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KCa3.1 Impairment Is Not Just a Slow Afterthought in Epilepsy

Protein Kinase A-Mediated Suppression of the Slow After Hyperpolarizing KCa3.1 Current in Temporal Lobe Epilepsy

Tiwari MN, Mohan S, Biala Y, Yaari Y. *J Neurosci.* 2019;39(50):9914-9926. doi: https://doi.org/10.1523/JNEUROSCI.1603-19.

Brain insults, such as trauma, stroke, anoxia, and status epilepticus (SE), cause multiple changes in synaptic function and intrinsic properties of surviving neurons that may lead to the development of epilepsy. Experimentally, a single SE episode, induced by the convulsant pilocarpine, initiates the development of an epileptic condition resembling human temporal lobe epilepsy (TLE). Principal hippocampal neurons from such epileptic animals display enhanced spike output in response to excitatory stimuli compared with neurons from nonepileptic animals. This enhanced firing is negatively related to the size of the slow after-hyperpolarization (sAHP), which is reduced in the epileptic neurons. The sAHP is an intrinsic neuronal negative feedback mechanism consisting normally of 2 partially overlapping components produced by disparate mechanisms. One component is generated by activation of Ca2+-gated K+ (KCa) channels, likely KCa3.1, consequent to spike Ca2+ influx (the KCa-sAHP component). The second component is generated by enhancement of the electrogenic Na+/K+ ATPase (NKA) by spike Na+ influx (NKA-sAHP component). Here we show that the KCa-sAHP component is markedly reduced in male rat epileptic neurons, whereas the NKA-sAHP component is not altered. The KCa-sAHP reduction is due to the downregulation of KCa3.1 channels, mediated by cAMP-dependent protein kinase A (PKA). This sustained effect can be acutely reversed by applying PKA inhibitors, leading also to normalization of the spike output of epileptic neurons. We propose that the novel "acquired channelopathy" described here, namely, PKA-mediated downregulation of KCa3.1 activity, provides an innovative target for developing new treatments for TLE, hopefully overcoming the pharmacoresistance to traditional drugs. Significance Statement: Epilepsy, a common neurological disorder, often develops following a brain insult. Identifying key molecular and cellular mechanisms underlying acquired epilepsy is critical for developing effective antiepileptic therapies. In an experimental model of acquired epilepsy, we show that principal hippocampal neurons become intrinsically hyperexcitable. This alteration is due predominantly to the downregulation of a ubiquitous class of potassium ion channels, KCa3.1, whose main function is to dampen neuronal excitability. KCa3.1 downregulation is mediated by the cAMP-dependent PKA signaling pathway. Most importantly, it can be acutely reversed by PKA inhibitors, leading to recovery of KCa3.1 function and normalization of neuronal excitability. The discovery of this novel epileptogenic mechanism hopefully will facilitate the development of more efficient pharmacotherapy for acquired epilepsy.

Commentary

The human brain contains billions of neurons with over 100 trillion synapses, which constantly process external and internal stimuli. Needless to say, these neurons must be tightly controlled to allow physiological functions such as coordinated behavior and learning without creating an electrical overload. Healthy neurons have multiple safeguards to prevent hyperexcitability and uncontrolled firing. In the epileptic brain, however, many of these safeguards are absent or defective, which impairs the brain's capability to prevent physiological neuronal excitation from getting out of control. Consequently, normally harmless neuronal activity or stimulation can turn into large-scale synchronous firing that leads to seizures. There is reasonable hope that a better understanding of these safeguards could lead to the identification of novel treatment targets for individuals with epilepsy that is unresponsive to currently available medications. In a new study published in the *Journal of Neuroscience*, Tiwari and coauthors address this challenge by revealing how defective regulation of the potassium channel KCa3.1, one of the channels dampening neuronal activity after stimulation, may contribute to hyperexcitability in epilepsy.[1]

Potassium channels play an important role in a neuron's safety net to prevent hyperactivity, because they—upon adequate stimulation—facilitate the exit of potassium ions out of the cell leading to a hyperpolarization of the neuronal membrane that helps reset a neuron to baseline levels. This mechanism is usually activated after an action potential and is called an afterhyperpolarization (AHP). Afterhyperpolarizations transiently incapacitate a neuron from generating a new action potential and therefore prevent continuous activity that could lead to synchronous firing of neuronal ensembles across the brain that develop into seizures. It is, thus, not surprising that potassium channelopathies, defects in potassium channel regulation, are frequent in epilepsy.[2,3] Tiwari et al have combined pharmacological manipulations with recordings in hippocampal slices from epileptic rats to elucidate a novel channelopathy that affects the KCa3.1 channel.

KCa3.1, an intermediate conductance calcium-activated potassium channel, may be of interest for epilepsy, as it is specifically activated after bursts of action potentials, a relatively strong physiological neuronal activity that must be tightly controlled to prevent neuronal hyperactivity. Bursts of action potentials lead to a slow form of afterhyperpolarization (sAHP), which, as the authors show, consists of an early, KCa3.1-mediated phase and a late, sodium/potassium ATPase (NKA)-mediated phase. Using combinations of multiple drugs and electrophysiological approaches in hippocampal slices from a rat model of temporal lobe epilepsy (TLE), the authors provide evidence that the KCa3.1-mediated (early) component of the sAHP is almost absent in epileptic brains, whereas the late, NKA-dependent phase is undisturbed. They further show that 2 different inhibitors of protein kinase A (PKA) restore the early sAHP, an effect that is largely occluded with TRAM-34, a KCa3.1 antagonist. These experiments provide strong rationale for a role of excessive PKA-mediated posttranslational modification of KCa3.1 in reducing the early phase of sAHP and increasing the intrinsic excitability of an epileptic hippocampus.

The authors used the rat pilocarpine model of acquired epilepsy, in which epilepsy develops over weeks after chemical induction of status epilepticus, mimicking epileptogenesis that turns a healthy brain into an epileptic brain after an insult, such as brain injury or a viral infection. They therefore were able to assess sAHP and intrinsic excitability over the course of epileptogenesis, 2 days after status epilepticus when no behavioral seizures are detectable, and 6 weeks later, when rats are expected to have spontaneous recurrent seizures. These experiments suggested that the observed changes in neuronal excitability and ion channel conductance were either weak or absent during early epileptogenesis, appearing only later. It is unclear, though, whether the occurrence of seizures is causally related to impaired sAHP, that is, part of the epileptogenic process leading to spontaneous seizures, or whether impaired sAHP is a consequence of the occurrence of repeated seizures. The authors postulate an interesting hypothesis regarding a potential causal role of reduced KCa3.1 in seizure generation: they suggest that the functional downregulation of KCa3.1 may make a brain more vulnerable to physiological fluctuations of NKA, which could, without KCa3.1 safeguarding the early phase of sAHP, increase the susceptibility to seizures after an otherwise benign neuronal stimulus. No seizures have been reported in KCa3.1 knockout mice,[4] but this has not been thoroughly assessed yet. The only published study assessing a potential causal relationship between reduced KCa3.1 and epileptogenesis has not supported this hypothesis, showing that acute pharmacological reduction of KCa3.1 function does not affect epileptogenesis in TLE models.[5] The 2 TLE models used in this study (amygdala kindling and electrical induction of self-sustained status epilepticus), though, were different than the pilocarpine model analyzed in Tiwari et al, making a direct comparison difficult. Further studies, using adult-onset genetic approaches to avoid compensation and off-target drug effects are needed to thoroughly test if impaired KCa3.1 causes increased susceptibility to seizures.

Two aspects that this study did not address are through which cell types KCa3.1 exerts its effects on intrinsic excitability in the hippocampus, and what role inflammation may play. Previous studies have shown that astrocytic KCa3.1 promotes astrogliosis and inflammation, and that KCa3.1 reduction, for example with TRAM-34, the same KCa3.1 inhibitor used here, is anti-inflammatory.[6] Inflammation has been suggested to contribute to epileptogenesis[7] but the present study suggests that reduction of KCa3.1 function, expected to reduce inflammation, promotes epilepsy. In vivo studies using cell-type specific knockout approaches paired with examination of neuroinflammation could help solve this apparent conundrum.

The intrinsic safety controls neurons employ to prevent hyperexcitability and excessive synchronous firing are attractive treatment targets in epilepsy. Harnessing endogenous mechanisms to control seizures might be safer than manipulating other mechanisms normally not involved in regulating neuronal excitability. The work by Tiwari et al suggests a potential avenue of increasing one of these endogenous safety nets, KCa3.1, by altering its posttranslational manipulation with PKA inhibitors; however, PKA is a ubiquitous protein kinase necessary for diverse functions throughout the body. Recent work has employed a different approach of increasing potassium channel function in the brain, namely through viral gene therapy targeted to the epileptic focus.[8] It will be interesting to assess if exogenous, for example, virally expressed KCa3.1 could provide a similar seizure-suppressing effect. Moreover, such an experiment may also further shed light on the controversy about KCa3.1's contributions to sAHP in the brain.[9,10] These studies could help circumvent off-target effects the drugs used here could have,[11] an inherent problem of virtually all pharmacological studies.

Notably, the observed defects in the early phase of sAHP may be significant beyond seizure susceptibility in epilepsy. Slow AHPs are believed to be important for spiking patterns of neurons, which are essential for neuronal communication and learning and memory. Epilepsy is often accompanied by cognitive impairments and mood disorders. KCa3.1 is an attractive candidate mechanism that may contribute to these comorbidities

and could be manipulated (pharmacologically or using gene therapy) to ameliorate these symptoms.

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