Epilepsy Currents
2023, Vol. 23(6) 372-374

DOI: 10.1177/15357597231199343
journals.sagepub.com/home/epi
Sage

Brake Early: RGS14 in CA2 Limits Seizures and Oxidative Stress After SE

RGS14 Limits Seizure-Induced Mitochondrial Oxidative Stress and Pathology in Hippocampus

Harbin NH, Lustberg DJ, Hurst C, Pare J, Crotty KM, Waters AL, Yeligar SM, Smith Y, Seyfried NT, Weinschenker D, Hepler JR. *Neurobiol Dis.* 2023;181:106128. doi:10.1016/j.nbd.2023.106128. PMID: 37075948

RGS14 is a complex multifunctional scaffolding protein that is highly enriched within pyramidal cells (PCs) of hippocampal area CA2. In these neurons, RGS14 suppresses glutamate-induced calcium influx and related G protein and ERK signaling in dendritic spines to restrain postsynaptic signaling and plasticity. Previous findings show that, unlike PCs of hippocampal areas CA1 and CA3, CA2 PCs are resistant to a number of neurological insults, including degeneration caused by temporal lobe epilepsy (TLE). While RGS14 is protective against peripheral injury, similar roles for RGS14 during pathological injury in hippocampus remain unexplored. Recent studies showed that area CA2 modulates hippocampal excitability, generates epileptiform activity and promotes hippocampal pathology in animal models and patients with TLE. Because RGS14 suppresses CA2 excitability and signaling, we hypothesized that RGS14 would moderate seizure behavior and early hippocampal pathology following seizure activity, possibly affording protection to CA2 PCs. Using kainic acid (KA) to induce status epilepticus (KA-SE) in mice, we show that the loss of RGS14 (RGS14 KO) accelerated onset of limbic motor seizures and mortality compared to wild type (WT) mice, and that KA-SE upregulated RGS14 protein expression in CA2 and CA1 PCs of WT. Our proteomics data show that the loss of RGS14 impacted the expression of a number of proteins at baseline and after KA-SE, many of which associated unexpectedly with mitochondrial function and oxidative stress. RGS14 was shown to localize to the mitochondria in CA2 PCs of mice and reduce mitochondrial respiration in vitro. As a readout of oxidative stress, we found that RGS14 KO dramatically increased 3-nitrotyrosine levels in CA2 PCs, which was greatly exacerbated following KA-SE and correlated with a lack of superoxide dismutase 2 (SOD2) induction. Assessing for hallmarks of seizure pathology in RGS14 KO, we unexpectedly found no differences in neuronal injury in CA2 PCs. However, we observed a striking and surprising lack of microgliosis in CA1 and CA2 of RGS14 KO compared to WT. Together, our data demonstrate a newly appreciated role for RGS14 in limiting intense seizure activity and pathology in hippocampus. Our findings are consistent with a model where RGS14 limits seizure onset and mortality and, after seizure, is upregulated to support mitochondrial function, prevent oxidative stress in CA2 PCs, and promote microglial activation in hippocampus.

Commentary

Changes in hippocampal physiology and pathology are a hallmark of temporal lobe epilepsy (TLE) and include neuronal injury, cell loss, gliosis, mossy fiber sprouting, as well as changes in ion channels, receptors, and regulatory proteins. The CA2 region of the hippocampus is resistant to seizure-related pyramidal cell loss in comparison to other hippocampal areas, though other pathophysiologic changes can occur in this region.[1,2] Status epilepticus (SE) in mice increases excitability of the CA2 region, with reductions in inhibitory input and increases in excitatory output.[3] Hyperexcitability in the CA2 region may therefore be an important pathologic outcome of SE. Further, seizure propagation via CA2 may be an important process, as projections from this region extend to hippocampal and extrahippocampal areas.[4] Pathogenic processes in this region include decreased inhibitory transmission, mossy fiber sprouting, and hyperexcitability.[3,5] Therefore, CA2 may be important in both maintaining hippocampal network activity in TLE as well as contributing to further pathogenesis. However, as CA2 has not been studied as extensively as other hippocampal regions, the mechanisms by which CA2 contributes to TLE pathophysiology is not as well understood.

Regulators of G-protein signaling (RGS) are important modulators of G-protein coupled receptors and are involved in a wide variety of cellular signaling pathways.[6] One isoform, RGS14, is expressed in the hippocampus, with the greatest amount of expression in CA2. Further, RGS14 may play an important role in restricting synaptic plasticity in this brain

region.[7] Harbin et al recently evaluated the impact of changes in RGS14 in the CA2 region in a mouse seizure model.[8] Their findings provide further insight into how RGS14 regulates hyperexcitability in the hippocampus. In addition, these studies shed light on the role of the CA2 region that has been relatively understudied for its role in epilepsy.

Mice lacking RGS14 (RGS14 KO) received intraperitoneal kainic acid (KA) to evaluate status epilepticus (SE) induction and RGS14 expression patterns. RGS14 is upregulated after SE, particularly in CA2. Loss of RGS14 facilitated entry into SE and increased mortality in the early stages of SE. This suggests that RGS14 may be important in dampening hyperexcitability in CA2. RGS14 may also be important for CA2 output and propagation of epileptiform activity as evidenced by mortality that may have arisen from propagation of seizures to brain regions controlling autonomic function. Furthermore, these data suggest the potential for targeting hippocampal RGS14 in the treatment of SE to reduce seizures and prevent mortality.

To better understand how RGS14 affects CA2 function, the authors performed proteomic analysis in hippocampal tissue from wild type (WT) and KO mice. While most data obtained suggest that RGS14 reduces intracellular signaling, several proteins and pathways were not explored and warrant further investigation. Importantly, the study utilized whole hippocampi for proteomic analysis and thus findings in RGS14 KO mice may not be limited to CA2. The researchers further focused on changes in metabolic proteins and pathways to better understand the role of RGS14. Electron microscopy revealed that RGS14 was localized to mitochondria in the CA2 region. Taken together with proteomic analysis in RGS14 KO mice, which had changes in several metabolic pathways, it is likely that loss of RGS14 contributes to aberrant cellular metabolism following SE. This was further explored in cultured cells expressing RGS14, which had a lower oxygen consumption rate. This may be an important process in CA2, where enhanced activity following seizures is associated with a greater metabolic demand.[9]

Oxidative stress is an important process following SE and in TLE. The authors explored the role of RGS14 in regulating responses to oxidative stress using immunohistochemistry for superoxide dismutase 2 (SOD2) and 3-nitrotyrosine (3-NT), a marker for oxidative stress. SOD2 induction and reduction of superoxide is an important post-SE process. The authors demonstrated that RGS14 is required for SOD2 induction and prevents 3-NT accumulation. Thus, one consequence of RGS14 affecting mitochondrial activity in CA2 may be the reduction of oxidative stress.

They next examined the pathological consequences of RGS14 in hippocampus. Surprisingly, they found no significant difference in early neuronal death in CA2 or other hippocampal regions following SE in RGS14 KO mice compared with WT mice, indicating that RGS14 does not play a critical neuroprotective role in SE-induced neuronal injury. Microgliosis during the acute post-SE period may serve a protective function.[10] The authors also evaluated gliosis following SE by studying microglia expression in WT and KO mice. Vehicle-treated RGS14 KO mice did not show an increase in microglia at baseline, in comparison to WT mice. However, in response to kainate-induced SE, RGS14 KO did not show an increase in markers of microglial activation seen in WT mice, suggesting that RGS14 may be necessary for microglial recruitment in response to SE.

In summary, the findings of Harbin et al suggest that RGS14 in CA2 may limit hyperexcitability and seizure propagation by preventing oxidative stress and facilitating microglial expression following SE, though the lack of effect on SE-induced neuronal death is somewhat unexpected. One application of knowledge gained from this study is the potential for targeting RGS14 as a disease-modifying therapy. Downregulation of RGS14 enhances long-term potentiation (LTP) and improves performance on learning and memory tasks,[6] and overexpression of CA2 RGS14 limits plasticity and dendritic spine volume.[7] Taken together, these data suggest that targeted upregulation of RGS14 may reduce synaptic plasticity that contributes to epileptogenesis. The mechanism by which RGS14 may be affecting signaling in CA2 may include extracellular regulated kinase (ERK) inhibition and subsequent decreased alpha-amino-3-hydroxy-5-methyl-4-isoxazole (AMPA) receptor trafficking.[6] Further, cytosolic RGS14 may be recruited to the synapse, better positioning it for regulation of LTP.[6] Despite this knowledge, the specific mechanisms whereby increasing RGS14 activity may reduce or prevent pathologic changes contributing to TLE are not fully understood. Such insight may further support the pursuit of RGS14 as a compelling target for potential therapy. Importantly, the majority of findings by Harbin et al centered around the 24-hours post-SE time point, suggesting that an RGS14 therapeutic strategy may need to be focused after an insult. This may provide a challenge for affecting RGS14 as a therapeutic strategy for TLE, where the ongoing contribution of RGS14 to pathogenesis is less clear. Therefore, future work may include evaluation of the effect of RGS14 KO on the development of spontaneous seizures in (eg, post-KA spontaneous recurring seizures). Such studies may also be beneficial in defining the timing and extent of RGS14 upregulation in other epilepsy models may provide insight into therapeutic strategies targeting this protein.

Cameron S. Metcalf, PhD
Department of Pharmacology & Toxicology, University of Utah

ORCID iD

Cameron S. Metcalf, PhD https://orcid.org/0000-0002-1510-0405

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

1. Munoz DG. The distribution of chromogranin A-like immunoreactivity in the human hippocampus coincides with the pattern of resistance to epilepsy-induced neuronal damage. *Ann Neurol*. 1990;27(3):266-275.
2. Wittner L, Huberfeld G, Clemenceau S, et al. The epileptic human hippocampal cornu ammonis 2 region generates spontaneous interictal-like activity in vitro. *Brain*. 2009;132(Pt 11):3032-3046.
3. Whitebirch AC, LaFrancois JJ, Jain S, et al. Enhanced excitability of the hippocampal CA2 region and its contribution to seizure activity in a mouse model of temporal lobe epilepsy. *Neuron*. 2022;110(19):3121-3138.e8.
4. Kilias A, Tulke S, Barheier N, Ruther P, Haussler U. Integration of the CA2 region in the hippocampal network during epileptogenesis. *Hippocampus*. 2023;33(3):223-240.
5. Piskorowski RA, Chevaleyre V. Hippocampal area CA2: interneuron disfunction during pathological states. *Front Neural Circuits*. 2023;17:1181032.
6. Gerber KJ, Squires KE, Hepler JR. Roles for regulator of G protein signaling proteins in synaptic signaling and plasticity. *Mol Pharmacol*. 2016;89(2):273-286.
7. Evans PR, Parra-Bueno P, Smirnov MS, et al. RGS14 restricts plasticity in hippocampal CA2 by limiting postsynaptic calcium signaling. *eNeuro*. 2018;5(3).ENEURO.0353-17.
8. Harbin NH, Lustberg DJ, Hurst C, et al. RGS14 limits seizure-induced mitochondrial oxidative stress and pathology in hippocampus. *Neurobiol Dis*. 2023;181:106128.
9. Farris S, Ward JM, Carstens KE, Samadi M, Wang Y, Dudek SM. Hippocampal subregions express distinct dendritic transcriptomes that reveal differences in mitochondrial function in CA2. *Cell Rep*. 2019;29(2):522-539.e6.
10. Eyo UB, Peng J, Swiatkowski P, Mukherjee A, Bispo A, Wu LJ. Neuronal hyperactivity recruits microglial processes via neuronal NMDA receptors and microglial P2Y12 receptors after status epilepticus. *J Neurosci*. 2014;34(32):10528-10540.