Losing Balance Over a Fatty Acid

Epilepsy Currents
2019, Vol. 19(2) 129-131

DOI: 10.1177/1535759719835664
journals.sagepub.com/home/epi
SAGE

Deficiency of AMPAR-Palmitoylation Aggravates Seizure Susceptibility

Itoh M, Yamashita M, Kaneko M, Okuno H, Abe M, Yamazaki M, Natsume R, Yamada D, Kaizuka T, Suwa R, Sakimura K, Sekiguchi M, Wada K, Hoshino M, Mishina M, Hayashi T. *J Neurosci*. 2018;38(47):10220-10235. doi:10.1523/JNEUROSCI.1590-18.2018. Epub 2018 Oct 24. PMID: 30355633.

Synaptic AMPAR expression controls the strength of excitatory synaptic transmission and plasticity. An excess of synaptic AMPARs leads to epilepsy in response to seizure-inducible stimulation. The appropriate regulation of AMPARs plays a crucial role in the maintenance of the excitatory/inhibitory synaptic balance; however, the detailed mechanisms underlying epilepsy remain unclear. Our previous studies have revealed that a key modification of AMPAR trafficking to and from postsynaptic membranes is the reversible, post-translational S-palmitoylation at the C-termini of receptors. To clarify the role of palmitoylation-dependent regulation of AMPARs *in vivo*, we generated GluA1 palmitoylation-deficient (Cys811 to Ser substitution) knock-in mice. These mutant male mice showed elevated seizure susceptibility and seizure-induced neuronal activity without impairments in synaptic transmission, gross brain structure, or behavior at the basal level. Disruption of the palmitoylation site was accompanied by upregulated GluA1 phosphorylation at Ser831, but not at Ser845, in the hippocampus and increased GluA1 protein expression in the cortex. Furthermore, GluA1 palmitoylation suppressed excessive spine enlargement above a certain size after long-term potentiation. Our findings indicate that an abnormality in GluA1 palmitoylation can lead to hyperexcitability in the cerebrum, which negatively affects the maintenance of network stability, resulting in epileptic seizures. Significance Statement: AMPARs predominantly mediate excitatory synaptic transmission. AMPARs are regulated in a post-translational, palmitoylation-dependent manner in excitatory synapses of the mammalian brain. Reversible palmitoylation dynamically controls synaptic expression and intracellular trafficking of the receptors. Here, we generated GluA1 palmitoylation-deficient knock-in mice to clarify the role of AMPAR palmitoylation *in vivo*. We showed that an abnormality in GluA1 palmitoylation led to hyperexcitability, resulting in epileptic seizure. This is the first identification of a specific palmitoylated protein critical for the seizure-suppressing process. Our data also provide insight into how predicted receptors such as AMPARs can effectively preserve network stability in the brain. Furthermore, these findings help to define novel key targets for developing antiepileptic drugs.

Commentary

Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors are crucial regulators of neuronal excitability and have been investigated extensively as treatment targets in epilepsy. In 2012, perampanel, the first antiepileptic drug selectively inhibiting AMPA receptors, was approved for treatment of partial-onset and primary generalized tonic-clonic seizures. Despite predominantly having a single mechanism of action, perampanel can induce severe side effects that may be caused by its widespread action on AMPA receptors throughout the brain.[1] If the underlying mechanisms of how AMPA receptors control the brain's excitability were understood better, more specific drugs could be developed to reduce side effects. A recent study by Itoh and coauthors points toward a potentially targetable mechanism that regulates AMPA receptor-mediated control of seizure susceptibility.

AMPA receptors function as ion channels in the membrane. Their membrane localization is highly dynamic and depends on protein binding partners and post-translational modifications. One of these modifications, palmitoylation, regulates membrane localization of AMPA receptors *in vitro* indicating an important role in controlling neuronal excitability.[2] Until now, it was unclear if and how this mechanism affects neuronal hyperexcitability in epilepsy. To get at this question, Itoh et al generated a mouse model that is deficient in palmitoylation of the AMPA receptor subunit GluA1.

Palmitoylation covalently adds a fatty acid, palmitate, to a cysteine residue on proteins. Palmitoylation is reversible and

sensitive to neuronal activity and external stimuli. It often serves as a "membrane anchor" but can have diverse functions depending on the protein and the location of the palmitoylation. GluA1 has two palmitoylation sites, one in a transmembrane domain that promotes accumulation of the receptor in the Golgi apparatus, and another at the C-terminus, which regulates its synaptic membrane localization. Previous studies in cultured neurons suggested that C-terminal palmitoylation of GluA1 reduces neuronal excitation after stimulation: mutations that prevent the C-terminal palmitoylation of GluA1 abolished activity-induced GluA1 internalization.[2] The transgenic mouse line generated by Itoh et al has the same mutation as used in these *in vitro* studies, replacing the C-terminal cysteine needed for palmitoylation with a serine, which prevents GluA1 palmitoylation specifically at this site.

A major finding of Itoh et al was that the GluA1 palmitoylation-deficient mice had a higher susceptibility to seizures induced by the gamma-aminobutyric acid (GABA) antagonist pentylenetetrazol (PTZ), and a heightened response to PTZ-induced kindling, a method in which repeated administration of low doses of PTZ eventually leads to convulsive seizures. The palmitoylation-impaired mice also showed stronger expression of the immediate early genes Arc/Arg3.1 and cFos upon seizure and were less responsive to several commonly used anticonvulsant drugs. These results suggest that GluA1 palmitoylation helps to prevent hyperexcitability in the brain. The study did not investigate basic electroencephalography (EEG) properties or the occurrence of spontaneous seizures. The effect on seizure susceptibility was modest, which argues against a spontaneous seizure phenotype, but it would be interesting to test if epileptogenesis after a precipitating event, such as status epilepticus, is increased. It is also unclear if the observed effect is selective for PTZ-induced seizures, or if other seizure-inducing methods, for example, kainic acid or electroconvulsive shock would produce the same phenotype.

The mice did not show any changes in basal neuronal properties and in two forms of hippocampal synaptic plasticity, long-term depression and long-term potentiation (LTP). This was surprising, because hippocampal phosphorylation of GluA1 at Serine 831 was increased under basal conditions. Phosphorylation at this site occurs during LTP and increases GluA1 ion conduction,[3] suggesting that AMPA receptor function was enhanced in the mice without affecting basal neuronal function. It is worth mentioning that the authors analyzed GluA1 phosphorylation in total cell lysates but not specifically at synapses. Compensatory mechanisms may have normalized GluA1 phosphorylation at synapses, which could have caused the lack of effects on basal neuronal function. The lack of an effect on basal neuronal function and LTP suggests that therapeutic interventions targeting GluA1 palmitoylation could leave normal brain function and cognition unaltered.

Although LTP was not altered in the mutant mice, the authors observed a significantly larger increase in dendritic spine volume after LTP in the mutant mice compared to wild type. Similar to the electrophysiological neuronal properties, there were no changes in dendritic spine size under basal conditions. This aligns with the idea that C-terminal palmitoylation of GluA1 specifically regulates stimulus-dependent mechanisms and should be further investigated. If synaptic plasticity-evoking activity increases dendritic spine volume beyond normal levels, one could expect long-term effects of recurring induction of synaptic plasticity. For example, exposing the mutant mice to repeated learning paradigms may produce enduring changes in neuronal morphology and function. Related, it will be important to assess if the mutation impairs cognition or behavior. A follow-up study of the authors supports a potential cognitive phenotype in the GluA1 palmitoylation-deficient mice: the distribution of Arc/Arg3.1 after stimulation is altered in the upper and lower blades of the dentate gyrus in these mice.[4] Arc/Arg3.1 is crucial for synaptic plasticity and learning and memory.[5] Its altered stimulus-induced distribution could therefore affect cognition. Notably, modulation of AMPA receptors improves social deficits in two mouse models of autism.[6] Autism is believed to be caused, at least partially, by an excitatory/inhibitory imbalance in the brain and is often comorbid with epilepsy; however, the role of GluA1 palmitoylation in autism is unknown. Analyzing cognition and behavior when GluA1 palmitoylation is impaired will be critical to evaluate the therapeutic value of Itoh and colleagues' findings.

In summary, the study supports the appealing hypothesis that loss of C-terminal palmitoylation of GluA1 shifts the brain balance toward a more excitable state without affecting basal function. Conversely, a method that increases palmitoylation of GluA1 at the C-terminus could move the brain toward a less excitable, and thus more stable state, suggesting a very specific novel treatment strategy for epilepsy with potentially few side effects. But how could this be achieved? So far, there are no drugs to modulate palmitoylation of specific proteins. The enzymes involved in palmitoylation—palmitoyltransferases and depalmitoylating enzymes—seem to be substrate-selective and could be promising drug targets, but more work is needed to understand the underlying mechanisms.[7] Many proteins are regulated by palmitoylation, including synaptic receptors and scaffolding proteins.[8] Interventions that alter overall palmitoylation in the brain may thus have widespread adverse effects. Indeed, Spinelli et al showed that enhanced palmitoylation may impair GluA1 trafficking and cognitive function in mice.[9] In this study, palmitoylation was upregulated by a typical western high-fat diet that increases the levels of saturated fatty acids like palmitate. A less dramatic increase of palmitoylation, though, may tip the scale toward a more stable brain, reducing seizure occurrence without affecting cognition. Itoh et al's findings that specifically blocking C-terminal palmitoylation of GluA1 affects seizures without altering neuronal function make this mechanism an attractive therapeutic target in epilepsy with potentially fewer side effects than current GluA1-modulating medication.

By Christina Gross

References

1. Tsai JJ, Wu T, Leung H, et al. Perampanel, an AMPA receptor antagonist: from clinical research to practice in clinical settings. *Acta Neurol Scand*. 2018;137(4):378-391.
2. Hayashi T, Rumbaugh G, Huganir RL. Differential regulation of AMPA receptor subunit trafficking by palmitoylation of two distinct sites. *Neuron*. 2005;47(5):709-723.
3. Derkach V, Barria A, Soderling TR. Ca2+/calmodulin-kinase II enhances channel conductance of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate type glutamate receptors. *Proc Nati Acad Sci USA*. 1999;96(6):3269-3274.
4. Itoh M, Okuno H, Yamada D, et al. Perturbed expression pattern of the immediate early gene Arc in the dentate gyrus of GluA1 C-terminal palmitoylation-deficient mice. *Neuropsychopharmacology Rep*. 2018. doi:10.1002/npr2.12044.
5. Plath N, Ohana O, Dammermann B, et al. Arc/Arg3.1 is essential for the consolidation of synaptic plasticity and memories. *Neuron*. 2006;52(3):437-444.
6. Kim JW, Park K, Kang RJ, et al. Pharmacological modulation of AMPA receptor rescues social impairments in animal models of autism. *Neuropsychopharmacology*. 2019;44(2):314-323.
7. Tabaczar S, Czogalla A, Podkalicka J, Biernatowska A, Sikorski AF. Protein palmitoylation: palmitoyltransferases and their specificity. *Exp Biol Med (Maywood)*. 2017;242(11):1150-1157.
8. Fukata Y, Fukata M. Protein palmitoylation in neuronal development and synaptic plasticity. *Nat Rev Neurosci*. 2010;11(3):161-175.
9. Spinelli M, Fusco S, Mainardi M, et al. Brain insulin resistance impairs hippocampal synaptic plasticity and memory by increasing GluA1 palmitoylation through FoxO3a. *Nat Commun*. 2017;8(1):2009.