Serotonin: The Anti-SuddenDeathAmine?

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Sudden unexpected death in epilepsy (SUDEP) is an exceptionally difficult condition to study in humans. Therefore, translational research in animal models has been very important in defining pathophysiological mechanisms of death and identifying potential treatments. These models are helping define whether the primary mechanism of death is cardiac or respiratory. They have also identified a link to the serotonergic system of the brainstem; this, in turn, led to recognition that SUDEP and sudden infant death syndrome (SIDS) may share a common final pathway in the sequence of events that lead to death.

SUDEP is responsible for 10 to 50 percent of deaths in those with chronic refractory epilepsy (1–4), but its sporadic occurrence is infrequent enough that it is hard to capture cardiovascular, respiratory, EEG, and other physiological data during actual SUDEP events. Our understanding of mechanisms in humans comes in part from those rare patients who died—or came close—while in epilepsy monitoring units (EMUs) (3, 5). It is not known if the small number of reported cases is representative of the rest of SUDEP deaths. Another clue to the mechanisms comes from human data showing that it is common for seizures to induce cardiovascular (1) and respiratory dysfunction (6–8), but these events are very common but rarely fatal. There is no evidence that having severe cardiovascular and respiratory changes with seizures predisposes a patient to SUDEP, but it seems likely to be the case. The human data are valuable and important, but with rare exceptions they are from epilepsy patients who did not die, so it is unclear whether the data are relevant to actual SUDEP.

Animal Models of SUDEP

Animal models have been widely used to study the pathophysiology of death induced by seizures and have led to significant insights into the human condition. Animal experiments have the advantage that a fatal seizure can be induced, and then physiological measurements can be made to define the mechanisms of death. New treatments can also be tested. For example, audiogenic seizures can be induced in DBA/1, DBA/2, and B6SAS mice with a loud sound; 90% of the time when a major seizure occurs, it causes respiratory arrest that is typically fatal (9, 10). More relevant chronic epilepsy SUDEP models that exhibit sudden death after spontaneous seizures are also common. Animals have other advantages: It is easier to treat fatal seizures with drugs that are potentially toxic, ob-

tain brain tissue samples for various assays, test large numbers of individuals, ensure genetic homogeneity, and ensure that subjects follow dietary and other guidelines.

Mice have rapidly become the species of choice for many animal experiments, including those for SUDEP. In addition to the above advantages, they offer the ability to manipulate their genetic material in ways that cannot be done in any other species. Just as mutations in humans have been identified that cause epilepsy, mouse strains have been generated with the same mutations (e.g., Dravet syndrome mice). In other cases, genes have been knocked out to better understand their function, and this has led to new models of SUDEP (e.g., the 5-HT_{2c} receptor knockout mouse) (11). As transgenic technologies have become progressively more sophisticated, it has become possible to delete genes in specific cell types (12). This has allowed the entire population of central 5-HT neurons to be deleted during development (13) or to be acutely silenced in adulthood in response to exogenous drugs (14). Many of these transgenic approaches have been or are currently being used to study various aspects of the pathophysiology of SUDEP.

Thus, there are many mouse models of seizure-induced death or cardiorespiratory dysfunction, each displaying some pathophysiological changes thought to occur during SUDEP. These models are now being used to identify potential mechanisms and treatments. For example, experiments in mice led to the hypothesis that an increase in 5-HT might prevent hypoventilation after a seizure (10, 11, 15–19) (see following), spurring a retrospective analysis of the effect of serotonin reuptake inhibitors (SSRIs) on postictal respiratory depression in humans (20). *Lmx1b^{fl/p}* mice in which there is genetic deletion of essentially all 5-HT neurons in the CNS (13) have complete absence of arousal in response to an increase in ambient CO₂, which may contribute to the pathogenesis of SUDEP (see following) (21). Further, mouse models of long QT syndrome and other ion channel defects have been described as having spontaneous seizures as well as malignant cardiac arrhythmias or altered cardiac autonomic tone that predispose to sudden death (22, 23).

These animal models have been vitally important in generating and testing hypotheses. However, there are important differences between these models and human SUDEP; for example, if not resuscitated, some animal models (e.g., DBA/2 mice) die nearly every time they have a generalized seizure (9). In contrast, some epilepsy patients who die of SUDEP might have hundreds of seizures pass uneventfully before a fatal event. It will be important to determine whether differences between animal models and human SUDEP point to different underlying mechanisms so that we learn how data from animals can be translated to humans.

What Is the Cause of Death in SUDEP?

The most active controversy in the SUDEP field is whether the primary cause of death is respiratory arrest or cardiovascular collapse. These are the only two ways to die. If either occurs, the other follows shortly, assuming there is no intervention. Of course, SUDEP is clearly a heterogeneous disorder, so there are likely some cases that are primary cardiac and others that are respiratory. However, it is likely that many cases share common mechanisms. If true, it is important to determine how a seizure can lead to cardiorespiratory dysfunction. One proposed mechanism is postictal generalized electroencephalographic suppression (PGES) (24). However, PGES reflects a change in cortical activity, so it could not cause respiratory arrest because the brainstem can generate normal breathing in the absence of any cortical input (25, 26). Thus, PGES could either be a biomarker of some other abnormality (e.g., cerebral dysfunction due to hypoxia (27)) or be caused by something that also causes death (e.g., loss of activity of the ascending arousal system, which simultaneously causes loss of drive to breathe (26)).

Respiratory Arrest

Since at least 1899, it has been known that seizures can cause apnea, as reported by Hughlings Jackson (6, 7, 28). However, it has only recently been recognized how commonly this occurs. This has come as a great surprise to many neurologists, who rarely monitor respiratory parameters in a standard epilepsy monitoring unit (EMU) except perhaps O_2 saturation (SpO_2). Respiratory monitoring is likely to become more important because as many as 35% of patients drop their SpO_2 below 85% during a seizure, usually due to central apnea (6). In a separate study it was found that 10% of all seizures cause a decrease in SpO_2 below 80%, and this is equally likely to occur with partial seizures as with generalized seizures (8). A small number of epilepsy patients have died in EMUs (5, 29). In two of these cases of SUDEP respiratory movements appeared to terminate before the heartbeat on EKG, so it was concluded that death was due to a primary defect in breathing followed by secondary cardiac arrest (5). In other cases breathing movements were reported by witnesses so that death was thought to be cardiac, but it is not possible to make this conclusion without making quantitative measurements of breathing.

Animal studies support the conclusion that SUDEP can arise from primary respiratory arrest. In a sheep model of status epilepticus, death was clearly due to severe hypoventilation, causing respiratory acidosis and hypoxia (30). However, this was not a model of SUDEP since these sheep

were in status epilepticus. In 5-HT_{2c} receptor knockout mice, there is obesity, seizures, and high mortality (11). Respiratory parameters and EKG were not measured, so there is some uncertainty about the primary cause of death in these mice; however, visual observation led to the conclusion that death was due to respiratory arrest. The DBA/2 strain of mouse is prone to audiogenic seizures, and almost all (>75%) of those with the most severe form of convulsion (tonic extension) have respiratory arrest after nearly every seizure (9, 10). Death can be prevented using oxygen administration (9) or mechanical ventilation (10). This SUDEP model has the advantage that its predictability and ability to resuscitate allows repeated testing to determine the mechanisms of respiratory arrest.

Cardiovascular Dysfunction

There has long been an appreciation that changes in cardiovascular output can be induced by seizures. As many as 99% of seizures cause tachycardia in humans (1, 6). Bradycardia and asystole have also been reported to occur in 15 to 37 percent of patients (1, 6, 31). Although slowed heart rate has been described by some as typically being in the context of a change in breathing (6), others report that it can be directly due to activation of autonomic circuitry (1).

Seizures can also induce lengthening of the QT interval, especially when there is hypercapnia, hypoxia, or sympathetic overactivity (3). If sufficiently prolonged, it is possible this could lead to a fatal tachyarrhythmia. This would be even more likely if it occurred in a patient with a mutation of a long QT syndrome gene. In fact, SUDEP has been associated with mutations in a variety of genes that cause long QT syndrome, (LQTS) including *SCN1A*, *SCN5A*, and *KCNH2* (32, 33). Seizure-induced death in mice has also been associated with the LQTS gene *KCNQ1* (22). However, it is not clear how many cases of SUDEP are due to arrhythmias in humans with LQTS gene mutations. Most of the LQTS genes are expressed in the brain, with many in the hippocampus (34), and some of these mutations cause epilepsy (22, 35). Most of the genes are also found in the medulla where they could affect cardiac output (22). If these genes are also expressed in respiratory neurons, then dysfunction of those neurons might disrupt respiratory output. It is possible that apnea or hypoventilation might be induced more easily after a seizure in these individuals (36). Alternatively, LQTS mutations could make the heart more likely to respond adversely to hypoxia induced by hypoventilation after a seizure. Thus, the association of LQTS mutations and SUDEP does not necessarily prove that a cardiac arrhythmia is the primary cause of death in all those cases. Data are needed to determine how often seizures induce fatal arrhythmias in patients with LQTS.

Role of Arousal

Some seizures might lead to respiratory depression severe enough by itself to cause death. However, hypoxemia would be more severe if respiratory depression occurred together with an impaired arousal response to airway obstruction. This could explain why many SUDEP cases are found in bed (averaging 60% across most studies, as high as 95% in one; (37)) and in those cases, they are typically in the prone position. In one study, 59% were found in bed, and 68% were prone (38). If

a patient is postictal and severely obtunded, and is face down on soft bedding, they would lack the protective reflex to wake up and turn their head to relieve the airway obstruction. The arousal deficit would make the hypoxia worse than if there were respiratory depression alone.

Thus, there is evidence for both respiratory depression and cardiovascular collapse in SUDEP, with the data suggesting that a primary respiratory mechanism may be the more common. Under some conditions respiratory, cardiac or arousal mechanisms may work together. This could explain why respiratory depression is as likely to occur with partial seizures as with generalized seizures, and yet 90% of witnessed SUDEP has been reported to occur after a generalized seizure. A generalized seizure is more likely than a partial seizure to cause a profound depression of consciousness that would make it hard to respond to airway obstruction from bedding. The significance of PGES remains unclear, but it may be a biomarker for severe postictal obtundation, or loss of the arousal response to hypercapnia, both of which may exacerbate hypoventilation.

SUDEP, Serotonin, and SIDS

There is emerging evidence that there is a link between 5-HT and SUDEP (10, 18–20). In mice, the subset of neurons within the medullary raphe nuclei that make and release 5-HT plays a prominent role in control of breathing (14, 16, 17, 26). These neurons are sensors of blood CO₂ and tissue pH (15, 39) and stimulate breathing to normalize these blood gases (16, 26). Those 5-HT neurons that are in the midbrain raphe are part of the ascending arousal system (40) and are also sensors of tissue CO₂ and pH (41). When all CNS 5-HT neurons are genetically deleted (13), there is a large reduction in the ventilatory response to increased CO₂ (45) and complete loss of the arousal that normally occurs when CO₂ levels rise (21). If there is a defect in 5-HT neurons in some epilepsy patients, that could explain why there is an inadequate ventilatory response to a rise in blood CO₂ levels (42) and also why many patients die in bed without waking to correct their airway obstruction.

Other evidence for a link between 5-HT and SUDEP includes: 1) Cyproheptadine (a nonselective 5-HT receptor antagonist) increases the percentage of DBA/2 mice that die from postictal respiratory arrest (10); 2) abnormalities of 5-HT receptor binding in DBA/2 mice (18); 3) acute treatment with the SSRI fluoxetine prevents seizure-induced death in DBA/2 mice (10); and 4) chronic treatment with SSRIs reduces seizure-induced oxygen desaturation in humans (20). It is not yet known if SSRIs prevent SUDEP.

These findings are interesting because there is also a link between 5-HT & SIDS, as discussed in detail in a recent review (43). There are many parallels between SUDEP and SIDS. In both cases, the diagnosis is one of exclusion. The autopsy must not show any clear explanation for death. Hypotheses for both syndromes include competing theories about cardiac versus respiratory causes, with a supporting role for defects in arousal. In both syndromes, there is a high incidence of individuals found prone in bed with their airway obstructed by soft bedding; in fact, this was the basis of the Back-to-Sleep campaign that has been so highly successful for SIDS (43). The parallels between SIDS and SUDEP suggest there could be

shared pathophysiological mechanisms involving serotonergic modulation of respiratory control.

Summary

An hypothesis to explain the existing data from humans and animal models is that some seizures, to varying degrees, invade nuclei in the brainstem involved in control of cardiovascular output, breathing, and/or maintenance of consciousness. These nuclei would include the raphe nuclei that contain 5-HT neurons and possibly other nuclei including those that contain other monoamine neurons. Depending upon the genetic background of the patient and the degree of invasion of the specific nuclei involved, the postictal state would include varying degrees of dysfunction of cardiorespiratory control or arousal. After some seizures, there may be relatively greater risk of death, especially if the patient is in a location, such as a bed, where airway obstruction might occur, and if that seizure caused a particularly deep level of postictal unresponsiveness. The next steps in determining the mechanisms of SUDEP include defining the CNS pathways involved in cardiorespiratory inhibition by seizures, how activation of those pathways inhibits brainstem neurons, and how SSRIs reduce mortality in mice and if they do the same in humans.

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