Inhibition Gets a New KAR Smell

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Ionotropic and metabotropic kainate receptor signaling regulates Cl^- homeostasis and GABAergic inhibition

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Potassium chloride cotransporter 2 (KCC2) plays a critical role in the regulation of chloride (Cl^-) homeostasis within mature neurons. The KCC2 is a secondarily active transporter that extrudes Cl^- from the neuron, which maintains a low intracellular Cl^-concentration [Cl^-]. This results in a hyperpolarized reversal potential of GABA (E_{GABA}), which is required for fast synaptic inhibition in the mature central nervous system. Potassium chloride cotransporter 2 also plays a structural role in dendritic spines and at excitatory synapses and interacts with "excitatory" proteins, including the GluK2 subunit of kainate receptors (KARs). Kainate receptors are glutamate receptors that display both ionotropic and metabotropic signaling. We show that activating KARs in the hippocampus hyperpolarizes E_{GABA}, thus strengthening inhibition. This hyperpolarization occurs via both ionotropic and metabotropic KAR signaling in the CA3 region, whereas it is absent in the GluK1/2$^{-/-}$ mouse, and is independent of zinc release from mossy fiber terminals. The metabotropic signaling mechanism is dependent on KCC2, although the ionotropic signaling mechanism produces a hyperpolarization of E_{GABA} even in the absence of KCC2 transporter function. These results demonstrate a novel functional interaction between a glutamate receptor and KCC2, a transporter critical for maintaining inhibition, suggesting that the KAR:KCC2 complex may play an important role in excitatory:inhibitory balance in the hippocampus. Additionally, the ability of KARs to regulate chloride homeostasis independently of KCC2 suggests that KAR signaling can regulate inhibition via multiple mechanisms. Activation of kainate-type glutamate receptors could serve as an important mechanism for increasing the strength of inhibition during periods of strong glutamatergic activity.

Commentary

The high prevalence of epilepsy, along with the troubling pervasiveness of pharmacoresistant epilepsies, highlights the critical importance of identifying new targets for potential treatments. One developing area of therapeutic research is the regulation of the intracellular chloride concentration ($[Cl^-]_i$) in neurons and subsequent impact on inhibitory efficacy. In mature neurons, the potassium chloride cotransporter 2 (KCC2)is the primary regulator of $[Cl^-]_i$, extruding chloride from cells such that the GABA reversal potential (E_{GABA}) remains hyperpolarized relative to the resting membrane potential and ensuring fast hyperpolarizing inhibition through activation of chloride-permeable $GABA_A$ receptors.[1] This effect is the basis for the canonical inhibitory actions of GABA in most mature neurons. If $[Cl^-]_i$ becomes sufficiently elevated, however, E_{GABA} can be set to a value more depolarized than the resting membrane potential, thus resulting in depolarizing, and potentially excitatory, actions of GABA. Recent clinical studies[2,3] suggest impaired KCC2 function and/or $[Cl^-]_i$ dysregulation in human epilepsy (for review, see the study by Moore et al[4]), and pharmacological inhibition of KCC2 can produce elevated $[Cl^-]_i$ and hyperexcitability of neural networks.[5] Neuronal KCC2 expression is controlled by multiple mechanisms and can be dynamically regulated in an activity-dependent manner.[4,6] Further identification of homeostatic regulatory mechanisms that restore $[Cl^-]_i$ to levels that facilitate inhibition may thus provide novel therapeutic targets.

Recent work from the Woodin Laboratory indicated a seemingly unlikely role for the kainate receptor (KAR) family of glutamate receptors in promoting the surface expression of KCC2 in hippocampus, subsequently regulating the strength of inhibition.[7] In this study, the same group describes a novel role for KARs in the homeostatic regulation of $[Cl^-]_i$ in hippocampal CA3 pyramidal neurons, calling further attention to KARs as potentially intriguing therapeutic targets in epilepsy. The authors first found that a pharmacological blockade of KARs resulted in a significant depolarization of E_{GABA} and decreased inward driving force for chloride, suggesting that KARs may modulate the activity of KCC2 in CA3 pyramidal cells. Conversely, KAR activation through application of 1 μM kainic acid for 5 minutes subsequently

hyperpolarized E_{GABA} and coincided with a depolarization of the resting membrane potential and an increased driving force for chloride. These results indicate that E_{GABA} responds in a homeostatic manner after KAR activation, possibly due to increased KCC2 activity.

Kainate receptors are unique in that despite being classified as ionotropic receptors and having a structure similar to that of AMPA and N-methyl-D-aspartate (NMDA) receptors, they can also signal through G protein-coupled metabotropic pathways.[8] Therefore, the authors sought to determine the mechanism underlying KAR-mediated hyperpolarization of E_{GABA}. A 5-minute bath application of a lower concentration (0.1 μM) of kainic acid, previously demonstrated as sufficient to activate metabotropic KAR signaling but with minimal ionic KAR currents, did not significantly hyperpolarize E_{GABA} during the application. However, E_{GABA} became hyperpolarized 10 minutes after the washout of KA, an effect possibly explained by the slower, longer lasting mechanism of action typical of metabotropic signaling. Next, the authors isolated the ionotropic actions by blocking KAR metabotropic signaling using 2 different G-protein inhibitors, NEM, and GDP-β-S. Application of 1 μM kainic acid (high enough to stimulate ionotropic KAR signaling) in the presence of NEM or GDP-β-S also significantly hyperpolarized E_{GABA} and increased the chloride driving force, although E_{GABA} returned to baseline levels following washout of NEM but not GDP-β-S. Additionally, the efficacy of 0.1 μM kainic acid (activating the metabotropic signaling pathway) to alter E_{GABA} or the chloride driving force was blocked in the presence of GDP-β-S. Taken together, these experiments demonstrate that activation of KARs can hyperpolarize E_{GABA} and alter the driving force for chloride via separate ionotropic and metabotropic mechanisms.

Lastly, to determine whether KAR-mediated hyperpolarization of E_{GABA} is dependent on KCC2 function, the authors tested the effects of activating either the ionotropic or metabotropic signaling pathways in the presence of a KCC2 antagonist. Interestingly, activation of the ionotropic signaling pathway by 1 μM kainic acid still resulted in a hyperpolarization of E_{GABA} and an increase in the chloride driving force. By contrast, the effect on E_{GABA} of activation of the metabotropic KAR signaling pathway by 0.1 μM kainic acid was lost. Together, these results suggest that downstream actions of KCC2 are required for the metabotropic KAR-mediated hyperpolarization of E_{GABA}, but not for ionotropic KAR-mediated regulation of E_{GABA}. Additionally, the authors determined that both signaling pathways depended on the GluK1 or GluK2 KAR subunits, as GluK1/2 knockout animals did not display any KAR-mediated hyperpolarization of E_{GABA}, supporting previous work demonstrating a strong interaction between GluK2 and KCC2.

Although the hypothesis of epilepsy as a manifestation of an improper excitation/inhibition ratio in the brain has prevailed, it is increasingly evident that this model is far too simplistic.[9] It has long been clear, however, that E_{GABA} depolarization is strongly associated with increased neural activity, and at least transiently depolarizing actions of GABA are often noted in epilepsy.[10] Therefore, the concept of an endogenous homeostatic mechanism of E_{GABA} and $[Cl^-]_i$ regulated by KAR activation is intriguing, particularly given that kainic acid administration in rodents is a commonly used preclinical model of seizure induction and epilepsy.[11] As a regulator of inhibition activated via glutamatergic transmission, KARs may be ideally primed to sense periods of excessive excitability and respond in a manner that bolsters inhibition. It is currently unknown whether KARs are capable of homeostatically driving inhibition during periods of hyperexcitability or whether this mechanism remains intact in models of epilepsy. Notably, the concentrations of kainic acid used here (μM) are low compared to concentrations commonly used in seizure models, as well as the concentration of glutamate present in synaptic clefts (~mM). In such saturated environments, particularly during seizures, it may prove difficult to endogenously harness these KAR-dependent pathways. Nonetheless, it would be interesting to investigate the potential role of KARs on the regulation of E_{GABA} and $[Cl^-]_i$ in animal models of epilepsy.

In theory, the therapeutic potential of KAR-activated hyperpolarization of E_{GABA} works on a similar principle as bumetanide, which blocks the Na-K-2Cl cotransporter responsible for importing chloride ions. In both cases, by keeping $[Cl^-]_i$ low, the inward driving force for chloride is increased such that it strongly promotes an inhibitory action of GABA through $GABA_A$ receptors, thus limiting potential negative impacts of depolarizing GABA responses. Additionally, the finding that KAR activation can modulate E_{GABA} independently of KCC2 function reveals a potential new mechanism of chloride homeostasis in neurons that bypasses the complexities associated with the KCC2 protein. For example, KCC2 expression levels may not necessarily correlate directly with functionality, as KCC2 is greatly impacted by post-translational modifications such as phosphorylation state.[4,6] Future work uncovering the mechanism underlying the KCC2-independent KAR-mediated hyperpolarization of E_{GABA} should lead to relevant insights into future therapies.

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References

1. Rivera C, Voipio J, Payne JA, et al. The K^+/Cl^- co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. *Nature*. 1999;397(6716):251-255.
2. Stödberg T, McTague A, Ruiz AJ, et al. Mutations in *SLC12A5* in epilepsy of infancy with migrating focal seizures. *Nat Commun*. 2015;6:8038.
3. Saitsu H, Watanabe M, Akita T, et al. Impaired neuronal KCC2 function by biallelic *SLC12A5* mutations in migrating focal seizures and severe developmental delay. *Sci Rep*. 2016;6:30072.

4. Moore YE, Kelley MR, Brandon NJ, Deeb TZ, Moss SJ. Seizing control of KCC2: a new therapeutic target for epilepsy. *Trends Neurosci*. 2017;40(9):555-571.
5. Sivakumaran S, Cardarelli RA, Maguire J, et al. Selective inhibition of KCC2 leads to hyperexcitability and epileptiform discharges in hippocampal slices and in vivo. *J Neurosci*. 2015;35(21):8291-8296.
6. Kaila K, Price TJ, Payne JA, Puskarjov M, Voipio J. Cation-chloride cotransporters in neuronal development, plasticity, and disease. *Nat Rev Neurosci*. 2014;15(10):637-654.
7. Mahadevan V, Pressey JC, Acton BA, et al. Kainate receptors coexist in a functional complex with KCC2 and regulate chloride homeostasis in hippocampal neurons. *Cell Rep*. 2014;7(6):1762-1770.
8. Lerma J, Marquez JM. Kainate receptors in health and disease. *Neuron*. 2013;80(2):292-311.
9. Staley K. Molecular mechanisms of epilepsy. *Nat Neurosci*. 2015; 18(3):367-372.
10. Miles R, Blaesse P, Huberfeld G, Wittner L, Kaila K. Chloride homeostasis and GABA signaling in temporal lobe epilepsy. In: Noebels JL, Avoli M, Rogawski MA, Olsen RW, Delgado-Escueta AV, eds. *Jasper's Basic Mechanisms of the Epilepsies*. Bethesda, MD: National Center for Biotechnology Information (US); 2012:581-590.
11. Lévesque M, Avoli M. The kainic acid model of temporal lobe epilepsy. *Neurosci Biobehav Rev*. 2013;37(10):2887-2899.