Serotonin in the Dorsal Raphe: As I Live and Breathe

Optogenetic Activation of 5-HT Neurons in the Dorsal Raphe Suppresses Seizure-Induced Respiratory Arrest and Produces Anticonvulsant Effect in the DBA/1 Mouse SUDEP Model.

Zhang H, Zhao H, Zeng C, Van Dort C, Faingold CL, Taylor NE, Solt K, Feng HJ. *Neurobiol Dis* 2018;110:47–58.

Sudden unexpected death in epilepsy (SUDEP) is a devastating epilepsy complication. Seizure-induced respiratory arrest (S-IRA) occurs in many witnessed SUDEP patients and animal models as an initiating event leading to death. Thus, understanding the mechanisms underlying S-IRA will advance the development of preventive strategies against SUDEP. Serotonin (5-HT) is an important modulator for many vital functions, including respiration and arousal, and a deficiency of 5-HT signaling is strongly implicated in S-IRA in animal models, including the DBA/1 mouse. However, the brain structures that contribute to S-IRA remain elusive. We hypothesized that the dorsal raphe (DR), which sends 5-HT projections to the forebrain, is implicated in S-IRA. The present study used optogenetics in the DBA/1 mouse model of SUDEP to selectively activate 5-HT neurons in the DR Photostimulation of DR 5-HT neurons significantly and reversibly reduced the incidence of S-IRA evoked by acoustic stimulation. Activation of 5-HT neurons in the DR suppressed tonic seizures in most DBA/1 mice without altering the seizure latency and duration of wild running and clonic seizures evoked by acoustic stimulation. This suppressant effect of photostimulation on S-IRA is independent of seizure models, as optogenetic stimulation of DR also reduced S-IRA induced by pentylenetetrazole, a proconvulsant widely used to model human generalized seizures. The S-IRA-suppressing effect of photostimulation was increased by 5-hydroxytryptophan, a chemical precursor for 5-HT synthesis, and was reversed by ondansetron, a specific 5-HT3 receptor antagonist, indicating that reduction of S-IRA by photostimulation of the DR is specifically mediated by enhanced 5-HT neurotransmission. Our findings suggest that deficits in 5-HT neurotransmission in the DR are implicated in S-IRA in DBA/1 mice, and that targeted intervention in the DR is potentially useful for prevention of SUDEP.

Commentary

Sudden unexpected death in epilepsy (SUDEP) is a major cause of concern for persons with epilepsy and their caregivers. This syndrome is a major public health burden, and is the second only to stroke as a cause of years of potential life lost (1). While SUDEP has been recognized since the 1970s, the last decade has seen an explosion of research into the mechanisms, and potential interventions for SUDEP.

First and foremost among the theories explaining SUDEP is seizure-induced respiratory or cardiovascular impairment, which have been reported both in clinical case studies (2) and preclinical animal models. In a recent study in *Neurobiology of Disease*, Zhang and colleagues extended our knowledge of the neural mechanisms regulating seizure-induced respiratory arrest (S-IRA) through selective optogenetic activation of serotonergic neurons in the Dorsal Raphe Nucleus (DRN). These data build on a now extensive preclinical literature linking serotonin signaling to SUDEP.

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The first clear link between serotonin and SUDEP came in the late 1990s from studies in mice lacking the 5-HT2c receptor; these mice, unlike their wild-type littermates, displayed pronounced acoustically evoked (audiogenic) seizures, marked by wild running, tonic extension of the hind limbs, respiratory arrest, and if animals were not artificially ventilated, death (3). A similar profile of audiogenic seizures followed by respiratory arrest has been reported in DBA/1 and DBA/2 mice, strains in which 5-HT2c receptor expression levels in the brainstem are decreased compared with seizure and SUDEP-resistant C57/Bl6 mice (4). Moreover, in these strains, serotonin-selective reuptake inhibitors, such as fluoxetine significantly reduces the incidence of respiratory arrest, whereas the nonselective serotonin antagonist cyproheptadine significantly increases the incidence of respiratory arrest (5).

While global genetic deletion of serotonin neurons in mice results in decreased seizure threshold, increased seizure induced mortality, and impaired respiratory function (6), the precise population of serotonin neurons mediating these effects remains unknown. The raphe nuclei are diverse and extend from the midbrain through the pons and medulla. Each cell group has discrete ascending and descending targets; for example, the dorsal raphe is a major source of forebrain

serotonin, while the raphe obscurus and raphe pallidus have extensive descending projections to brainstem and spinal cord.

In the context of epilepsy, neurons in the dorsal raphe are regulators of both limbic seizure threshold (7) and are anatomically positioned to modulate brainstem network–mediated seizures, such as the wild running and tonic extension seen in audiogenic seizure models. Through projections to both the caudal periaquaductal gray and broadly to the pontine reticular formation (8), dorsal raphe modulation may impact tonic extensor seizures (9).

In their study, Zhang and colleagues generated DBA/1 mice expressing cre-recombinase under the control of the tryptophan hydroxylase promoter. This enabled selective targeting of serotonin neurons for channelrhodopsin expression, the fidelity of this expression was confirmed through immunofluorescence. The authors reported that optogenetic activation of serotonin neurons in the DRN prior to induction of either audiogenic or chemoconvulsive seizures produced a "dose"-dependent suppression of seizure induced respiratory arrest. Longer periods of preseizure optogenetic activation were associated with greater protection, as was higher intensity light delivery. This effect was striking, with approximately 95% of mice protected. Further supporting the link to serotonin, systemic pretreatment of the animals with the serotonin precursor 5-hydroxytryptophan, potentiated the effect of stimulation, whereas the 5-HT3 antagonist ondansetron, attenuated the effect.

In addition to suppressing respiratory arrest, the authors reported a significant attenuation of tonic seizure activity following pretreatment with optogenetic activation. Seizure-induced respiratory arrest typically occurs following tonic, but not clonic seizures across models, raising the possibility that the protection from the seizure induced respiratory arrest/SUDEP phenotype in this study was at least in part due to a suppression of tonic seizures. However, it is worth noting that 40% of animals protected from S-IRA following photostimulation still displayed tonic seizures, suggesting that these effects are at least partially separable.

These findings open the door to several new areas of exploration. First, what circuit mechanisms mediate the protection they observed? The degree to which divergent projections from the dorsal raphe mediate effects on respiratory function as compared with suppression of tonic seizures is of particular importance—narrowing in on the pathways supporting respiratory function may open new avenues for pharmacotherapy. The fact that protective effects of dorsal raphe stimulation were abolished by pretreatment with a 5-HT3 antagonist suggests that areas of 5-HT3 expression may be important targets. While the 5-HT3 receptor is widely expressed in the CNS, some of the densest expression of this receptor occurs in the level of the brainstem, in areas adjacent to the facial nucleus (10). Major respiratory neurons in the Botzinger complex are located just posterior to the facial nucleus. Similarly, 5-HT3 expression has been reported in the hypoglossal nucleus, which is directly involved in inspiration.

Second, another recent study has shown that hippocampal-evoked seizures, which are not typically associated with tonic extension, produces a robust suppression in firing of medullary serotonergic neurons coincident with depressed cardiorespiratory function (11). In this study, effects in the dorsal raphe, by contrast, were mixed: while population activity in the dorsal raphe was decreased, single unit activity of serotonin neurons was not. Thus, the degree to which similar optogenetic stimulation of the medullary raphe, would exert similar effects is of interest. However, the brain networks engaged by limbic seizures as compared with audiogenic seizures differ, and the profile of modulation of activity in the raphe may likewise differ between these seizure types.

Third, one of the intriguing features reported by Zhang and colleagues is that pretreatment was required to protect animals against respiratory arrest, and that this protective effect waned slowly over the course of several days. How does transient activation of the dorsal raphe translate into lasting protection against respiratory arrest? Clearance of synaptic serotonin is a highly efficient process, and it thus seems unlikely that this effect is due to prolonged elevation of synaptic serotonin. The degree to which long-term adaptation in firing of pre- or postsynaptic cells, alterations in receptor expression, or other mechanisms mediate the lasting effects of dorsal raphe stimulation remains an open area of investigation.

Together, these data solidify a central role for serotonin in seizure-induced respiratory arrest. These data may be a launching point for future anatomic and functional studies of the dorsal raphe in SUDEP. While perhaps a long-term goal, elucidating the mechanistic basis for the observed protection against S-IRA translates into successful interventions for SUDEP.

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