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Regulation of AMPA receptor gating and pharmacology by TARP auxiliary subunits

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Abstract

Presynaptic glutamate release elicits brief waves of membrane depolarization in neurons by activating AMPA receptors. Depending on its precise size and shape, current through AMPA receptors gates downstream processes like NMDA receptor activation and action potential generation. Over a decade of research on AMPA receptor structure and function has identified binding sites on AMPA receptors for agonists, antagonists and allosteric modulators as well as key residues underlying differences in the gating behavior of various AMPA receptor subtypes. However, the recent discovery that AMPA receptors are accompanied in the synaptic membrane by a family of auxiliary subunits known as transmembrane AMPA receptor regulatory proteins (TARPs) has revealed that the kinetics and pharmacology of neuronal AMPA receptors differ in many respects from those predicted by classical studies of AMPA receptors in heterologous systems. Here, we summarize recent work and discuss remaining questions concerning the structure and function of native TARP–AMPA receptor complexes.

Introduction

Ionotropic neurotransmitter receptors mediate fast communication between neurons by opening a selective ion-permeable pore in response to the presynaptic release of a neurotransmitter. At most excitatory synapses in the mammalian brain, the neurotransmitter released is glutamate, and the postsynaptic receptors belong to a subclass of glutamate receptors known as AMPA receptors. AMPA receptor subunits come in four varieties (GluR1 to GluR4), and each consists of an extracellular ligand-binding domain, three transmembrane domains, a re-entrant loop and an intracellular C-terminal domain (C-tail). Functional AMPA receptors are tetramers that are typically heteromeric combinations of two of the four possible subunits. The functional properties as well as the number of AMPA receptors clustered at synapses dictate the strength and timing of synaptic transmission, and, therefore, determining the factors that control the gating and trafficking of AMPA receptors is crucial to understanding how neurons process and encode information [1].

Until recently it was thought that the trafficking of AMPA receptors to the plasma membrane and their localization at synapses could be accounted for entirely by interactions between the C-tails of AMPA receptors and various cytoplasmic proteins like PICK1, GRIP1, NSF and SAP97 [2]. However, synaptic scaffolding proteins like PSD-95 and PSD-93 do not interact

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directly with the C-tails of AMPA receptors, and, yet, their expression levels in neurons have been shown to determine the number of AMPA receptors at synapses [3–5]. This discrepancy was resolved with the discovery that AMPA receptors interact with a four-pass transmembrane protein called 'stargazin' that directly interacts with PSD-95 through a C-terminal PDZ-binding domain to control the synaptic targeting of AMPA receptors [6–8]. In the *stargazer* mutant mouse, loss of stargazin protein expression nearly abolishes the surface and synaptic expression of AMPA receptors in cerebellar granule neurons, resulting in severe ataxia [9,10]. It has since been realized that other neuronal cell types express homologs to stargazin that also are crucial for the trafficking of AMPA receptors to surface and synaptic membranes [11] (K. Menuz *et al.*, unpublished). These stargazin-like proteins are now considered to be auxiliary subunits to AMPA receptors and are referred to as transmembrane AMPA receptor regulatory proteins (TARPs).

In addition to the prototypical TARP [stargazin (or γ-2)], the TARPs γ-3, γ-4, γ-7 and γ-8 are expressed in distinct but overlapping populations of neurons, where they are specifically associated with native AMPA receptors [12–15]. Although it has been shown that residues in the TARP C-tail other than the PDZ-binding domain constitute a necessary and sufficient signal for the delivery of AMPA receptors to the plasma membrane [16,17], the relevant protein interaction remains unidentified. Experiments conducted on the null background of cerebellar granule cells cultured from *stargazer* mice demonstrated that each TARP subtype is sufficient to mediate both the surface and synaptic trafficking of AMPA receptors [18]. However, several residues in the intracellular domains of TARPs differ across TARP subtypes. Whether this variation imparts differential regulation of AMPA receptor trafficking to different TARP subtypes has not been studied and could represent an exciting new direction for the field.

Several recent studies have demonstrated that, in addition to their roles in AMPA receptor trafficking, TARPs also profoundly modulate the functional properties of AMPA receptor channels [16,18–23] and it is this property of TARPs that truly classifies them as auxiliary subunits of AMPA receptors. Recent work also has revealed that TARPs influence AMPA receptor pharmacology, modulating the actions of several drugs that commonly are used to probe neuronal AMPA receptors *in vitro* and *in vivo*. In this article we summarize recent findings concerning the effects of TARPs on AMPA receptor gating and pharmacology from the perspective that the majority of synaptic AMPA receptors in the brain are associated with TARPs and, therefore, an understanding of synaptic AMPA receptor structure and function must be attained in the context of modulation by TARP auxiliary subunits. Finally, we will infer from existing data a structural model to account for TARP modulation of AMPA receptor function.

Synaptic AMPA receptor gating

Initial studies coexpressing AMPA receptors and TARPs in heterologous systems indicated that the degree to which TARPs enhanced steady-state agonist-evoked currents was larger than would be expected from a trafficking mechanism alone [24]. Subsequently, several groups investigated the effects of TARPs on the gating of AMPA receptors [16,18–23]. The consensus from these studies is that TARPs slow AMPA receptor activation, deactivation and desensitization (see Box 1), the functional relevance of which is to increase the total charge transfer through AMPA receptors during synaptic transmission. Figure 1a shows examples of outside-out patches from HEK cells expressing the AMPA receptor subunit GluR1, with and without TARPs, in response to a 1 ms pulse of glutamate. It also is apparent in Figure 1a that different TARP subtypes vary in their ability to modulate the kinetics of AMPA receptors [18,22,23], with γ-4 slowing the rise and deactivation of GluR1 to a greater extent than γ-2. These differences between TARP subtypes also are apparent with native synaptic AMPA receptors in cerebellar granule cells cultured from *stargazer* mice. Figure 1b shows that

synaptic events rescued by expression of γ-4 rise and decay more slowly than those rescued by γ-2 [18]. Furthermore, native synaptic AMPA receptors in acute brain slices from the striatum that are normally associated with γ-4 have faster rise and decay kinetics in a *cacng4* (γ-4) knockout mouse [18] (Figure 1c). This demonstrates that TARP-subtype-specific gating of AMPA receptors contributes to heterogeneity in native AMPA receptor kinetics across neuronal cell types; this idea previously had been attributed entirely to differences in the subunit composition of the AMPA receptors themselves [25].

Box 1. AMPA receptor channel kinetics

When an AMPA receptor is challenged with a brief (1 ms) pulse of glutamate, the rise time of the resulting current reflects the binding of glutamate to the ligand-binding domains of the AMPA receptor and a conformational change that leads to opening of the ion channel pore. The decay time reflects the opposite process, referred to as receptor deactivation, during which the ion channel closes, the receptor changes conformation and glutamate unbinds (these gating processes are depicted diagrammatically in Figure 3a and example currents are shown in Figure 1a). When the glutamate pulse is extended to 100 ms, the persistent rebinding of glutamate and reopening of the channel drives a separate process with a different time course, known as desensitization. Although glutamate stays bound during desensitization, AMPA receptors enter a distinct closed-channel conformation known as the desensitized state (Figure 3a). The relative contributions of deactivation and desensitization to the shape of synaptic currents varies across different synapses in the brain and depends on many factors, including the specific geometry of the synapse and the rate of clearance of glutamate by diffusion and active transport [25].

Native AMPA receptor pharmacology

Partial agonist: kainate

In heterologous systems AMPA receptors respond to glutamate with large peak currents that decay to a small steady-state owing to rapid and almost complete desensitization. They also respond to a structurally related compound, kainate, with small, nondesensitizing currents [26,27]. TARP association with AMPA receptors, in expression systems and natively in neurons, increases the efficacy of kainate, resulting in nondesensitizing currents that are similar in amplitude to peak glutamate currents [16,20,28]. This is demonstrated in Figure 2a, which compares the responses of GluR1 in HEK cells to local application of glutamate and kainate with and without TARP γ-2 [28]. The effect of TARPs on kainate efficacy manifests both as an increased apparent affinity and an increased maximal response to saturating doses of kainate [18,20,29].

Competitive antagonist: CNQX

Competitive antagonists, such as CNQX and NBQX, bind AMPA receptors with high affinity at the glutamate-binding site, thereby precluding their activation by glutamate. Thus, these drugs have been indispensable in elucidating the cell biology and pathophysiology of AMPA receptors [30,31]. However, CNQX also has a paradoxical excitatory action on neurons that was presumed to be an off-target effect [32]. This discrepancy was resolved by the demonstration that CNQX directly activates AMPA receptor channels that are associated with TARPs [33] (Figure 2b). Accordingly, CNQX application to brain slices depolarizes a variety of neuronal cell types, particularly when desensitization is pharmacologically inhibited [33]. This partial-agonist activity of CNQX was not observed with the related compound NBQX or the noncompetitive antagonist GYKI 53655, which remain viable alternatives for chronic blockade of neuronal AMPA receptors.

Noncompetitive antagonist: spermine

Neurons express endogenous polyamines, such as spermine and spermidine, that interact specifically with calcium-permeable AMPA receptors lacking the GluR2 subunit. These positively charged molecules block open AMPA receptor channels upon membrane depolarization, conferring inward rectification to the current-voltage relationship of GluR2-lacking AMPA receptors [34–36]. Accordingly, measuring rectification of synaptic currents in the presence of intracellular spermine has become a standard assay for the presence or absence of GluR2-lacking AMPA receptors in neurons [37,38]. However, recent work has shown that TARP association reduces AMPA receptor affinity for spermine such that GluR2-lacking AMPA receptors display only intermediate, rather than complete, rectification [20, 23,39] (Figure 2c). This effect is particularly surprising given that TARPs are known to increase the frequency of AMPA receptor channel openings [16], which would be expected to facilitate block of the open channel. One possible explanation, which we discuss in detail in the next section, is that TARPs disrupt the binding site for spermine by altering the shape of the AMPA receptor pore itself (Figure 3e).

Allosteric modulator: cyclothiazide

As inhibitors of AMPA receptor desensitization, benzothiadiazines like cyclothiazide and trichloromethiazide often are used to increase steady-state currents to facilitate detection of surface AMPA receptors, measure rectification (see above) or determine the efficacy of novel AMPA receptor antagonists [16,26,40]. Research into the mechanism of action of cyclothiazide also has provided a valuable insight into AMPA receptor structure and function. Cyclothiazide binds AMPA receptors within an extracellular domain that is regulated by splice variation known as the flip/flop cassette [41–43]. The fact that flip AMPA receptors desensitize more slowly than flop AMPA receptors immediately suggests a direct role for this domain in the mechanism of AMPA receptor desensitization [27,44,45]. Indeed, structural studies have shown that residues contained in the flip/flop splice cassette participate in an unstable protein–protein interaction between the ligand-binding domains of adjacent AMPA receptor subunits [43,46]. During desensitization AMPA receptors undergo a conformational change that disrupts this ‘dimer interface’ between AMPA receptor subunits, decoupling ligand binding from channel opening [43,47] (Figure 3a). By interacting at this bridge between AMPA receptor subunits, cyclothiazide prevents the conformational change required for desensitization [43,48].

Might TARPs also modulate AMPA receptor gating by influencing their conformation? This was addressed by taking advantage of the fact that benzothiadiazine binding depends on the intact dimer interface to infer information about the conformation of AMPA receptors associated with TARPs [20]. TARPs speed the association rate of trichloromethiazide with AMPA receptors, suggesting that TARPs increase the time AMPA receptors spend in the nondesensitized conformation. TARPs do not appear to alter the affinity of AMPA receptors for benzothiadiazines because the dissociation rate of trichloromethiazide does not change [20]. However, TARPs do alter the efficacy of benzothiadiazines, causing a leftward shift in the dose–response relationship for cyclothiazide on AMPA receptors [49] (Figure 2d). Also, although cyclothiazide is more effective at modulating flip AMPA receptors than flop AMPA receptors [50], TARPs substantially increase the potency of cyclothiazide on flop receptors [49]. These data demonstrate that, although both TARPs and benzothiadiazines stabilize the nondesensitized state of the AMPA receptor, TARPs also facilitate the stabilizing action of benzothiadiazines. Although it is clear that the influence of TARPs on AMPA receptor gating depends largely on a TARP extracellular domain [16,18,20,23], it is unknown whether that domain interacts directly with the dimer interface (depicted in Figure 3d) or other structural elements of the AMPA receptor (Figure 3b,c). Furthermore, whether TARPs act solely by influencing the stability of pre-existing receptor conformations, by fundamentally altering

those conformations or even by forming new conformations will undoubtedly require high-resolution structural data.

Beyond the ligand-binding domain

Structural and functional studies of AMPA receptors have shown that ligand binding involves closure of a clamshell-like binding cleft, which exerts tension on the linker domains that connect the binding cleft to the AMPA receptor pore. This tension can be relieved either by channel opening or by desensitization (see above) (Figure 3a) [43,46,47,51]. The degree of cleft closure induced by various ligands corresponds to their relative efficacies, providing a structural basis for partial agonism by kainate and competitive antagonism by DNQX [51, 52]. Indeed, recent evidence suggests that the stability of the closed-cleft conformation is a crucial determinant of AMPA receptor kinetics and agonist efficacy [53,54].

One possibility is that TARPs influence AMPA receptor gating by increasing the amount of cleft closure induced by ligand binding (Figure 3b). The increased tension this would place on the linker domains could well account for the increased efficacy of kainate and partial agonism by CNQX. However, although CNQX does not induce current through AMPA receptors in the absence of TARPs [33] (Figure 2b), CNQX does induce significant cleft closure in crystals of the isolated AMPA receptor ligand-binding domain [33]. Although it cannot be ruled out that TARP association further increases cleft closure, another possibility is that TARPs facilitate the translation of partial cleft closure into channel opening by interacting directly with the linker domains (Figure 3c). Consistent with this model, mutation or transplantation of AMPA receptor linker domains profoundly alters AMPA receptor gating and renders CNQX a partial agonist, similar to TARP association [55–58]. Furthermore, mutation of a transmembrane residue immediately adjacent to a linker domain (known as the lurcher mutation) abolishes the influence of TARPs on both AMPA receptor gating and trafficking [59].

Interestingly, when AMPA receptor complexes are purified and imaged by single-particle electron microscopy, the most evident contribution by TARPs to AMPA receptor structure is not extracellular but, rather, transmembrane [14]. Whether TARP transmembrane residues directly contribute to the inner lining of the AMPA receptor pore has not been determined. However, the fact that TARPs disrupt the polyamine-binding site inside the AMPA receptor pore suggests that TARPs at least indirectly alter its conformation (Figure 3e). Further supporting this possibility, TARPs increase the average single-channel conductance of AMPA receptors [16,39]. The structural models presented here are not necessarily mutually exclusive, and it is probable that some combination of these possible mechanisms underlie the varied influences of TARPs on AMPA receptor gating.

Conclusion

Initially appreciated for their role in trafficking AMPA receptors, it is now clear that TARP auxiliary subunits fundamentally alter the gating and pharmacology of neuronal AMPA receptors. Interestingly, the invertebrate homologs of TARPs specifically modulate glutamate receptor gating, but not trafficking, suggesting that these functions of TARPs could be evolutionarily distinct [60]. Although studies of AMPA receptor structure have focused primarily on static crystals of the isolated AMPA receptor ligand-binding domain, it is clear that typical neuronal AMPA receptors are heteromeric, TARP associated and incredibly dynamic. Accordingly, further study of the interaction between TARPs and AMPA receptors undoubtedly will provide insight into how changes in native AMPA receptor conformation mediate synaptic transmission.

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Glossary

AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid.
NMDA	N-methyl-D-aspartic acid
CNQX	6-cyano-7-nitroquinoxaline-2,3-dione.
NBQX	2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione.

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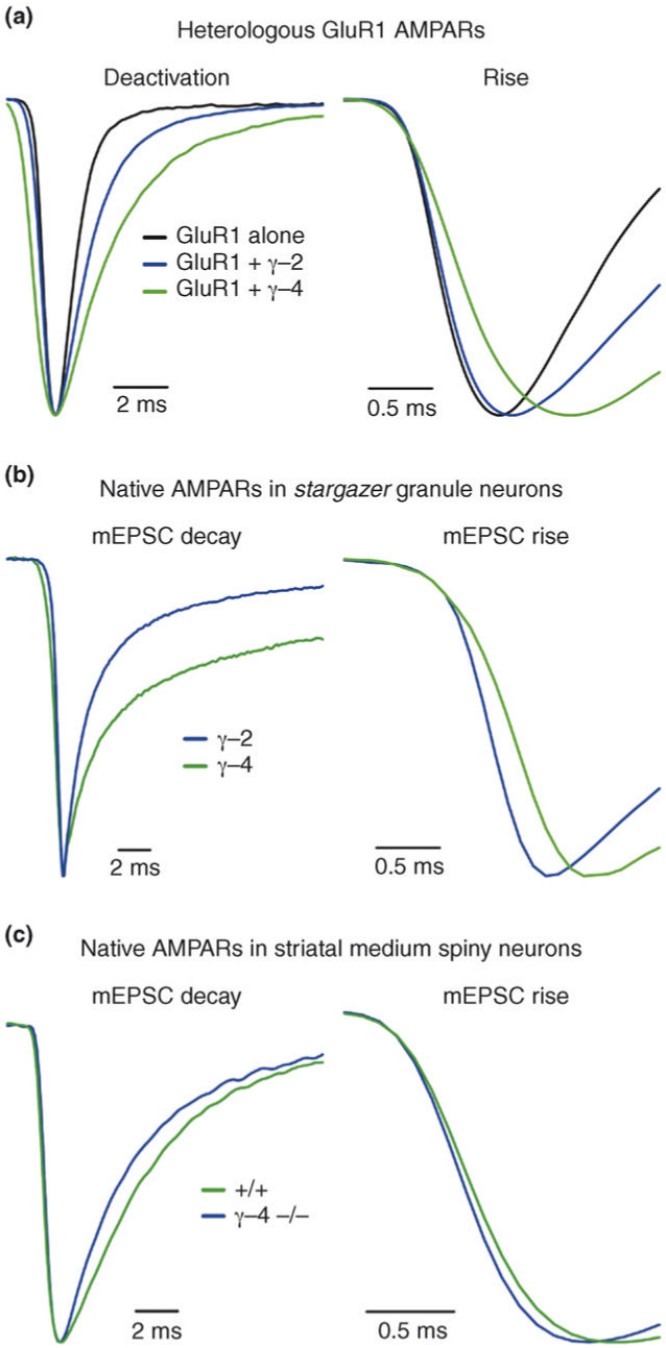

Figure 1.

TARP subtypes differentially modulate AMPA receptor (AMPAR) gating. (**a**) 1 mM glutamate was applied for 1 ms to outside-out patches of HEK cells expressing recombinant GluR1 with or without TARPs γ-2 and γ-4, and the resulting responses were normalized to the peak. The trace on the left is aligned to the peak and demonstrates that TARPs slow the deactivation time course of GluR1. The effect of TARP γ-4 is more pronounced than that of γ-2. The trace on the right is aligned to the 10% rise point and demonstrates that TARP γ-4, but not γ-2, slows the activation time course of GluR1. (**b**) Although *stargazer* cerebellar granule cells lack synaptic AMPA receptors, miniature excitatory postsynaptic currents (mEPSCs) are rescued in cells expressing TARPs. mEPSCs rescued by γ-4 decay and rise more slowly than those

rescued by γ-2. (**c**) Medium spiny neurons in acute slices of the striatum normally express TARP γ-4. mEPSCs from these neurons rise and decay faster in mice lacking TARP γ-4. These panels are reproduced, with permission, from Ref. [18].

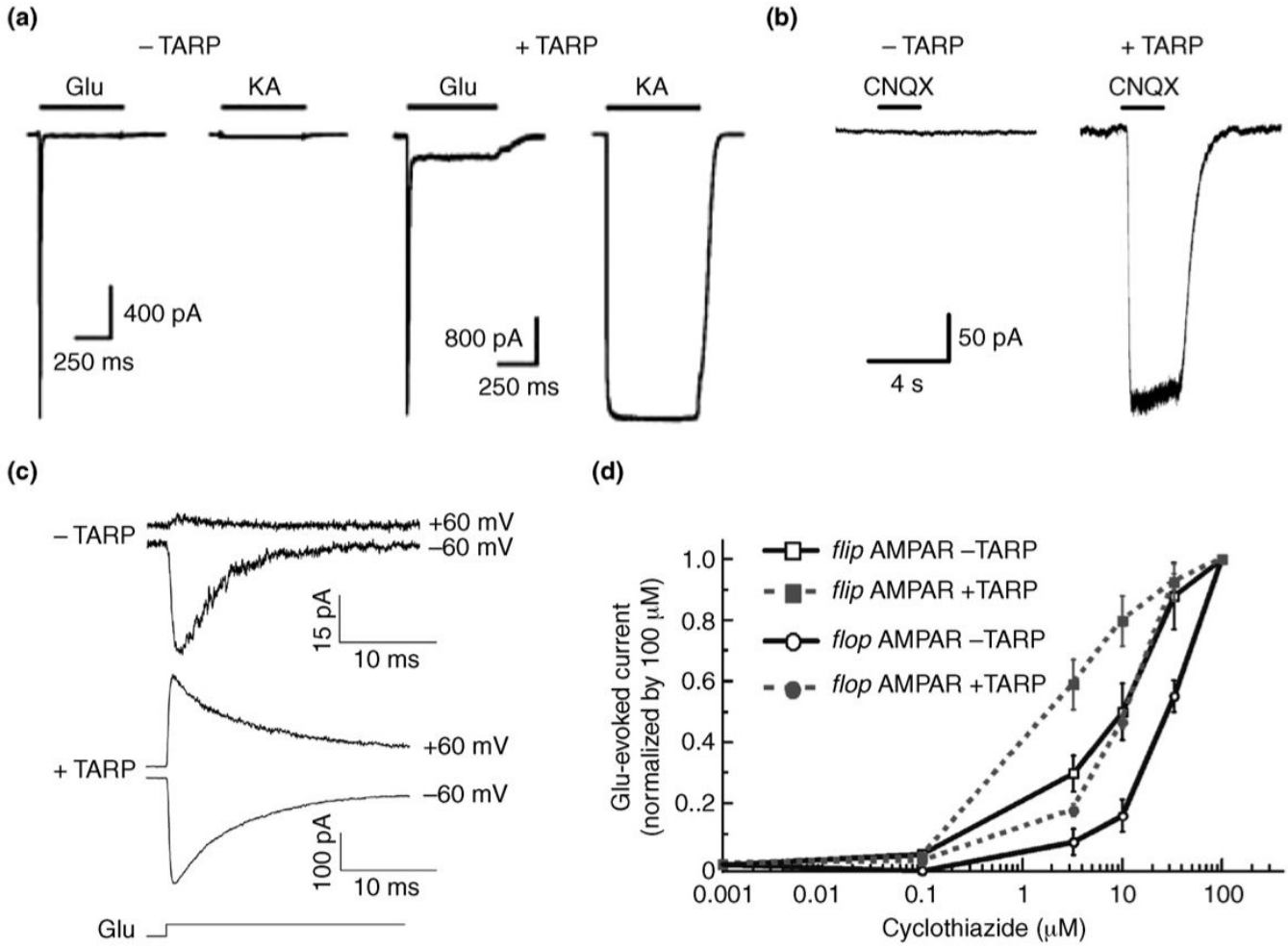

Figure 2.

TARPs modulate AMPA receptor pharmacology. (**a**) TARP γ-2 increases the efficacy of the partial agonist kainate (KA) on GluR1 expressed in HEK cells and reduces the amount of steady-state desensitization induced by glutamate (Glu). (**b**) In the presence of TARP γ-2, GluR1 expressed in HEK cells directly responds to the application of the competitive antagonist CNQX with an inward current, demonstrating that TARPs convert CNQX into a partial agonist of AMPA receptors. (**c**) TARP γ-2 reduces the voltage-dependent block of GluR4 in tsA201 cells by spermine. (**d**) TARP γ-2 increases the efficacy of cyclothiazide on GluR1 flip and GluR1 flop, as evidenced by a leftward shift in the dose–response relationships of cyclothiazide on these receptors expressed in ooctyes. Panel (a) is reproduced, with permission, from Ref. [20]. Panel (b) is reproduced, with permission, from Ref. [33]. Panel (c) is reproduced, with permission, from Ref. [39]. Panel (d) is reproduced, with permission, from Ref. [49].

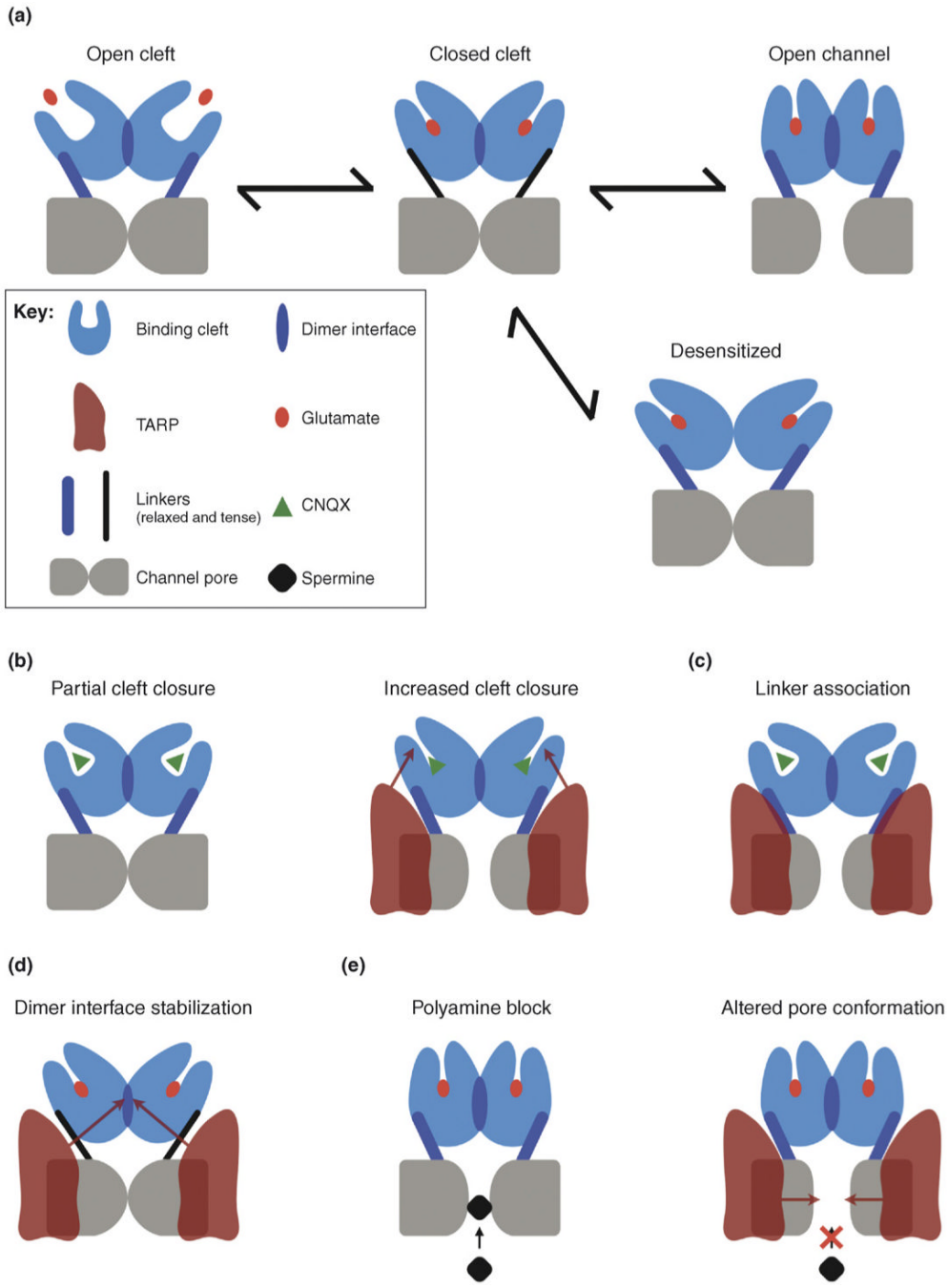

Figure 3.

Structural models of TARP modulation of AMPA receptor gating. (**a**) A simplified model of AMPA receptor gating. Two AMPA receptor subunits, each with a ligand-binding domain, and the interaction between the two subunits (dimer interface) are represented. Glutamate binding involves closure of the binding clefts, which places tension on the linker domains. Linker tension can be relieved either by entering an open-channel conformation or by breaking the dimer interface and entering a desensitized conformation. (**b**) Increased cleft closure model: diagram depicts TARP extracellular domains interacting directly with the AMPA receptor ligand-binding clefts, increasing the degree of cleft closure to account for the partial-agonist activity of CNQX. (**c**) Linker association model: diagram depicts TARP extracellular domains

interacting directly with the AMPA receptor linker domains, facilitating the translation of partial cleft closure into channel opening to account for the partial agonist activity of CNQX. (**d**) Dimer interface stabilization model: diagram depicts TARP extracellular domains interacting directly with the AMPA receptor dimer interface to account for increased stability of the nondesensitized conformation and increased benzothiadiazine efficacy. (**e**) Altered pore conformation model: diagram depicts TARP transmembrane domains directly contributing to the shape of the AMPA receptor pore to account for reduced spermine affinity.