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# Cardiorespiratory Dysfunction Induced by Brainstem Spreading Depolarization: A Potential Mechanism for SUDEP

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Review of Jansen et al.

Sudden unexpected death in epilepsy (SUDEP) is the main cause of death in people with refractory epilepsy (Massey et al., 2014; Buchanan, 2019), but the mechanisms responsible for SUDEP are still unclear. Evidence from animal and human studies suggests that SUDEP is related to an impairment in the central control of respiration, cardiac function, and arousal (Ryvlin et al., 2013; Massey et al., 2014; Buchanan, 2019). Given that all of these functions are essentially controlled by the brainstem, it is reasonable to postulate that the brainstem is involved in the pathophysiology of SUDEP. Indeed, some data indicate that cardiorespiratory arrest observed in experimental models of SUDEP is related to brainstem dysfunction (Aiba and Noebels, 2015). Nonetheless, its underlying mechanisms need further investigation.

A recent study published in *The Journal of Neuroscience* by Jansen et al. (2019) provides new insights into the potential mechanisms underlying SUDEP. The authors used a transgenic mouse model (*Cacna1a*$^{S218L}$ mice) carrying a homozygous S218L missense mutation that leads to gain of function of voltage-gated CaV2.1 $Ca^{2+}$ channels and increased risk for spontaneous fatal seizures, and which has been proposed as a SUDEP model (Loonen et al., 2019). The main finding of Jansen et al. (2019) was that that cardiorespiratory dysfunction related to spontaneous brainstem seizures in Cacna*1a*$^{S218L}$ mice is caused by medullary spreading depolarization (SD). *Cacna1a*$^{S218L}$ mice displayed lethal and nonlethal spontaneous seizures. All lethal seizures were accompanied by SD in the brainstem (oral pontine reticular nucleus and ventrolateral medulla), followed by suppression of neuronal activity in these areas, bradypnea, brainstem hypoxia, and bradycardia. In contrast, brainstem SD occurred in only a minority of nonlethal seizures, and, when it occurred, the brainstem DC potential and multiunit activity spontaneously recovered, the respiratory activity was rapidly restored, and heart rate did not significantly change. These findings, along with previous findings reported by this research group (Loonen et al., 2019), indicate that the *Cacna1a*$^{S218L}$ mouse might be a useful model to study the mechanisms underlying SUDEP and that brainstem SD is a key feature for the cardiorespiratory dysfunction associated with lethal seizures in this model.

Jansen et al. (2019) also performed experiments to determine a causal relationship between brainstem SD and brainstem hypoxia. Because hypoxia can promote an SD-like event called anoxic depolarization (Ayata and Lauritzen, 2015), the authors wanted to ensure that SD is a cause and not a consequence of hypoxia during seizures. To accomplish this, the authors induced seizures by electrical stimulation of the inferior colliculus (IC) in *Cacna1a*$^{S218L}$ and wild-type mice. This stimulation triggered ipsilateral brainstem SD in *Cacna1a*$^{S218L}$ but not in wild-type mice. Brainstem SD in *Cacna1a*$^{S218L}$ mice preceded apnea and brainstem hypoxia, demonstrating a causative role for brainstem SD in the initiation of seizure-related apnea and subsequently hypoxia.

Another notable finding by Jansen et al. (2019) was that mechanical ventilation after seizures induced by electrical stimulation of the IC in *Cacna1a*$^{S218L}$ mice was able to prevent death in seven of nine cases, suggesting that respiratory dysfunction plays an essential role in SUDEP. It is important to highlight that the ventilatory support was initiated at least 60 s (65–79 s) after brainstem SD onset to ensure that mechanical ventilation was applied in cases of potentially lethal seizures. Even though the ventilatory support was initiated relatively late, it was sufficient to restore heart rate, ventrolateral medulla DC

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potential and multiunit activity, breathing, and ventrolateral medulla $PO_2$, preventing death in the majority of cases. These data may have important implications for the clinical management of seizures, suggesting that interventions to restore ventilation in patients with perictal respiratory disturbances (hypoventilation/apnea) might be a valuable strategy to prevent death.

Finally, because the excitatory amino acid glutamate and its NMDA receptors are importantly implicated in the generation and propagation of SD (Ayata and Lauritzen, 2015), Jansen et al. (2019) tested whether NMDA receptor antagonism could prevent seizure-related deaths. The authors found that pharmacological interventions with NMDA receptor antagonists (MK-801 or memantine hydrochloride) administered intraperitoneally 30 min before IC stimulation prevented fatal outcomes in all cases and prevented brainstem SD in all except one case (one of eight animals treated with memantine hydrochloride). These results may encourage future clinical studies for the development of preventive strategies for SUDEP, especially on the potential benefits of NMDA antagonism in patients with a history of seizures associated with brainstem SD.

In the study by Jansen et al. (2019), analysis of the vigilance/sleep state of animals during fatal and nonfatal seizures could provide additional insights into the mechanisms involved in SUDEP. Most cases of SUDEP in humans occur at night during sleep (Lamberts et al., 2012; Ryvlin et al., 2013; Sveinsson et al., 2018). The mechanisms underlying this relationship are still unclear, but some studies have proposed that impaired $CO_2$-induced arousal is involved (Nobili et al., 2011; Zhan et al., 2016; Buchanan, 2019). $CO_2$-induced arousal is an important protective reflex mechanism that depends on brainstem serotonergic neurons (Smith et al., 2018). Mice with genetic deletion of serotonergic neurons (*Lmx1b*$^{f/f/p}$ mice) do not arouse after stimulation of dorsal raphe nucleus (DRN) with acidosis; in addition, pharmacological or optogenetic acute inactivation of DRN serotonergic neurons abolish the $CO_2$-induced arousal from sleep (Smith et al., 2018). In this regard, it has been suggested that abnormal brainstem serotonergic function could play an important role in SUDEP by impairing $CO_2$-induced arousal during ictal and postictal periods (Zhan et al., 2016; Smith et al., 2018), likely aggravating apnea, hypoxia, and acidosis. Thus, it is plausible that the brainstem SD observed in *Cacna1a*$^{S218L}$ mice (Jansen et al., 2019) and other SUDEP mouse models (Aiba and Noebels, 2015) depresses the activity of DRN serotonergic neurons, impairing $CO_2$-induced arousal from sleep, and, ultimately, contribute to fatal outcomes by worsening apnea, hypoxia, and acidosis during seizures.

The possible role of brainstem serotonergic neurons in SUDEP is not restricted to its function in $CO_2$-induced arousal from sleep. Brainstem serotonergic neurons located in the medullary raphe play an important role in the control of breathing more generally (Richerson, 2004). These neurons are central chemoreceptors and, in conditions of increased $CO_2$, provide a potent stimulatory drive to breath by sending excitatory projections to important respiratory areas such as the ventral respiratory column (VRC) and the retrotrapezoid nucleus (Richerson, 2004; Ptak et al., 2009). Therefore, the suppression of medullary raphe serotonergic inputs to respiratory nuclei would result in an inability to increase ventilation during situations of seizure-related apnea with elevated $CO_2$ resulting in catastrophic outcomes. Of note, in addition to potentially contributing to SUDEP by controlling arousal and breathing mechanisms, serotonergic neurons can regulate seizure threshold (Buchanan et al., 2014) by affecting the excitability of seizure networks, perhaps including those in the brainstem.

Although Jansen et al. (2019) found that mechanical ventilation prevented death from seizures induced by IC stimulation indicating the essential role of respiratory dysfunction in SUDEP, the contribution of cardiovascular disturbances to SUDEP pathophysiology cannot be ruled out, especially since *Cacna1a*$^{S218L}$ mice displayed both apnea and bradycardia during all fatal seizures. Under normal conditions, heart rate is maintained by tonic activity of preganglionic parasympathetic neurons, most of which reside in the nucleus ambiguus (NA; Dergacheva et al., 2010). At rest, a major glutamatergic projection arising from nucleus tractus solitarius (NTS) excites the cardiac preganglionic parasympathetic neurons, while GABAergic and glycinergic inputs from respiratory nuclei (VRC and pontine nuclei) inhibit the neurons during each inspiration, generating a physiological phenomenon called respiratory sinus arrhythmia (Neff et al., 2003; Dergacheva et al., 2010). Given that Jansen et al. (2019) found SD and sustained neuronal suppression in the ventral respiratory column during fatal seizures, it could be that the inhibitory inputs to NA were suppressed, allowing the excitatory input from NTS to strongly excite cardiac preganglionic parasympathetic neurons, resulting in profound bradycardia. Moreover, during fatal seizures, the excitatory input from NTS to NA might be further strengthened by the ongoing hypoxia, thus aggravating bradycardia and eventually causing cardiac arrest and death. Therefore, it could be possible that in the study by Jansen et al. (2019), treatment with NMDA receptor antagonists (MK-801 or memantine hydrochloride) prevented seizure-related death by inhibiting this NTS-to-NA glutamatergic pathway. More studies are needed to test these hypotheses.

In conclusion, Jansen et al. (2019) revealed the crucial role of brainstem SD in the cardiorespiratory dysfunction during spontaneous and induced seizures in *Cacna1a*$^{S218L}$ mice, providing new insights into the potential neural mechanisms underlying SUDEP. Future studies may help to identify the factors contributing to brainstem hyperexcitability; the role of specific respiratory, cardiovascular, and arousal mechanisms; and the precise brainstem nuclei and pathways involved in the pathophysiology of SUDEP in *Cacna1a*$^{S218L}$ mice and other SUDEP models. Finally, it is noteworthy to highlight the translational potential of the findings reported by Jansen et al. (2019), which should direct future research and may help to develop preventive strategies for SUDEP.

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