



Tuning the Wave: Controlling Spreading Depolarization with Activation/Inactivation of Kv7.2

Kcnq2/Kv7.2 Controls the Threshold and Bihemispheric Symmetry of Cortical Spreading Depolarization

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Spreading depolarization (SD) is a slowly propagating wave of massive cellular depolarization associated with acute brain injury and migraine aura. Genetic studies link depolarizing molecular defects in Ca^{2+} flux, Na^+ current in interneurons, and glial $\text{Na}^+ - \text{K}^+$ ATPase with SD susceptibility, emphasizing the important roles of synaptic activity and extracellular ionic homeostasis in determining SD threshold. In contrast, although gene mutations in voltage-gated potassium ion channels that shape intrinsic membrane excitability are frequently associated with epilepsy susceptibility, it is not known whether epileptogenic mutations that regulate membrane repolarization also modify SD threshold and propagation. Here, we report that the Kcnq2/Kv7.2 potassium channel subunit, frequently mutated in developmental epilepsy, is an SD modulatory gene with significant control over the seizure-SD transition threshold, bihemispheric cortical expression, and diurnal temporal susceptibility. Chronic DC-band cortical EEG recording from behaving conditional Kcnq2 deletion mice ($\text{Emx1}^{\text{cre/+}}::\text{Kcnq2}^{\text{flox/flox}}$) revealed spontaneous cortical seizures and SD. In contrast to the related potassium channel deficient model, Kv1.1-KO mice, spontaneous cortical SDs in Kcnq2 cKO mice are tightly coupled to the terminal phase of seizures, arise bilaterally, and are observed predominantly during the dark phase. Administration of the nonselective Kv7.2 inhibitor XE991 to Kv1.1-KO mice reproduced the Kcnq2 cKO-like SD phenotype (tight seizure coupling and bilateral symmetry) in these mice, indicating that Kv7.2 currents directly and actively modulate SD properties. *In vitro* brain slice studies confirmed that Kcnq2/Kv7.2 depletion or pharmacological inhibition intrinsically lowers the cortical SD threshold, whereas pharmacological Kv7.2 activators elevate the threshold to multiple depolarizing and hypometabolic SD triggers. Together these results identify Kcnq2/Kv7.2 as a distinctive SD regulatory gene, and point to SD as a potentially significant pathophysiological component of KCNQ2-linked epileptic encephalopathy syndromes. Our results also implicate KCNQ2/Kv7.2 channel activation as a potential adjunctive therapeutic target to inhibit SD incidence.

Commentary

Spreading depolarization (SD) is a wave of cellular depolarization that slowly propagates leading to usually reversible inhibition.¹ SD is perhaps best known for its role in migraine, but it is also seen with focal insults including traumatic brain injury (TBI), hemorrhage, stroke, and epileptogenesis associated with brain tumors.² In at least 1 mouse model, it was found to contribute to seizure-related death akin to sudden unexpected death in epilepsy (SUDEP).³ In this case, SD is proposed to contribute to seizures reaching cardiac and/or respiratory control centers to contribute to the cardiorespiratory demise underlying the pathophysiology of SUDEP.^{3,4} Understanding how seizures spread to contribute to cardiorespiratory consequences that lead to SUDEP would be of great value in developing preventive strategies both as a putative biomarker for risk and in informing development of prophylactic treatments. More broadly, if SD is an integral part of the mechanism by which epileptogenesis occurs following acute lesions, then mitigating this may prevent a variety of symptomatic epilepsies.

In the current study,⁵ the authors examine the role of the potassium (K^+) channel subunit Kv7.2, encoded by Kcnq2, in regulating SD. To begin, they generated a novel conditional knock out mouse in which Kv7.2 was eliminated in cortical neurons. Mice were instrumented for EEG and chronic direct current (DC)-band recording. Kcnq2-cKO mice were found to display spontaneous seizures. 97% of spontaneous seizures were associated with SD. SD occurred bilaterally, with a short offset between the 2 hemispheres. SD was always associated with seizure. They assessed the time of day of seizure occurrence and found that most seizures occurred during the dark/night phase. The 2 deaths they observed occurred around the dark to light transition.

To compare to Kcnq2-cKO mice, seizures and SD were examined in mice with knockout of the voltage-gated K^+ channel Kv1.1 (Kv1.1-KO) encoded by Kcna1. These mice are well known to display severe epilepsy with a high rate of SUDEP.⁶ In contrast to Kcnq2-cKO mice, most seizures (88%) in Kv1.1-KO mice were not associated with SD, SD was seen independently from seizures in 7.4% of cases, and SD always





arose unilaterally. About 5% of the seizures were associated with SD. They again assessed time of day of seizure occurrence and somewhat surprisingly saw more seizures during the light phase. Notably, all seizures that were associated with SD occurred during the light/daytime phase. While they noted a low death rate for these mice, the 2 deaths occurred during the dark/night phase were consistent with previous findings in this model.⁷ Application of the Kv7.2 inhibitor, XE991, triggered bilateral seizures with SD in Kv1.1-KO, but not wildtype (WT), mice, suggesting a critical role for Kv7.2.


In vitro, in layer 2/3 pyramidal neurons in cortical slices from Kcnq2-cKO mice, there was a shorter latency to SD, lower threshold for SD, and faster SD propagation following bath application of KCl to increase $[K^+]$ compared to slices from WT mice. In contrast, in Kv1.1-KO mice, there was no difference in $[K^+]$ threshold or propagation rate. The amplitudes of spontaneous excitatory post-synaptic currents (sEPSC) were increased in both Kcnq2-cKO and Kv1.1-KO mice compared to WT controls. Amplitudes of spontaneous inhibitory post-synaptic currents (sIPSC) were increased in Kcnq2-cKO, but not in Kv1.1-KO mice. There was no difference in frequencies of sEPSCs or sIPSCs in Kcnq2-cKO or Kv1.1-KO mice compared to controls. This suggests excitation/inhibition balance was tipped toward excitation in Kv1.1-KO mice, but there was a neutral synaptic effect in Kcnq2-cKO mice.

Application of the Kv7.2 inhibitor XE991 to WT cortical slices did not affect propagation rate, but reduced $[K^+]$ SD threshold. Application of Kv7.2 activators, retigabine or ML213, reduced propagation rate in WT slices. Retigabine also increased $[K^+]$ SD threshold in WT slices. Retigabine had no effect on propagation rate in cortical slices from Kcnq2-cKO mice, suggesting that the effect on WT slices was specific to Kv7.2 activation. The gamma-aminobutyric acid A (GABA_A) receptor antagonist, gabazine, increased propagation rate in WT slices. This effect was reversed with retigabine. Retigabine also reduced SD propagation in anesthetized WT cortex in vivo.

Here, the authors shed light on molecular mechanisms of SD. They importantly implicate Kv7.2 in SD propagation and demonstrate that targeting Kv7.2 pharmacologically may be a feasible means to reduce SD. Heterotetramers of Kv7.2 generate a noninactivating hyperpolarizing M-type K^+ current that sets the resting membrane potential. These channels are always open and can become more open with depolarization. Inhibition of these currents leads to tonic firing of neurons, and perhaps in this case promoted SD. Kv1.1 mediates a delayed rectifier current which serves to regulate action potential shape, propagation, and timing of repetitive firing. Elimination of these channels contributes to excess excitation, but seemingly has little role in propagation of SD. Elimination of either Kv7.2 or Kv1.1 makes the brains of the animals hyperexcitable, causing them to display spontaneous seizures; however, the 2 different models have distinct SD profiles. More work will be needed to understand how loss of Kv7.2 leads to the nearly 100% association of seizures with SD and to SD occurring only following seizures.

If SD is found to be critical for SUDEP, then prophylactic therapy targeting Kv7.2 currents may be prudent and may also be beneficial to other diseases in which SD is prominent. For instance, activating Kv7.2 could have a role in abortion of migraine or in prophylaxis against epileptogenesis associated with stroke, TBI, and tumors. It would be useful to know if SD-reducing drugs reduced seizures themselves or if their role was only in modulating SD threshold and propagation rate. The drug employed to activate Kv7.2, retigabine, is an approved anti-seizure medication; however, it has not been in production for several years.

It is important to note that here they were primarily considering cortical SD. While they saw a high, nearly 100%, association of seizure with SD in the Kcnq2-cKO mouse, there were only 2 deaths. Thus, though there is suggestion that SD may precipitate SUDEP, this likely requires spread beyond the cortex into the brainstem or other structures. There were also only 2 deaths in Kv1.1-KO mice in this study. This mouse is known to have a high mortality rate⁶ with progressive cardiorespiratory sequelae.⁸ It would be interesting to know if more seizures would be associated with SD, SD would become more severe/widespread, and whether SD spread would be associated with cardiorespiratory sequelae as these animals age and approach death. Brainstem spread of SD was suggested to contribute to death in a model of Dravet syndrome, an epileptic encephalopathy with high incidence of SUDEP.³ Spread into the brainstem could be expected to be associated with cardiac and respiratory changes, as was seen in a rat model.⁹ Spread into the amygdala would also be expected to be associated with respiratory changes.¹⁰ The present study demonstrates an important role for Kv7.2 in generation and propagation of SD. More work is needed to appreciate the full impact of modulating Kv7.2 in treatment and prevention of disease.

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References

1. Herreras O, Makarova J. Mechanisms of the negative potential associated with Leao's spreading depolarization: A history of brain electrogenesis. *J Cereb Blood Flow Metab.* 2020;40:1934-1952.
2. Kramer DR, Fujii T, Ohiorhenuan I, Liu CY. Cortical spreading depolarization: Pathophysiology, implications, and future directions. *J Clin Neurosci.* 2016;24:22-27.
3. Aiba I, Noebels JL. Spreading depolarization in the brainstem mediates sudden cardiorespiratory arrest in mouse SUDEP models. *Sci Transl Med.* 2015;7:282ra46.
4. Ryvlin P, Nashef L, Lhatoo SD, et al. Incidence and mechanisms of cardiorespiratory arrests in epilepsy monitoring units (MORTEMUS): a retrospective study. *Lancet Neurol.* 2013;12:966-977.



5. Aiba I, Noebels JL. Kcnq2/Kv7.2 controls the threshold and bi-hemispheric symmetry of cortical spreading depolarization. *Brain* 2021. doi:[10.1093/brain/awab141](https://doi.org/10.1093/brain/awab141).
6. Iyer SH, Aggarwal A, Warren TJ, et al. Progressive cardiorespiratory dysfunction in Kv1.1 knockout mice may provide temporal biomarkers of pending sudden unexpected death in epilepsy (SU-DEP): The contribution of orexin. *Epilepsia*. 2020;61:572-588.
7. Moore BM, Jerry Jou C, Tatalovic M, Kaufman ES, Kline DD, Kunze DL. The Kv1.1 null mouse, a model of sudden unexpected death in epilepsy (SUDEP). *Epilepsia*. 2014;55:1808-1816.
8. Glasscock E, Yoo JW, Chen TT, Klassen TL, Noebels JL. Kv1.1 potassium channel deficiency reveals brain-driven cardiac dysfunction as a candidate mechanism for sudden unexplained death in epilepsy. *J Neurosci*. 2010;30:5167-5175.
9. Lertwittayanon W, Devinsky O, Carlen PL. Cardiorespiratory depression from brainstem seizure activity in freely moving rats. *Neurobiol Dis*. 2020;134:104628.
10. Dlouhy BJ, Gehlbach BK, Kreple CJ, et al. Breathing inhibited when seizures spread to the amygdala and upon amygdala stimulation. *J Neurosci*. 2015;35:10281-10289.