice [22,26], a similar phenotype to that observed in mice lacking the GluA1 AMPAR subunit (GluA1 KO) [27]. These similarities indicate that the reduction of AMPAR activity in γ -8 KO mice may result from the loss of the γ -8 or indirectly from the severe reduction in AMPAR expression. To discern between these possibilities, γ -8 knock-in (KI) mice lacking the c-terminal PDZ binding motif (TTPV; γ -8 $\otimes$) were generated [28]. In the γ -8 Δ 4 KI mice, both AMPAR-mediated EPSCs and synaptic AMPAR protein levels were reduced to a similar extent as those observed in γ -8 KO mice, despite a slight reduction in total AMPAR expression [28]. These results suggest that the four c-terminal amino acids of the γ -8 (-TTPV) regulate the abundance of synaptic AMPAR, and the remainder of the γ -8 protein stabilizes AMPAR expression in the brain [28].

Importantly, 70% of AMPAR-mediated EPSCs were still detected in the γ -8 KO and the γ -8 Δ 4 KI mice [22,28]. As hippocampal CA1 pyramidal cells also express the γ -2/3/4 TARPs [8,20], it remains unclear whether or not all AMPAR at synapses require binding to TARPs for normal function. Triple KOs of γ -2/3/4, γ -2/3/8 and γ -2/4/8 are lethal at early developmental stage [29], therefore region-specific conditional TARP KO mice will be

useful to study the contribution of TARPs to AMPAR-mediated neurotransmission in the adult brain.

Lipid molecules have been identified to modulate the synaptic localization of the AMPAR/TARP complex (Figure 1). Negatively-charged lipids and lipid bilayers interact with positively-charged arginine clustered regions in the TARP cytoplasmic domain [30]. Electron crystallographic reconstruction of recombinant γ -2 revealed the c-terminal interaction of TARPs with lipid bilayers [31]. Interestingly, lipid interactions with TARPs inhibit the binding of TARPs to PSD-95 [30]. Furthermore, TARPs are phosphorylated in the brain [32,33], and TARP phosphorylation inhibits its ability to interact with lipids, and is followed by the localization of the AMPAR/TARP complex to the synapse [30]. The γ -2 phospho (all nine phosphorylatable serine residues to aspartic acid) and non-phospho (all nine phosphorylatable serine residues to alanine) knockin mice were generated [30]. The amplitudes of AMPAR-mediated EPSCs at cerebellar mossy fiber-granule cell synapses are larger in γ -2 phospho-mimic KI mice (stargazin^{SD}) than in γ -2 nonphospho-mimic KI mice (stargazin^{SA}) [30]. In primary cultured neurons, phospho-mimic γ -2 is more stable than non-phospho-mimic γ -2 [28,34]. However, γ -8 Δ 4 KI mice showed reduction in AMPAR-mediated EPSCs in basal transmission, but no changes in LTP [28]. This result suggests that the PDZ binding motif on TARPs is not required for LTP. Another modulation, the calpain-mediated cleavage of the TARP cytoplasmic domain upon NMDA stimulation, has been demonstrated [35]. Therefore, the post-translational modification of TARPs might contribute to their ability to modulate synaptic strength.

TARPs regulate channel properties of AMPA receptors

In addition to the modulation of AMPAR localization, TARPs regulate the channel properties of AMPARs (Figure 2) [4-6,10-12]. The presence of TARPs slows the kinetics of AMPAR deactivation (channel closure upon glutamate removal) and desensitization (channel closure upon glutamate binding) [36-38]. Stargazer mice (γ -2^{stg/stg}) show no AMPAR activity at cerebellar mossy fiber - granule cell synapses, and no miniature EPSCs (mEPSCs) was observed in primary cerebellar granule cell cultures [16,21]. Overexpression of type I TARPs (γ -2/stargazin, γ -3, γ -4, and γ -8) restores mEPSCs in primary cerebellar granule cells cultures from Stargazer [8,16].

However, the decay kinetics of mEPSCs in neurons expressing type Ia TARPs (γ -2 and γ -3) are faster than those in neurons expressing type Ib TARPs (γ -4 and γ -8) [39,40]. This isoform-specific modulation of AMPAR kinetics is also observed in heterologous cells [39,40]. In addition, TARPs also modulate ion permeability of AMPARs. The presence of TARPs reduces the rectification of AMPARs, rendering them more calcium permeable [25,39,41,42]. TARPs are also involved in CaMKII-mediated modulation of AMPAR conductance [43].

TARPs also modulate the pharmacology of AMPARs. TARPs make the AMPAR partial agonist, kainate, more efficacious in heterologous cells, and similar kainate pharmacology was observed for native, neuronal AMPAR [36,37,44]. Potentiators of AMPAR represent a class of reagents that increase AMPAR signaling by blocking channel closure. TARPs alter the affinity and efficacy of AMPAR potentiators such as cyclothiazide, PEPA, and CX546 [45,46]. In addition, TARPs alter the actions of CNQX from those of a competitive AMPAR antagonist, to those of a partial agonist [47,48]. Kainate, cyclothiazide, and CNQX bind to the extracellular domain of AMPARs; this binding localization suggests that the modulatory effects of TARPs may also occur at the extracellular domains of AMPARs. In support of this concept, the extracellular loop 1 of TARP has been shown to be both necessary and sufficient for the modulation of AMPAR kinetics [36,39,40,49]. On the other hand, the γ -2/

stargazin cytoplasmic domain was shown to mediate surface expression of AMPAR/TARP complex [36], and other studies have also recently shown that the cytoplasmic domains of some TARP isoforms modulate AMPAR properties [50,51]. In addition to affecting the properties of pharmacological reagents that bind to the AMPAR extracellular domain, TARPs modulate the actions of the AMPAR blocker, philanthotoxin-74, which presumably binds near the channel pore [52]. Interestingly, TARPs have been shown to functionally uncouple from AMPARs upon AMPAR desensitization, which auto-inactivates the AMPAR/TARP complex [53]. However, since this modulation is reversible, AMPAR/TARPs are unlikely to be physically dissociated; rather they are functionally decoupled without dissociation. Future structural studies will be necessary to reveal the precise molecular movements of the AMPAR/TARP complex upon agonist or antagonist binding.

Roles of CNIH2/3 in regulating AMPA receptors in the brain

CNIH2/3 were identified by proteomic analysis of native AMPAR complexes [7]. The expression of CNIH2/3 is substantially reduced in the hippocampus of γ -8 KO mice, and the CNIH2/3 protein can be co-immunoprecipitated with AMPARs with the use of an anti-TARP antibody [54]. These results suggest that CNIH2/3 forms a tripartite complex with AMPAR and γ -8 in the hippocampus, and that γ -8 stabilizes the CNIH/AMPA complex in this brain region (Figure 1). Although CNIH2 expression enhances the surface expression of AMPARs in heterologous cells [7], the surface expression of CNIH2 is reduced in the hippocampus of γ -8 KO mice [55]. One interpretation of these results is that CNIH2/3 drives AMPAR to the cell surface, where γ -8 stabilizes the CNIH/AMPA complex. Alternatively, CNIH2/3 might enhance AMPAR trafficking to the cell surface in heterologous cells, but not in hippocampal neurons. Further analysis of CNIH2/3 KO mice is needed to distinguish these two possibilities.

In a similar manner to the TARPs, CNIH2 slow AMPAR decay kinetics and alters the pharmacology of kainate and AMPAR potentiators [7,54,56,57]. Furthermore, CNIH2 modulates the decay kinetics of the AMPAR/TARP γ -8 complex [54,56]. Overexpression of CNIH2 alone in stargazer granule cells did not restore AMPAR-mediated mEPSCs [54,56], whereas the co-expression of CNIH2 together with γ -8 did restore AMPAR-mediated mEPSCs [54]. Cells co-expressing CNIH2 and γ -8 had slower mEPSC decay kinetics than cells expressing γ -8 alone [54]. In addition, CNIH2 expression is reduced in the PSD fraction of hippocampi from γ -8 Δ 4 KI mice, which lack AMPARs at the synapse [28]. In contrast, CNIH2 overexpression did not alter mEPSC kinetics from neurons expressing γ -2/stargazin [56]. The discrepancy between the actions of CNIH2 on mEPSC kinetics in neurons expressing γ -2 vs. γ -8 remains unexplained. It is possible that CNIH2/3 differentially modulates channel properties of AMPARs with the different TARP isoforms. A systematic analysis of the CNIH modulation of various combinations of AMPAR subunits and TARP isoforms should be conducted.

CNIH2/3 has also been shown to regulate the assembly and stoichiometry of native AMPAR complexes in the hippocampus. The numbers of TARP on each AMPAR varies depending on the expression levels of TARPs in heterologous cells [58,59]. Biochemical studies have shown that there may be one to four TARP molecules per each AMPAR tetramer in heterologous cells, whereas native AMPAR complex contains a minimum of one TARP [59]. On the other hand, one functional study showed that native AMPAR complexes may contain 2 to 4 TARP molecules, and the TARP stoichiometry was variable between neurons [56]. AMPARs containing four γ -8 molecules have unique channel properties, e.g., resensitization, which was not observed in native AMPAR complex in hippocampal CA1 pyramidal cells, indicating that the AMPARs in the hippocampal CA1 pyramidal cells typically contain fewer than four γ -8 molecules [54,55]. In the tripartite complex formed by

CNIH2/AMPA/TARP [54], CNIH2 and TARPs show a competitive interaction with the AMPAR [55]. These results indicate that CNIH2/3 may displace TARPs on AMPARs to control TARP stoichiometry. Further analysis of CNIH2/3 KO mice will likely elucidate the roles of CNIH proteins in AMPAR function.

Do all AMPARs in the brain contain TARPs or CNIHs? Comparison of molecular weight of AMPAR complex in cerebella from wild type and stargazer mice using BN-PAGE showed that almost all AMPARs in wild type cerebellum contain γ -2/stargazin [59,60]. On the other hand, antibody shift assay on BN-PAGE showed that anti γ -2/3 antibody only shift 30% of native AMPAR in hippocampus, whereas anti CNIH2/3 antibody shifted almost all AMPARs except AMPAR associated with γ -2/3 [7]. This result indicates that CNIH2/3 and γ -2/3 form distinct complex with AMPARs. However, CNIH2 was shown to form a tripartite complex with AMPAR and γ -8 in hippocampus [54]. Therefore, it remains still unclear what % of AMPAR contains TARPs or CNIHs in hippocampus and other brain regions.

Roles of Neto auxiliary subunits in KAR function

In comparison with the AMPAR and NMDAR, the KAR exhibits very slow decay kinetics and a distinct distribution revealed by [3 H] kainate binding in the brain [61-63].

The Neto2 protein was identified as an auxiliary subunit of KARs by the biochemical purification of the KAR complex using anti-KAR antibodies from cerebella [64]. The presence of Neto2 slows the decay kinetics of KARs expressed heterologously (Figure 2) [64-66]. Unlike the modulation of AMPAR trafficking by the TARPs, the presence of the GluK2 subunit of the KAR enhances the surface expression of Neto2 in the brain and in cRNA-injected oocytes, whereas Neto2 does not enhance the surface expression of GluK2 in cRNA-injected oocytes (Figure 1) [64]. However, Neto2 was found to enhance the surface expression of the GluK1 subunit in transfected HEK cells [65]. The surface expression of different KAR isoforms should be examined in Neto2 KO mice to determine the specific effects of Neto2 on KAR trafficking.

The Neto2 homologue, Neto1, also slows the decay kinetics of GluK2-containing KARs expressed in heterologous cells, but these kinetics are still faster than those of native KAR-mediated EPSCs at hippocampal mossy fiber-CA3 pyramidal cell synapses [67]. These results indicate that the expression solely of Neto1/2 and GluK2 is not sufficient to reconstruct native KARs. However, the decay kinetics of KAR-mediated EPSCs in Neto1 KO mice are similar to those of native AMPARs [67,68], suggesting that Neto1 is required for the slow decay kinetics characteristic of native KARs [61,62].

In the hippocampus stratum lucidum, where mossy fibers form synapses with CA3 pyramidal neurons, a strong signal for [3 H] kainate binding is observed [63]. This signal is completely abolished in GluK2 KO mice [69] and is also substantially reduced in Neto1 KO mice without any change in GluK2 distribution [67]. These findings indicate that Neto1 may determine the [3 H] kainate-binding characteristics, but not the distribution, of the KAR. Indeed, Neto1 modulates the kainate-binding affinity of KARs in the brain and in heterologous cells expressing GluK2 and GluK5 [67]. The molecular mechanisms by which KARs are stabilized at synapses remains unclear, and future studies will be needed to address this important question.

Conclusions

The different auxiliary subunits of the iGluRs play distinct roles in their trafficking and function. The TARPs modulate both the trafficking and the channel properties of AMPARs,

whereas the Netos and CNIH2/3 modulate the channel properties of KARs and AMPARs, respectively. The identification of novel auxiliary subunits is rapidly expanding out knowledge of the molecular machinery that regulated iGluRs in the brain. However, numerous questions remain unanswered. For example, what roles do these subunits play in LTP? How does KAR localize to the synapse? The AMPAR and KAR both have auxiliary subunits; does NMDAR have one? By combining the power of various experimental approaches, the molecular mechanisms that underlie glutamatergic synaptic transmission will be elucidated hopefully soon.

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Highlights

- >Native glutamate receptors contain pore subunits and auxiliary subunits.
- >Different glutamate receptors bind to distinct auxiliary subunits.
- >Auxiliary subunits modulate trafficking and/or channel properties.
- >Each of the auxiliary subunits uniquely modulates glutamate receptors.

Box1: Components of Native Receptor Complexes

Native ionotropic receptor complexes consist of two principal types of subunits.

>**Pore subunit** form a channel pore by itself, and line the pathway for ion flow.

>**Auxiliary subunits** associate directly and stably with pore subunits, and modulate their trafficking and/or channel properties.

Figure 1. Trafficking of Glutamate Receptor Complexes

(a) Schematic domain structure of pore-forming subunits and auxiliary subunits, ATD (amino terminal domain), LBD (ligand binding domain, CUB (Complement C1r/C1s, Uegf, Bmp1 domain), LDL (LDL-receptor class A domain), and globular structure at C-terminus indicates PDZ binding motif. (b) AMPAR associates with CNIH and TARPs in hippocampus, and TARPs mediate surface expression and synaptic localization of AMPAR complex (red arrows). The AMPAR complex is stabilized at postsynaptic density (PSD) via interaction of the TARP PDZ ligand with the PDZ domain of PSD-95. Phosphorylated TARPs dissociate from negatively-charged lipid bilayers and interact with PSD-95, whereas non-phosphorylated TARPs associate with negatively-charged lipid bilayers and stays at extrasynaptic sites. (c) Surface expression of KAR complex depends on low-affinity GluK1/2/3 KAR subunits (blue arrow), and the mechanism of synaptic targeting for KAR complex is unknown.

Figure 2. Modulation of Channel Kinetics by Auxiliary Subunits

(a, b): In heterologous cells, TARPs and Neto2 slow both deactivation and desensitization of AMPAR (a) and KAR (b), respectively. However, each isoform of auxiliary subunits modulates channel kinetics at different degree. Bottom panel: At the cerebellar mossy fiber - granule cell synapses, AMPAR-mediated EPSCs are absent in *Stargazer*-mice (γ -2^{stg/stg}) (c), due to loss of AMPARs at synapses. On the other hand, overexpression of various TARP isoforms rescued loss of mEPSCs in stargazer granule cells with distinct mEPSC kinetics, demonstrating that decay kinetics of synaptic AMPAR is also controlled by TARPs as synaptic localization (c). In contrast, Neto1 KO mice show KAR-mediated EPSCs at mossy fiber - CA3 pyramidal cells. However, decay kinetics of KAR-mediated EPSCs is significantly fastened in Neto1 KO mice (d). Schematic drawings are modified from publications [39,67].