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ABCDEF…SUDEP: Action Potential Barrage in (Superior) Colliculus Causes (Spreading) Depolarization Leading to Epilepsy Fatality

Hyperexcitable Superior Colliculus and Fatal Brainstem Spreading Depolarization in a Model of Sudden Unexpected Death in Epilepsy

Cain SM, Bernier LP, Zhang Y, Yung AC, Kass J, Bohnet B, Yang Y, Gopaul R, Kozlowski P, MacVicar BA, Snutch TP. *Brain Commun.* 2022;4(2):fcac006. doi:10.1093/braincomms/fcac006

Cardiorespiratory arrest and death in mouse models of sudden unexpected death in epilepsy occur when spreading depolarization (SD) is triggered by cortical seizures and then propagates to the brainstem. However, the critical brain regions and the specific changes required to allow SD to propagate to the brainstem under the relatively rare circumstances leading to a fatal seizure are unknown. We previously found that following cortical seizure-inducing electrical stimulation, SD could occur in both the superior and inferior colliculi in Cacna1a^{S218L} mice, but was never observed in wild-type animals or following non-seizure-inducing stimuli in Cacna1a^{S218L} mice. Here, we show that optogenetic stimulation of the superior/inferior colliculi in Cacna1a^{S218L} mice induces severe seizures, and resulting SD in the superior/inferior colliculi that propagates to the brainstem and correlates with the respiratory arrest followed by cardiac arrest. Further, we show that neurons of the superior colliculus in Cacna1a^{S218L} mice exhibit hyperexcitable properties that we propose underlie a distinct susceptibility to SD. Our data suggest that the susceptibility of the superior colliculus to elicit fatal SD is a result of either genetic or seizure-related alterations within the superior colliculus that may involve changes to structure, connectivity and/or excitability.

Commentary

Intractable seizures are associated with a high risk of sudden unexpected death in epilepsy (SUDEP). SUDEP is in fact the leading cause of death in this patient population.[1] While it is well accepted that seizures lead to cardiorespiratory arrest to cause SUDEP in susceptible individuals, precisely how this happens is not well understood.[2] One putative mechanism could be spreading depolarization (SD) triggered at the end of the seizure.[3] Spreading depolarization is a wave of depolarization and cellular swelling that moves through neurons and glia.[4] While widely appreciated as a critical aberrant mechanism in migraine,[5] it has been garnering interest in other neurological diseases including traumatic brain injury, stroke, and epilepsy.[3] Understanding the mechanisms of how seizures lead to death, including not just the etiologies of death, but also the sites involved and in what manner the sites are affected will lend important insights into mitigating this awful consequence of some seizures.

Mice (Cacna1a^{S218L}) with a point mutation in the *Cacna1a* gene that encodes the P/Q type voltage gated calcium channel ($Ca_V2.1$) demonstrate SD following an induced seizure. This mutation is found in patients with familial hemiplegic migraine (FHM) and some patients with epilepsy. Using this model, Cain and colleagues set out to understand the circuitry involved in seizure-induced SD.[6] They previously determined that cortically induced seizures in these mice lead to SD in the superior colliculus (SC) and thalamus.[7] Here they wanted to know how important of a role these structures play in SD that is ultimately fatal.

First, they optogenetically stimulated the SC in wild type (WT) and Cacna1a^{S218L} mice. They injected an adeno-associated virus (AAV) allowing expression of the excitatory opsin, channelrhodopsin2 (ChR2) in cells, into the SC. They determined that the AAV spread to the inferior colliculus (IC) and thus ChR2 was also expressed there, and these cells were also stimulated when light directed at the SC/IC region was shined through the skull. Superior colliculus/IC stimulation caused seizures in most Cacna1a^{S218L} mice, but in none of the WT. Five of 8 Cacna1a^{S218L} mice died following the seizure. The other 3 died after subsequent seizures.

Second, they sought to evaluate SD caused by SC/IC stimulation. For this they coupled optogenetic stimulation of

the SC/IC with diffusion-weighted magnetic resonance imaging using an innovative methodology that they originally developed to track cellular swelling associated with SD in FHM.[8] They determined that SC/IC stimulation triggered SD that propagated to cortex, returned through SC/IC, and then travelled to brainstem to incite respiratory arrest, followed by cardiac arrest and death. This demonstrates in yet another model that respiratory arrest precedes cardiac arrest to cause death.

Third, since this group previously observed that seizures resulted in SD that spread to the thalamus, they wanted to determine whether thalamic stimulation could cause brainstem SD. For this they injected the AAV and implanted an optic fiber into the thalamus. Thalamic stimulation caused SD that was restricted to the cortex and hippocampus, without brainstem involvement. SD caused by thalamic stimulation was never fatal in this study. These findings suggest that though seizures may lead to SD in the thalamus, this is insufficient to trigger brainstem SD.

To determine whether SC or IC may be playing a greater role in triggering brainstem SD, neuronal excitability was assessed in acute brain slices from Cacna1a^{S218L} mice. There was increased tonic firing of SC neurons from Cacna1a^{S218L} mice compared to WT when recording in current clamp mode. They also found reduced amplitude and increased frequency of spontaneous excitatory post synaptic currents indicative of increase glutamatergic synaptic activity. None of these changes were seen in IC, suggesting that in this model, hyperexcitability in the SC is responsible for triggering SD.

This study nicely illuminates a putative pathway for distant effects of seizure that lead to mortality. It is intriguing that stimulation of SC alone was sufficient to cause a seizure, and that this could incite SD leading to fatal respiratory arrest. Though their optogenetic method was insufficient to differentiate between SC and IC activation, the follow-up electrophysiological studies implicate SC.

SD seems to have a dichotomous role in epilepsy. On one hand, seizures trigger a wave of depolarization that sweeps through the brain leading to terminal respiratory arrest when it reaches brainstem respiratory control centers. On the other hand, disruption of electrochemical gradients across cell membranes disallows action potentials from firing. Thus, when this occurs in the peri-ictal area, SD prevents seizures from continuing. It is not entirely clear how these gradients are disrupted, that is, whether it is due to cellular swelling, or if the swelling is triggered by dysregulation of channels or pump function. Certainly, in this model, the point mutation in Cacna1a, which encodes the $Ca_V2.1$ P/Q type voltage gated calcium channel, leads to hyperexcitability in SC cells that facilitates SD. In many instances the wave of SD is associated with impaired Na^+/K^+ ATPase activity.[9]

Seizures certainly are capable of producing enough local hypoxia to trigger cell swelling and disruption of electrochemical gradients to produce SD. The question remains as to whether SD is always the mechanism for SUDEP. Or does SD only arise in some cases, for instance in those with an underlying susceptibility to SD as is seen in this genetic model of FHM. And perhaps in other cases, long distance effects of seizures are propagated along fiber tracts. There is ample evidence that seizures also propagate through existing pathways and engage known and abnormal networks. Some of these may involve amygdala as an intermediary.[10-12] Perhaps the SC is involved in this pathway. However, in the authors' previous work, spread propagated via extra-synaptic means.[7] This raises the question of whether there should be subcategories of SUDEP to reflect different baseline susceptibilities and specific underlying mechanisms, and if so, how should these be defined?

There is ongoing debate regarding whether respiratory arrest or cardiac arrest is the initial inciting event for SUDEP. More recent, careful studies assessing both breathing and cardiac function in patients and in animal models suggests terminal apnea precedes terminal asystole.[2] It is intriguing that in this model respiratory arrest preceded cardiac arrest prior to death.

This study sheds light on potential mechanisms underlying the pathophysiology of SUDEP. It will be important to determine if these findings will translate to other animal models used to study SUDEP. While more work is needed to understand the precise mechanisms and identity of specific cell types and nodes, this starts us propagating in a good direction.

Gordon F. Buchanan, MD, PhD, FAES, FANA
Department of Neurology, University of Iowa Roy J and Lucille A Carver College of Medicine

ORCID iD

Gordon F. Buchanan https://orcid.org/0000-0003-2371-4455

Declaration of Conflicting Interests

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