



## O Brother, Wherefore Are Thou? Calcium-Permeable AMPA Receptors Make an Appearance in Adult Status Epilepticus

### Calcium-Permeable AMPA Receptors Are Expressed in a Rodent Model of Status Epilepticus.

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**OBJECTIVE:** A study was undertaken to characterize the plasticity of AMPA receptor (AMPA)-mediated neurotransmission in the hippocampus during status epilepticus (SE). **METHODS:** SE was induced by pilocarpine, and animals were studied 10 minutes (refractory SE) or 60 minutes (late SE) after the onset of the first grade 5 seizures. AMPAR-mediated currents were recorded from CA1 pyramidal neurons and dentate granule cells (DGCs) by voltage clamp technique. The surface expression of GluA2 subunit on hippocampal membranes was determined using a biotinylation assay. GluA2 internalization and changes in intracellular calcium ( $[Ca]_i$ ) levels were studied in hippocampal cultures using immunocytochemical and live-imaging techniques. AMPAR antagonist treatment of SE was evaluated by video and electroencephalography. **RESULTS:** AMPAR-mediated currents recorded from CA1 neurons from refractory and late SE animals were inwardly rectifying, and philanthotoxin-sensitive; similar changes were observed in recordings obtained from DGCs from refractory SE animals. GluA2 subunit surface expression was reduced in the hippocampus during refractory and late SE. In cultured hippocampal pyramidal neurons, recurrent bursting diminished surface expression of the GluA2 subunit and enhanced its internalization rate. Recurrent bursting-induced increase in  $[Ca]_i$  levels was reduced by selective inhibition of GluA2-lacking AMPARs. GYKI-52466 terminated diazepam-refractory SE. **INTERPRETATION:** During SE, there is rapid, ongoing plasticity of AMPARs with the expression of GluA2-lacking AMPARs. These receptors provide another source of  $Ca^{2+}$  entry into the principal neurons. Benzodiazepam refractory SE can be terminated by AMPAR antagonism. The data identify AMPARs as a potential therapeutic target for the treatment of SE.

### Commentary

The  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA)-type glutamate receptors (AMPA) are normally assembled from GluA subunits 1–4 into tetrameric heteromers containing GluA2 within the endoplasmic reticulum. Prior to their assembly, GluA2 mRNA is edited by ADAR2 at a critical region within the pore. Editing is thought to be complete from birth onward. Edited GluA2 subunits, when associated with other GluA subunits, confer calcium impermeability while GluA2-lacking receptors flux calcium. Calcium-permeable AMPAR—GluA2-lacking or GluA2-unedited—can be detected by their resulting inward rectification (relative lack of current at positive versus negative holding potentials) conferred by endogenous or exogenous polyamines such as spermine, unique sensitivity to externally applied polyamine toxins such as PhTx433, faster kinetics and larger single-channel conductance (1, 2).

Following assembly and packaging into endosomes, AMPAR are trafficked to synapses where they exist in three simplified pools: synaptic, extrasynaptic, and endosomal (subsynaptic) pools. Synaptic GluA2-lacking AMPAR are generally a feature of early development, largely disappearing after the first post-natal week in CA1 hippocampus (3); later > 80% of synaptic and > 95% extrasynaptic receptors contain GluA2 (4). However, enrichment of synaptic GluA2-lacking receptors (presumably GluA1 homomers) has been transiently detected in both “normal” and pathological conditions. Post-synaptic changes in GluA subunit numbers or properties are thought to underlie synaptic modification in long-term potentiation, and depression (LTP, and LTD) (5). This has resulted in postulated AMPA receptor “subunit rules”: 1) synaptic removal of GluA2 subunits underlies LTD, 2) GluA1 not associated with GluA2 act independently, and 3) insertion and/or modification of GluA1 underlies LTP (6).

A rapid, selective trafficking of GluA2-lacking receptors into synapses has been shown to participate in the early phase of hippocampal CA1 LTP (7). Calcium influx through these receptors triggers a later phase swap of GluA2-lacking for GluA2-containing receptors. This swap appears to be critically dependent on the nature—size, extent, and temporal



features—of the calcium accumulation. The swap is mediated in part by the calcium sensor PICK1. This feature is crucial, as alternative calcium accumulations may trigger PICK1 to remove GluA2s in LTD (8).

Calcium permeable AMPAR are also a pathological feature. Down-regulation of GluA2, up-regulation of GluA1, loss of GluA2 editing, and selective GluA1 trafficking could each potentially lead to more calcium-permeable AMPAR. The former contributed to the “GluA2 hypothesis” (9) whereby preferential decrease in expression and subsequent loss of synaptic GluA2 (with no changes in GluA1) could lead to AMPAR that flux calcium. GluA1 up-regulation has been found in an adult model of electroconvulsive therapy (10) and after hypoxic seizures in immature rats (11). GluA2 knock-down studies have shown that down-regulation of GluA2 can lead to seizures and hippocampal injury (12). Clinical evidence from pathological studies might support up-regulation of GluA1 in epileptic tissue (13). Loss of GluA2 mRNA editing by down-regulation of the editing enzyme ADAR2, leading to greater calcium permeability, has been demonstrated after hypoxia (14). The present study now sheds light on alterations in GluAs associated with status epilepticus.

Refractory status epilepticus (RSE) (longer than 60–90 minutes) is a worst-case clinical scenario. Patients experience prolonged seizures, and nothing short of pentobarbital-induced coma may (or may not) stop the seizures and prevent the associated morbidity and mortality. Despite the clinical impact of 60,000–150,000 patients per year with approximately 55,000 deaths (15), the mechanisms underlying the transition from self-limited seizure to prolonged, medically refractory seizure are not fully understood. Utilizing animal models, prior work has detailed alterations in GABA receptors that begin to explain the resistance of RSE to benzodiazepines (16). Here, the pilocarpine model of status epilepticus has been used as a rodent model of RSE. The pilocarpine model is especially attractive in this regard as it represents the severe end of the spectrum of rodent models of status epilepticus; it is associated with significant mortality and morbidity, including later epilepsy.

The authors prepared hippocampal slices from rats during RSE. Using whole-cell patch-clamp recordings in CA1 and dentate gyrus, they found electrophysiological evidence for synaptically activated GluA2-lacking/calcium-permeable AMPAR. Using a biochemical technique, they found a nearly twofold loss of surface (synaptic plus extrasynaptic) GluA2 and a twofold gain of surface (synaptic plus extrasynaptic) GluA1. Given that the time course and nature of these alterations could not be readily studied in an intact preparation, the authors used hippocampal cultures. Brief treatment of hippocampal cultures with low magnesium resulted in sustained burst-firing, thought to represent an *in vitro* correlate of RSE. Here, the authors measured the rate of decline of surface GluA2 which paralleled the internalization (endosomal) and accumulation of GluA2 with a time-constant of ~6 minutes. Further, the authors measured calcium accumulations in burst-firing cultures and determined that these could be attenuated with selective antagonism of GluA2-lacking receptors. The authors did not investigate whether synaptic GluA2-lacking receptors were present, nor did they investigate accumula-

tion of surface GluA1 receptors in burst-firing cultures. These findings might be necessary to further tightly link their *in vivo* findings with their *in vitro* model of RSE. Nevertheless, these findings highlight the role of AMPAR in RSE. To strengthen this point, the authors found that the selective AMPAR antagonist GYKI-52466 stopped RSE in a dose-dependent manner *in vivo*.

Thus, the authors provide strong evidence for the therapeutic benefit of AMPAR antagonism to combat RSE. The authors' findings do raise several important questions to be addressed by future work to better understand the impact of AMPAR in mediating RSE: While synaptic pools of AMPAR are affected by RSE, it is not clear whether extrasynaptic pools are altered as well. In other words, not all shifts in surface AMPAR impact the synapse. This distinction is important in RSE, as the normally tight control of glutamate within the synaptic cleft is potentially lost by alterations in glutamate uptake (17). Activation of extrasynaptic AMPAR may lead to activation of additional signaling pathways that mediate RSE, as is the case for extrasynaptic NMDA-receptor activation in models of hypoxia (18) and Huntington disease (19). To make parallel comparisons to LTP, understanding why synaptic GluA1 homomers stay increased without a switch back to GluA1/2 heteromers is important. While transient calcium-mediated PICK1 signaling mediates the normal late phase subunit switch, prolonged signaling may underlie the further removal of GluA2 in a parallel comparison to LTD. This may further exacerbate calcium accumulations in a positive feedback loop. This is also important, as it represents perhaps another therapeutic target for RSE. Further, does RSE actually lead to a potentiation of synaptic responses? Does GluA1-mediated excessive synaptic drive further underlie RSE? In other words, how do all of the changes in AMPAR mediate RSE? Has ADAR2 modulation been ruled out? Finally, GluA2-lacking receptor function is further modulated by associated proteins called TARPs; alterations in TARP-AMPA interactions could contribute to some of the changes in rectification seen with RSE (20).

by Tim Benke, MD, PhD

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