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Review Article

AMPA receptors as a molecular target in epilepsy therapy

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Epileptic seizures occur as a result of episodic abnormal synchronous discharges in cerebral neuronal networks. Although a variety of non-conventional mechanisms may play a role in epileptic synchronization, cascading excitation within networks of synaptically connected excitatory glutamatergic neurons is a classical mechanism. As is the case throughout the central nervous system, fast synaptic excitation within and between brain regions relevant to epilepsy is mediated predominantly by AMPA receptors. By inhibiting glutamate-mediated excitation, AMPA receptor antagonists markedly reduce or abolish epileptiform activity in *in vitro* preparations and confer seizure protection in a broad range of animal seizure models. NMDA receptors may also contribute to epileptiform activity, but NMDA receptor blockade is not sufficient to eliminate epileptiform discharges. AMPA receptors move into and out of the synapse in a dynamic fashion in forms of synaptic plasticity, underlying learning and memory. Often, the trigger for these dynamic movements is the activation of NMDA receptors. While NMDA receptor antagonists inhibit these forms of synaptic plasticity, AMPA receptor antagonists do not impair synaptic plasticity and do not inhibit memory formation or retrieval. The demonstrated clinical efficacy of perampanel, a high-potency, orally active non-competitive AMPA receptor antagonist, supports the concept that AMPA receptors are critical to epileptic synchronization and the generation and spread of epileptic discharges in human epilepsy.

M. A. Rogawski

Department of Neurology, School of Medicine and Center for Neuroscience, University of California, Davis, Sacramento, CA, USA

Key words: AMPA receptor; competitive AMPA receptor antagonist; epilepsy; neuronal synchronization; NMDA receptor; non-competitive AMPA receptor antagonist; seizures

Michael A. Rogawski, Department of Neurology, School of Medicine, University of California, Davis, 4860 Y Street, Suite 3700, Sacramento, CA 95817, USA
e-mail: rogawski@ucdavis.edu

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Introduction

Epilepsy is characterized by recurring, unprovoked seizures, representing the abnormally synchronous activity of neurons in a focal area of the brain or throughout the entire brain. Antiepileptic drugs (AEDs) protect against seizures by interacting with various molecular targets in the brain. The known molecular targets for clinically used AEDs include voltage-gated sodium and calcium channels, $GABA_A$ receptors, the GAT-1 GABA transporter, the GABA catabolic enzyme GABA transaminase, and the synaptic vesicle protein SV2A (1). By altering the functional activity of these diverse targets, some AEDs suppress the occurrence of abnormal hypersynchronous activity in localized brain regions so that seizures fail to occur. Other AEDs may permit localized, clinically insignificant hypersynchronous (epileptiform) discharges but prevent these discharges from spreading. Thus, the discharges fail to encompass a neural aggregate that is large enough to result in a clinically significant behavioral seizure.

The central role of neuronal synchronization in epilepsy

Various pathological mechanisms lead to the abnormal neuronal synchronization in epilepsy (2). These include impaired inhibition, loss of

after-hyperpolarization, increased extracellular potassium, enhanced excitatory synaptic transmission, and depolarizing GABA responses. These mechanisms occur in the epileptic brain as a result of one or more of the following chronic pathologies: alterations in the intrinsic excitability properties of neurons (e.g., due to a genetic or acquired ion-channel dysfunction), changes in synaptic properties (e.g., altered synaptic receptors, most notably $GABA_A$ receptors), altered neuronal connectivity (e.g., mossy fiber sprouting associated with enhanced excitatory neurotransmission), changes in the function of astrocytes, and loss of neurons. In the presence of these pathological changes in the normal structure and function of the brain, diverse interactions between neurons lead to epileptic synchronization. These interactions are both non-synaptic and synaptic. Surprisingly, in some experimental circumstances, seizure-like events can be generated in the absence of synaptic transmission (3). The mediators of synchronization under these circumstances include field effects, fluctuations in extracellular ions (including potassium and calcium) and, in some cases, gap junctions. Under ordinary circumstances, synaptic mechanisms interact with these non-synaptic mechanisms in the generation of pathological epileptic synchronization.

Excitatory and inhibitory synaptic connections underlie epileptic activity

Although various non-conventional mechanisms have been implicated in epileptic synchronization, for three decades, cascading excitation within networks of synaptically connected excitatory neurons has been the best accepted mechanism (4, 5). This has been most often studied in the hippocampus, where recordings in hippocampal slices and computer modeling have led to the concept that bursts of action potentials in even one excitatory pyramidal neuron can recruit additional pyramidal neurons in a network through sequential activation of excitatory neurons via excitatory synaptic connections (6). Pyramidal neurons within the hippocampus possess profusely arborizing axon collaterals that make excitatory synapses onto neighboring pyramidal cells, onto neurons in distant regions of the hippocampus (e.g., CA3 neurons excite CA1 neurons) and onto hippocampal inhibitory interneurons. When synaptic inhibition is suppressed, the synchronous firing of a large population of hippocampal neurons can be entrained by a single excitatory neuron (6) (Fig. 1). This occurs as a result of the spread of activity through the entire population via recurrent excitatory connections (7). Excitation within the network is divergent so that each neuron excites multiple follower neurons (8). However, the probability that any two neurons are synaptically connected is low, perhaps 1–2% (9). When synaptic inhibition is suppressed, more excitatory neuron pairs are functionally connected, and latent polysynaptic pathways are revealed. As inhibition becomes progressively blocked, these various effects cause the network to exhibit seizure-like behavior (4). The minimum 'epileptic aggregate' necessary to sustain synchronized epileptic discharges in the case of the CA3 region of the hippocampus is ~1000–2000 neurons. This represents a 'critical mass' of neurons analogous to the critical mass of a nuclear chain reaction.

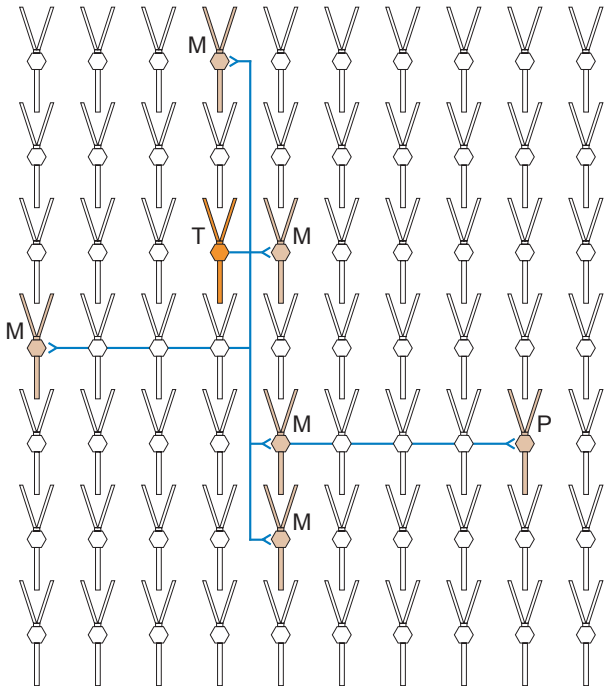

Figure 1. Model neuronal network as in the CA3 region of the hippocampus, showing excitatory connections between pyramidal neurons that generate synchronous epileptiform behavior. Each pyramidal neuron is schematically illustrated as a soma (central hexagon) with a basal dendrite (extending below the soma) and a branching apical dendrite (extending above the soma). The individual neurons are capable of bursting in response to brief excitation. Excitatory inputs are onto basal and apical dendrites but for simplicity are shown near the somata. The orange neuron (T) is assumed to trigger the synchronized discharge. Like every other neuron in the neural aggregate, it sends outputs via axon collaterals (blue lines) to multiple (on average, five) neurons, but it connects to only a small subset of neurons (the actual fraction in the CA3 region of the hippocampus is estimated to be 1%). Inhibitory neurons are not shown, as GABA-mediated inhibition is assumed to be eliminated. Under such conditions of reduced GABAergic inhibition, the number of monosynaptic connections (M) is increased, and polysynaptic (P) connections are uncovered.

The mechanism of excitatory neurotransmission

In the presence of chronic alterations in synaptic inhibition, which are hypothesized to be a key pathological mechanism in epilepsy (10), excitatory connections in the hippocampus and other brain regions, most notably the neocortex, are crucial to the generation of epileptic activity. The neurotransmitter glutamate generates 'fast excitatory synaptic potentials' (EPSPs) that are responsible for excitatory connectivity between principal neurons (pyramidal neurons) and between principal neurons and interneurons. Following its exocytotic release at excitatory synapses, glutamate diffuses across the synaptic cleft and binds with ionotropic glutamate receptors in the synaptic membrane of the post-synaptic neuron to generate an EPSP. EPSPs arriving at individual neurons summate to trigger action potentials, and synchronous EPSPs in groups of neighboring neurons are responsible for epileptic field potentials.

Fast glutamatergic neurotransmission is mediated by several types of ionotropic receptor localized to the post-synaptic membrane. AMPA and NMDA receptors are the predominant type at most excitatory synapses. An understanding of the roles of these receptors was enabled by the discovery of selective pharmacological antagonists. Soon after the first selective blockers of non-NMDA glutamate receptors were identified in 1988 (11), it was shown that these antagonists markedly reduce or abolish epileptiform activity in the hippocampus *in vitro* (12–14). By contrast, selective blockade of NMDA receptors alone has little effect on epileptiform discharges, although NMDA receptor blockers can, in some cases, reduce the duration of the discharges (Fig. 2) (15). This indicates distinct roles for the two types of ionotropic glutamate receptors in mediating epileptiform activity and a critical requirement for AMPA receptors in the generation of epileptic discharges. The conclusion that AMPA receptors are of primary importance in initiating epileptiform discharges was confirmed by studies in other brain regions relevant to epilepsy, including the rat (16) and human (17) neocortex and rat entorhinal cortex (18).

The role of AMPA receptors was reinforced by computer modeling of the hippocampal CA3 region similar to that described previously, in which the physiological properties of AMPA and NMDA receptors were included in the model (Fig. 3) (15). As before, the model generated seizure discharges that resembled those recorded in hippocampal slices treated with a $GABA_A$ receptor blocker. Simulated blockade of AMPA receptors in the model prevented synchronized firing, whereas blockade of NMDA receptors shortened bursts but did not eliminate them. It was possible to conclude that epileptic discharges are generated by the intrinsic excitability properties of neurons but the discharges are initiated and synchronized by recurrent excitatory collateral connections primarily involving AMPA receptors. Within tens of milliseconds or less of activation of even one of the neurons, all of the neurons fire and mutually excite each other via AMPA receptors to produce a synchronized burst; the burst is prolonged by the activity of NMDA receptors. These studies demonstrate the critical role of AMPA receptors in mediating epileptic synchronization and provide a scientific basis for the use of AMPA receptor antagonists to prevent seizures.

Distinct roles of AMPA and NMDA receptors

Although glutamate was found to excite neurons in the mammalian brain in the late 1950s, it is only in the last few decades that it has been accepted as the major neurotransmitter released from excitatory neurons responsible for fast syn-

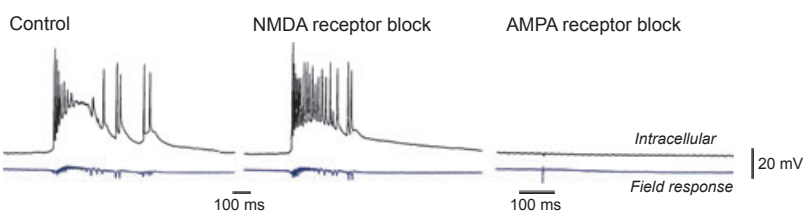

Figure 2. Electrophysiological recording from a guinea pig hippocampal slice in the presence of 50 μM picrotoxin. Top (black) traces are intracellular recordings from CA3 pyramidal neurons; bottom (blue) traces are extracellular recordings from the *stratum pyramidale*. Bath application of the NMDA receptor antagonist DL-2-amino-5-phosphonovaleric acid (APV, 200 μM) eliminates late bursting but does not affect the triggering of the initial epileptiform discharge. However, when the AMPA receptor antagonist CNQX (20 μM) is added, epileptiform activity is eliminated, indicating that AMPA receptors alone are sufficient to bring about the synchronized bursting underlying the primary burst (in the presence of blockade of $GABA_A$ receptors and in neurons that have intrinsic bursting properties of CA3 pyramidal neurons). Adapted from (15), with permission of John Wiley and Sons.

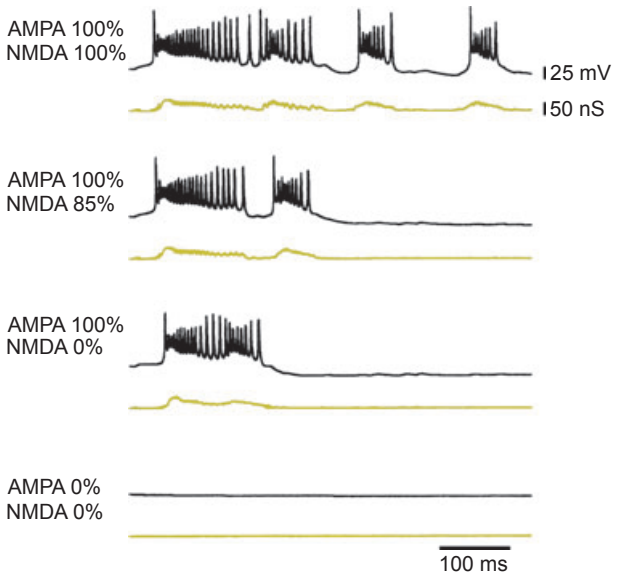

Figure 3. Computer simulation based on a network such as that shown in Fig. 1, illustrating the different contributions of AMPA and NMDA receptors to epileptiform activity. Top (black) traces represent the voltage response of the neuron; bottom (colored) traces represent the conductance of AMPA receptors in the apical dendrite. As the conductance due to NMDA receptors is reduced, the late burst is progressively eliminated, but the initial burst response is largely unaffected. Setting the conductance of NMDA receptors and AMPA receptors to zero abolishes synchronized activity. Compare the simulation with the experimental data shown in Fig. 2. Together, the results indicate that initiation of bursting involves spread of firing involving AMPA receptors. Adapted from (15), with permission of John Wiley and Sons.

aptic excitation (19). As discussed previously, AMPA and NMDA are the main types of ionotropic glutamate receptor mediating fast glutamatergic neurotransmission, but AMPA receptors mediate ordinary synaptic signaling. A key difference between NMDA and AMPA receptors is that NMDA receptors are relatively stable at synapses and undergo only minimal recycling, while AMPA receptors move into and out of the post-synaptic membrane dynamically in an activity-dependent manner (20).

Most AMPA receptors are permeable to sodium and potassium but not calcium; opening of the AMPA receptor channel causes depolarization of the membrane potential, predominantly due to the influx of sodium ions. By contrast, NMDA receptors, which are permeable not only to sodium and potassium but also to calcium, generate current only when excitatory synapses are strongly activated, for example, by high-frequency stimulation. This is because NMDA receptors are blocked by magnesium, which is only relieved when the neuron is strongly depolarized. However, when NMDA receptors are activated, the calcium that flows into neurons can initiate intracellular signaling cascades that lead to alterations in the functional properties of synapses (21). For example, some calcium binds to calmodulin, and the bound complex activates several protein kinases, including calcium-/calmodulin-dependent protein kinase II (CaMKII), which phosphorylates AMPA receptors, thus increasing their conductance. In addition, CaMKII promotes movement of AMPA receptors into the post-synaptic membrane. The overall effect is a lasting change in the strength (amplitude) of EPSPs, which is hypothesized to be a major mechanism underlying learning and memory. Note, however, that AMPA receptors (in contrast with NMDA receptors) do not ordinarily play a role in mediating the calcium entry that triggers persisting changes in synaptic strength, although they can do this in some circumstances (22, 23). The phenomenon of long-term potentiation (LTP) is believed to be a cellular mechanism underlying memory formation (24). In LTP, a brief tetanic stimulation causes a persistent increase in the amplitude of the EPSP generated by activation of an excitatory pathway. NMDA receptor gating is required for the induction of LTP, and NMDA receptor antagonists eliminate LTP induction. By contrast, blockade of AMPA receptors does not affect LTP induction, even though synaptic transmission in the pathway may be inhibited by the AMPA receptor antagonist (25). In line with these *in vitro* observations, in animal models, AMPA receptor antagonists were not found to impair memory formation or retrieval, even at doses that affected motor performance (26, 27), whereas NMDA receptor antagonists were found to suppress memory acquisition in these and many other studies (28).

The molecular architecture of AMPA receptors

AMPA receptors are glutamate-gated ion channels formed as tetramers from combinations of the protein subunits GluA1, GluA2, GluA3, and GluA4 (formerly GluR1–4). The GluA2 subunit plays a special role. When the pre-mRNA for this subunit is edited at Q/R-site 607 by ADAR2 (adenosine deaminase acting on RNA) so that it codes for arginine (R) instead of glutamine (Q), receptors formed from the subunit are calcium impermeable. Virtually, all GluA2 subunits are edited. At most excitatory synapses onto principal (excitatory) neurons – which are localized to dendritic spines – AMPA receptors contain the GluA2 subunit. However, AMPA receptors exist not only in the post-synaptic membrane of principal neurons, but also in inhibitory interneurons

and some glia. These AMPA receptors may not contain GluR2. Such GluR2-lacking AMPA receptors are calcium permeable. Calcium-permeable AMPA receptors also exist in the immature brain, in certain pathological conditions, and are formed after plasticity-inducing stimuli (29, 30).

AMPA receptors are complexed with a group of proteins known as TARPs (transmembrane AMPA receptor regulatory proteins (31). The first TARP to be identified was stargazin (TARP γ-2). In rodents, there are eight stargazin-related proteins, γ-1–γ-8. TARPs play a major role in AMPA receptor trafficking and their expression at synapses. They also influence the kinetics of AMPA receptor currents and, in some cases, the drug sensitivity of the receptor (32–34). A mutation in the mouse stargazin gene causes seizures, reminiscent of absence epilepsy in humans, as well as other neurological abnormalities. Moreover, human genetic studies have suggested that TARPs can underlie certain familial epilepsies (34). Mutations in many AED targets, including voltage-gated sodium and potassium channels and $GABA_A$ receptors, have been associated with specific epilepsy syndromes. While mutations in AMPA receptors *per se* have not yet been identified as a cause for epilepsy, the observation that mutations in TARPs do cause epilepsy is consistent with the view that AMPA receptors play a fundamental role in the disorder.

Distribution of AMPA receptors

AMPA receptors are distributed widely in the central nervous system and are present in all areas relevant to epilepsy, including the cerebral cortex, amygdala, and thalamus (Fig. 4) (36). Although there are regional differences in the expression of the four subunits, GluA1, GluA2, and GluA3 are the most abundant subunits in the forebrain, with the exception of some thalamic nuclei, where GluA4 is also abundant. Approximately 80% of AMPA receptors at excitatory synapses on CA1 hippocampal pyramidal neurons are GluA1/GluA2 heteromers (37). AMPA receptor EPSPs in this and other brain regions are largely mediated by AMPA receptors of this subunit composition, although GluA3/

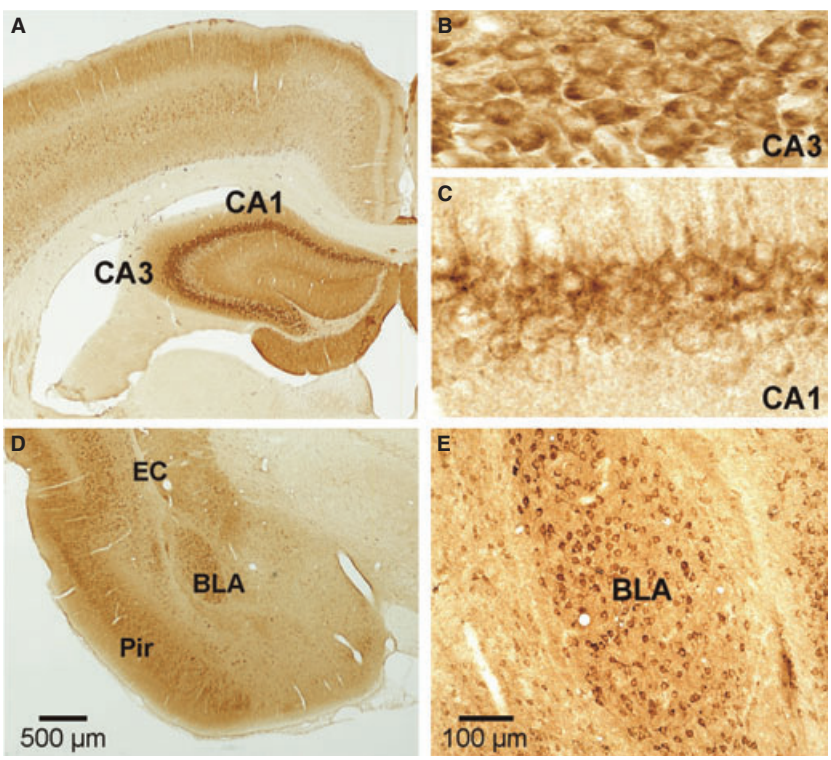

Figure 4. Localization of AMPA receptors in a coronal section of the rat brain showing distribution in areas relevant to epilepsies. Immunostaining was carried out with the immunoperoxidase method, using a specific antibody to the AMPA receptor GluA2 subunit. (A) Low-power view of hippocampus and surrounding neocortex. There is dense staining of pyramidal cells in the CA3 and CA1 subfields. (B) and (C) Higher-power view of stained neuronal cell bodies and processes in CA3 (B) and CA1 (C). (D) Low-power view of the anterior portion of the amygdala showing the external capsule (EC), basolateral amygdala (BLA), and piriform cortex (Pir). (E) Higher-power view of the BLA showing prominent staining of neuronal cell bodies. Scale in (D) also applies to (A). Reproduced from (35), with permission of John Wiley and Sons.

GluA2 heteromers contribute as well. AMPA receptors are located to the post-synaptic membrane at synapses, as illustrated in Fig. 5, but they can move laterally within the membrane into and out of synapses (20, 38). Insertion and internalization of AMPA receptors occur dynamically at extrasynaptic sites (39). At some synapses, AMPA receptors are located on presynaptic axon terminals, where they regulate neurotransmitter release (40).

Antagonist pharmacology of AMPA receptors

Understanding the physiological roles of AMPA receptors was enabled by the discovery in the 1980s of selective pharmacological antagonists (41). Rapid advances in the pharmacology were undoubtedly driven by a myriad of potential clinical applications (42). Because AMPA receptors are largely [although not exclusively; see (40)] located in the central nervous system, AMPA receptor antagonists generally do not produce side effects involving other organs or the peripheral nervous system. The first potent and selective AMPA receptor antagonists were quinoxalinediones, such as CNQX (43), which exert their blocking action by competing for agonist binding at the glutamate recognition site. Gating of the AMPA receptor channel occurs by binding of agonists, including the natural ligand glutamate or the synthetic but more selective ligand AMPA, to a pocket produced by two globular protein domains forming the ligand-binding core that close around the agonist in a Venus-flytrap or hinged-clamshell-type mechanism (44). The movement of lobe 2 toward lobe 1 by ~20° is the initial conformation change that triggers receptor activation (45) (Fig. 6). Quinoxalinediones occupy the ligand-binding core but produce minimal domain closure (~5°); their blocking action is competitive because it can be surmounted by agonists. Early quinoxalinediones exhibited poor *in vivo* activity, due to a lack of blood–brain barrier permeability but later analogs, such as NBQX, were found that did exhibit systemic activity (46), including anticonvulsant activity in diverse seizure models (47–50). Additional quinoxalinediones with *in vivo* anticonvulsant activity were described, such as YM872 (51, 52), YM90K (53, 54), ZK 200775 (MPQX, fanapanel) (55), and AMP397 (becampanel) (40, 56). Some structurally dissimilar competitive AMPA receptor antagonists with *in vivo* anticonvulsant activity were also identified, including the pyrazine derivative RPR117824 (57, 58); isatin oximes, such as NS1209 (SPD 502) (59, 60), which also inhibits GluK1 kainate receptors; a series of quinazoline-2,4-dione sulfonamides (61, 62); and the quinazolin-2-one Ro 48-8587 (40). NS1209 is not orally active and was investigated as an intravenous agent for the treatment of status epilepticus (63). NS1209 was reported to have good tolerability in human volunteers, but the results in status epilepticus have not been disclosed. Decahydroisoquinoline LY 293558 (tezampanel) is an additional structurally novel competitive AMPA receptor antagonist with *in vivo* anticonvulsant activity (64, 65). Although LY 293558 was originally believed to be a selective AMPA receptor antagonist, it inhibits GluK1 kainate receptors with even greater potency.

In the early 1990s, the 2,3-benzodiazepines, exemplified by GYKI 52466, were characterized

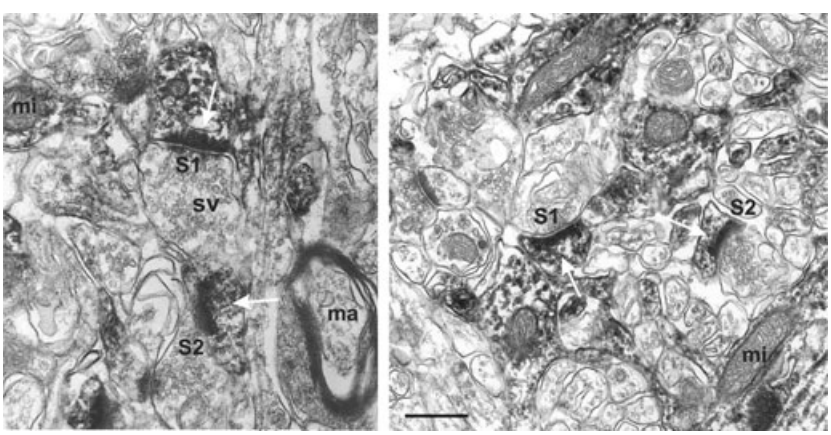

Figure 5. Electron microscopic localization of AMPA receptors to synapses (S1, S2) in rat hippocampus subfields CA3 (left panel) and CA1 (right panel). White arrows indicate electron-dense reaction product corresponding to AMPA receptors in the post-synaptic density. The stained post-synaptic elements are apposed to unstained presynaptic terminals containing synaptic vesicles (sv). Scale bar represents 500 nm for the right panel and 390 nm for the left panel. Reproduced from (35), with permission of John Wiley and Sons.

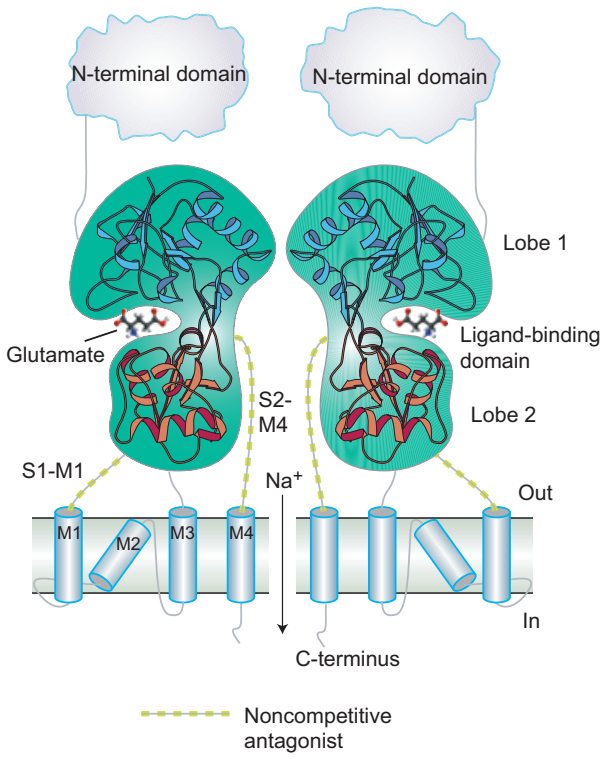

Figure 6. Model of AMPA receptor illustrating the domain structure of two subunits with linker segments that bind non-competitive antagonists; for simplicity, two subunits of the tetrameric AMPA receptor are not shown. Each subunit consists of an amino (N)-terminal domain; a two-lobe ligand-binding core that serves as the binding site for competitive antagonists; three linker segments; a transmembrane domain that forms the ion channel, which is composed of three membrane-spanning segments (M1–M3, where M3 lines the pore) and a pore loop (M2); and a carboxy (C)-terminal cytoplasmic domain. Agonists, including glutamate and the synthetic amino acids AMPA and kainate, bind to lobe 1 and then the flexible lobe 2 moves toward lobe 1. This movement is transmitted through the linkers to the transmembrane domain leading to a conformational change ('gating' of the channel) permitting flow of ions. Na^+ is illustrated entering the cell through the central pore, which leads to depolarization; however, AMPA receptors are permeable to both Na^+ and K^+. Binding of non-competitive antagonists to the S1–M1 and S2–M4 linkers stabilizes the closed conformation. This prevents gating of the channel when agonist is bound to the ligand-binding domain.

as a novel class of selective AMPA receptor antagonists (66). In contrast with previously described AMPA receptor antagonists, 2,3-benzodiazepines are non-competitive antagonists that act as negative allosteric modulators at a site that is distinct from the glutamate recognition site (67, 68). Such compounds were demonstrated to have broad spectrum anticonvulsant activity in diverse animal models (47, 49, 69, 70). The GYKI 52466 analog talampanel (GYKI 53773, LY300164) demonstrated efficacy in a clinical trial for the treatment of refractory partial seizures, although the interpretation was confounded by various drug–drug interactions (63). Adverse events near peak plasma concentrations of talampanel included sedation, mild-to-moderate ataxia, and dizziness (71). The plasma terminal half-life time of talampanel is about 6 h, so that administration three times daily is required for therapeutic use. The multiple peaks in plasma concentration throughout the day might unfavorably influence the safety profile.

2,3-Benzodiazepine AMPA receptor antagonists, such as talampanel, are selective for AMPA receptors. They do not inhibit NMDA receptors and only weakly inhibit kainate receptors. Domain swapping and site-directed mutagenesis have demonstrated that the antagonists bind to peptide segments of AMPA receptor subunits that link the transmembrane spanning regions and the ligand-binding core, as illustrated in Fig. 6 (72). Binding of antagonists disrupts the ability of conformational changes in the ligand-binding domains induced by agonist binding to open the channel. Certain non-competitive antagonists appear to bind most potently to receptors that do not have agonists bound (72), whereas others bind preferentially to the ligand bound open state (68, 73).

In addition to 2,3-benzodiazepines, several other structurally dissimilar non-competitive AMPA receptor antagonists have been described, including the phthalazine YM928 (74, 75), the imidazole GYKI 47261 (76), and the quinazolin-4-one CP-465,022 (77, 78). More recently, the highly potent non-competitive AMPA receptor antagonist perampanel was discovered by a rational drug discovery program. In clinical epilepsy trials in patients with partial-onset seizures, perampanel was found to have acceptable safety and significant efficacy (79–81). The pharmacology of perampanel is described in a companion article (82).

Conclusions

A quarter century of research supports the central role of AMPA receptors in epileptic synchronization (9), and studies in *in vitro* preparations and animal models demonstrate the potential utility of AMPA receptors as a target for seizure protection (83). The evidence that perampanel is efficacious as adjunctive therapy in the treatment of human partial-onset seizures (79–81) validates the AMPA receptor as a novel target for epilepsy therapy. This development will undoubtedly stimulate interest in the investigation of other drugs that target AMPA receptors for the treatment of epilepsy and a broad range of other central nervous system disorders.

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Conflict of interest

The author has served as a consultant to Eisai Inc.

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