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ARTICLE

Postictal serotonin levels are associated with periictal apnea

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Abstract

Objective

To determine the relationship between serum serotonin (5-HT) levels, ictal central apnea (ICA), and postconvulsive central apnea (PCCA) in epileptic seizures.

Methods

We prospectively evaluated video EEG, plethysmography, capillary oxygen saturation (SpO_2), and ECG for 49 patients (49 seizures) enrolled in a multicenter study of sudden unexpected death in epilepsy (SUDEP). Postictal and interictal venous blood samples were collected after a clinical seizure for measurement of serum 5-HT levels. Seizures were classified according to the International League Against Epilepsy 2017 seizure classification. We analyzed seizures with and without ICA (n = 49) and generalized convulsive seizures (GCS) with and without PCCA (n = 27).

Results

Postictal serum 5-HT levels were increased over interictal levels for seizures without ICA (p = 0.01), compared to seizures with ICA (p = 0.21). In patients with GCS without PCCA, serum 5-HT levels were increased postictally compared to interictal levels ($p < 0.001$), but not in patients with seizures with PCCA (p = 0.22). Postictal minus interictal 5-HT levels also differed between the 2 groups with and without PCCA (p = 0.03). Increased heart rate was accompanied by increased serum 5-HT levels (postictal minus interictal) after seizures without PCCA (p = 0.03) compared to those with PCCA (p = 0.42).

Conclusions

The data suggest that significant seizure-related increases in serum 5-HT levels are associated with a lower incidence of seizure-related breathing dysfunction, and may reflect physiologic changes that confer a protective effect against deleterious phenomena leading to SUDEP. These results need to be confirmed with a larger sample size study.

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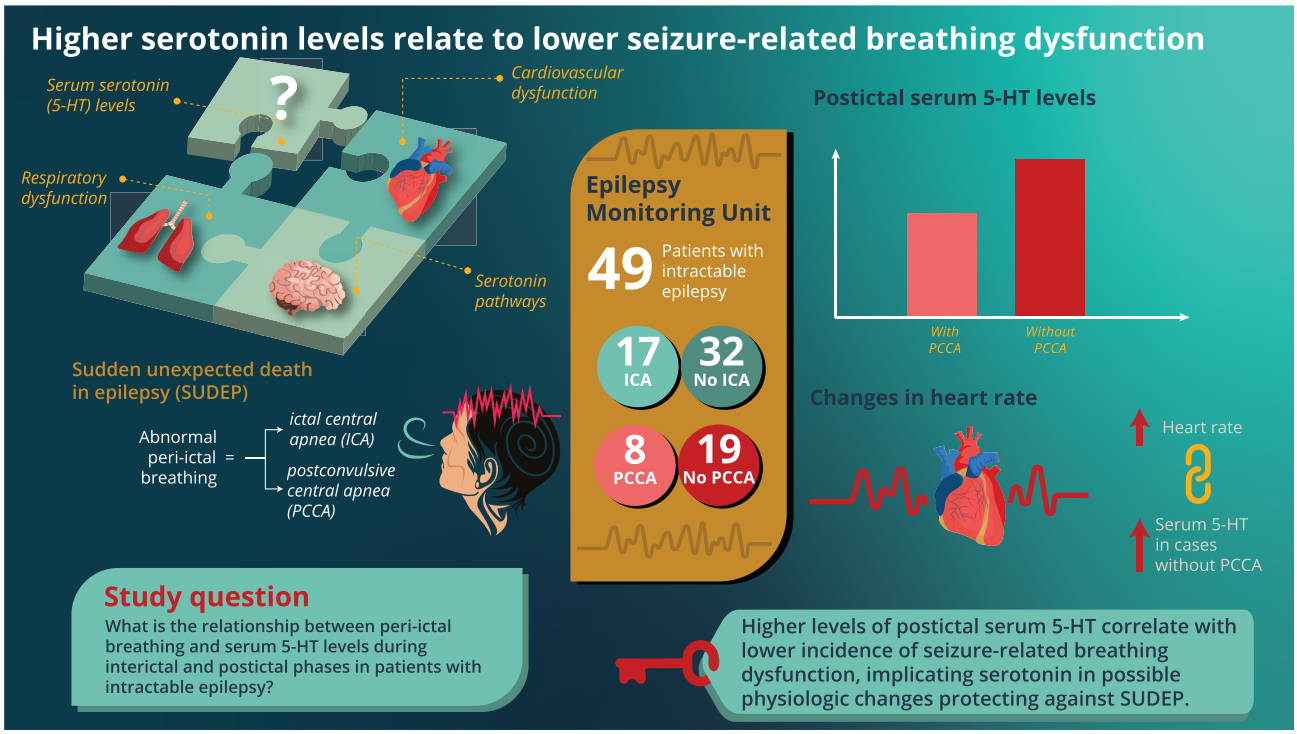
Glossary

BBB = blood–brain barrier; **BMI** = body mass index; **GCS** = generalized convulsive seizures; **HR** = heart rate; **ICA** = ictal central apnea; **PCCA** = postconvulsive central apnea; **PGES** = postictal generalized EEG suppression; **SRI** = serotonin reuptake inhibitor; **SUDEP** = sudden unexpected death in epilepsy; **VEEG** = video EEG.



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Sudden unexpected death in epilepsy (SUDEP) is second only to stroke as a leading cause of years of potential life lost in patients with neurologic disorders.[1] Agonal neural pathways, with or without preceding seizures, are characterized by profound cardiovascular or respiratory dysfunction.[2,3] Peri-ictal breathing compromise includes prolonged ictal central apnea (ICA),[4] and postconvulsive central apnea (PCCA) with or without bradycardia/asystole.[5] Increasing evidence from animal and human studies suggest that SUDEP-related breathing dysfunction may involve serotonergic pathways[6,7]; brainstem respiratory rhythm generators that control breathing are stimulated by serotonin (5-HT) brainstem raphe neurons,[8] which contribute to respiratory and arousal responses to hypercapnia.[9,10] Serotonergic fibers project to cerebellar Purkinje and deep nuclei, both of which serve to dampen expression of apnea and blood pressure, with the deep nuclei having a significant chemosensitive role.[11] These midline serotonergic neurons, in addition to somatostatin and neurokinin-1 receptor expressing neurons in the ventrolateral medulla, are substantially reduced in SUDEP patients compared to controls.[12] Additional support for brainstem contributions to SUDEP comes from imaging evidence of significant atrophy in pontomedullary areas known to modulate breathing (a clinical brainstem marker of seizure severity), tonic posturing during generalized convulsive seizures (GCS),[12,13] and specific neuroimaging changes in a SUDEP animal model.[14] In a previous study, increased 5-HT levels from baseline occurred immediately after seizures in patients but the degree of 5-HT increase was negatively correlated with the duration of the tonic seizure phase, suggesting an association between more severe seizures and lower serotonergic tone in the postictal state.[15] In the current study, we examined the relationship between peri-ictal central apnea and serum 5-HT levels in the interictal and postictal phases in patients with intractable epilepsy.

Methods

Standard protocol approvals, registrations, and patient consents

The study was approved by the Institutional Review Board at the University Hospital, Cleveland, Ohio. Written informed consent was provided by all patients enrolled in the research study.

Patient selection

A prospective cohort of patients with intractable epilepsy aged 18 years and above, admitted to the Epilepsy Monitoring Unit

during January 2015 to April 2018, were consented to participate in an institutional review board–approved multicenter autonomic and imaging SUDEP biomarker project (National Institute of Neurological Disorders and Stroke U01-090407) study. Forty-nine seizures were recorded in 49 patients (1 seizure per patient). Seizures were classified according to the 2017 International League Against Epilepsy seizure classification based on the most prominent clinical signs. The seizures were classified into 2 major groups: GCS, which included generalized tonic-clonic seizures of genetic generalized epilepsy and focal to bilateral tonic-clonic seizures, and focal seizures, which did not generalize.

Video EEG (VEEG) and cardiorespiratory monitoring

Standard surface VEEG using the 10–20 International Electrode System and multichannel ECG were acquired using the Nihon-Kohden system (Tokyo, Japan). Pulse oximetry was used to capture peripheral capillary oxygen saturation (SpO_2) and heart rate (HR) (Nellcor OxiMax N-600x, Covidien, Dublin, Ireland). Chest and abdominal excursions were recorded using inductance plethysmography (Ambu, Ballerup, Denmark). Both ICA and PCCA were defined as ≥1 missed breath based on breathing rhythm in the previous 30 seconds, with a minimum duration of 5 seconds without alternative explanation (e.g., movement, speech, or intervention). PCCA was also defined as absence of breathing for at least 5 seconds after GCS end. In addition, we diagnosed ICA in patients with generalized onset motor tonic-clonic seizures as cessation of breathing movements lasting from ≥5 seconds in the absence of generalized tonic or clonic movements, since such movements invariably produced movement artifact in breathing channels. We preferred the term PCCA rather than postictal central apnea because electrographic seizure discharges continued for varying durations after convulsions ended in some patients.

Data collection

Phenotypic and electroclinical data were collected, including sex, age, body mass index (BMI), age at epilepsy onset, epilepsy duration, respiratory comorbidities, cardiac comorbidities, epileptogenic zone, presence and duration of ICA and PCCA, presence and duration of hypoxemia, sleep state, baseline (2 minutes before seizure onset) HR, HR at GCS onset, maximum or minimum HR up to the immediate 3 minutes postictal period based on R-R interval, seizure phase and duration (tonic, clonic, jittery), and presence and duration of postictal generalized EEG suppression (PGES). Postictal and interictal venous blood samples were collected in serum-separator tubes (Z Serum Sep Clot activator tubes from Greiner-Bio-One North America, Inc., Monroe, NC) for a cleaner separation of serum from blood cells. Since no commercial assays are available for measuring whole blood 5-HT using platelet-rich and platelet-poor plasma preparations, we opted for a diagnostic assay to measure the 5-HT levels in the serum since our objective was to determine if peripheral levels of 5-HT in the blood change after seizures. All samples were processed within 30 minutes of collection, spun down, and serum was frozen. They were shipped in frozen state by courier to reference laboratory LabCorp (Burlington, NC; labcorp.com/) for analysis. Serum 5-HT levels were measured using high-performance liquid chromatography with electrochemical detection. The reference range reported by LabCorp for serum 5-HT was 0–420 ng/mL. Collection of interictal samples was only carried out after successful collection of postictal samples at the earliest feasible time as there was no guarantee of seizure occurrence during admission. Interictal samples were always obtained at rest after at least 12 hours since the last recorded clinical seizure.

Statistical analysis

Statistical analysis was done using SPSS software version 24.0 (IBM, Armonk, NY). Summary statistics were reported as mean ± SD (range). Statistical significance for intergroup differences was assessed with the Pearson χ^2 test and Fisher exact test for dichotomous or nominal variables. Mann-Whitney *U* test was used for continuous variables since they did not follow a normal distribution (Kolmogorov-Smirnov test). Binary logistic regressions were used to assess the association between dichotomous variables and other variables and combinations. Kruskal-Wallis H test was used to determine differences in continuous variables. Spearman correlation was used to determine correlation between continuous variables. A nonparametric Wilcoxon paired *t* test was used to compare interictal and postictal 5-HT levels in groups with and without ICA and PCCA. $p < 0.05$ was considered statistically significant. A multivariate linear regression analysis was used to model the relationship of change in 5-HT levels with other independent variables of age, sex, age at epilepsy onset, and time to postictal blood draw for generalized seizure groups with PCCA or ICA.

Data availability

The datasets used and analyzed during the current study are available from the corresponding author on request.

Results

We analyzed a total of 49 seizures (27 GCS, which included both generalized tonic-clonic seizures of genetic generalized epilepsy and focal to bilateral tonic-clonic seizures, and 22 focal seizures) in 49 patients (29 female) with mean age of 42.0 ± 14.4 years (range 20–77). Age at epilepsy onset was 25.2 ± 18.4 years, mean epilepsy duration was 16.8 ± 15.1 years, and BMI was 28.9 ± 7.8. ICA was seen in 17/49 (34.7%) seizures whereas PCCA was found in 8/27 (29.6%) seizures.

Characteristics of patients with and without ICA

Electroclinical characteristics of seizures in the presence and absence of ICA are presented in table 1. A total of 12/17 (71%) patients with ICA were female, compared to 17/32

Table 1 Clinical characteristics of seizures with and without ictal central apnea (ICA)

Demographics	ICA (n = 17)	No ICA (n = 32)	*p* Value
Sex			0.23
Male	5 (29.4)	15 (47.0)	
Female	12 (70.6)	17 (53.0)	
Age, y	46.5 ± 14.3	39.6 ± 14.0	0.10
BMI	26.9 ± 6.0	29.9 ± 8.4	0.20
History			
Age at epilepsy onset, y	34.2 ± 20.4	20.5 ± 15.5	0.01
Epilepsy duration, y	12.3 ± 12.8	19.2 ± 15.8	0.13
Epileptogenic zone			
Generalized	—	7 (21.8)	
Temporal	14 (82.3)	10 (31.3)	
Frontal	2 (11.8)	10 (31.3)	
Parietal	—	2 (6.3)	
Unknown	1 (5.9)	3 (9.4)	
Seizure semiology[a]			
Generalized onset motor tonic-clonic	6 (35.3)	12 (37.5)	
Focal to bilateral tonic-clonic	3 (17.6)	6 (18.8)	
FOIA nonmotor onset	4 (23.5)	4 (12.5)	
FOA motor onset automatisms	—	3 (9.4)	
FOIA motor onset hyperkinetic	4 (23.5)	5 (15.6)	
FOIA clonic	—	2 (6.3)	
Seizure measures			
Clinical seizure duration, s	50.4 ± 14.9	55.5 ± 20.7	0.37
ICA duration, s	16.1 ± 11.3	—	
ICA blood draw, minutes after seizure end	5.5 ± 2.9	6.7 ± 6.5	0.32

Abbreviations: BMI = body mass index; FOA = focal onset aware; FOIA = focal onset impaired awareness.
Values are given as n (%) or mean ±SD.
[a] International League Against Epilepsy classification of seizures.

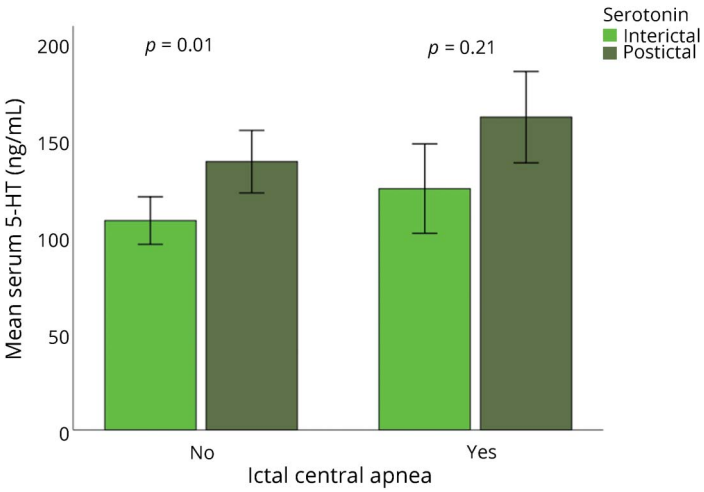
(53%) in the non-ICA group. Patients with ICA tended towards older age groups, but without statistical significance (p = 0.10). Duration of epilepsy appeared to be shorter for patients with ICA but not significantly so. BMI was similar in both groups. However, patients with ICA were older at onset of epilepsy (p = 0.01).

5-HT levels and ICA in seizures

The relationship between 5-HT levels and ICA is shown in figure 1. Mean interictal 5-HT levels in patients without ICA (n = 32) were 109.1 ± 70.1 ng/mL (range 8–343) compared to 125.8 ± 96.1 ng/mL (range 2–416) in patients with ICA. Postictal levels in patients without ICA were 139.8 ± 92.2 ng/mL (range 11–386) compared to 163.0 ± 98.2 ng/mL (range 7–306) in patients with ICA. The rise in postictal serum 5-HT was seen in both ICA and non-ICA groups but proved significant only in the latter (p = 0.01), possibly due to difference in sample size. There was no significant difference between the ICA and non-ICA group when interictal levels, postictal levels, or the postictal to interictal difference in serum 5-HT were compared.

Average time elapsed between seizure end and postictal 5-HT blood draw was 5.5 ± 2.9 minutes for those with ICA and 6.7 ± 6.5 minutes for those without ICA (p = 0.32). There was no correlation between blood draw time and postictal 5-HT levels or change in 5-HT levels between postictal and interictal levels in patients with and without ICA.

Figure 1 Serum 5-HT levels and ictal central apnea (ICA) after generalized convulsive and focal seizures



The mean serum interictal 5-HT levels are shown in light green bars and postictal 5-HT levels (ng/mL) are shown in dark green bars for the 2 groups; the left 2 columns without ICA (n = 32) and the right 2 columns with ICA (n = 17). The levels of postictal serum 5-HT in the absence of ICA was higher when compared to interictal levels (p = 0.01). However, in the presence of ICA, no significance was observed (p = 0.21).

Characteristics of patients with and without PCCA

Electroclinical characteristics of patients with GCS in the presence and absence of PCCA are shown in table 2. Patients with PCCA (7/8 [87.5%]) were more likely to be female compared to the non-PCCA group (8/19 [42%]; p = 0.05). Patients with PCCA tended to be younger, but not significantly so. Average age at epilepsy onset was earlier in patients with PCCA compared to non-PCCA but was not different (p = 0.16). BMI, duration of epilepsy, and cardiac or respiratory comorbidities were similar in both groups.

Seizure semiology and epileptogenic zones for both groups are shown in table 2. The clinical seizure duration was similar in both groups. SpO_2 nadir (%) did not differ between the 2 groups. There was no significant difference between the 2 groups for other clinical features of the seizure including durations of hypoxemia, tonic phase, clonic phase, jittery phase, or PGES. There was also no difference in HR at baseline, generalized clinical onset, or maximum HR within 3 minutes of seizure end. The average time elapsed between postictal blood draw and seizure end was 11.0 ± 9.3 minutes for those with PCCA and 7.8 ± 5.1 minutes for those without PCCA (p = 0.26).

5-HT levels and PCCA in GCS

Postictal central apnea was only seen after GCS and was referred to as PCCA. In seizures with PCCA, mean interictal and postictal 5-HT levels were 92.9 ± 69.8 ng/mL (range 16–227) and 124.8 ± 104.9 ng/mL (range 11–306), respectively (figure 2A). In the non-PCCA group, the mean interictal 5-HT level was 116.4 ± 62.8 ng/mL (range 8–198) and postictal level was 189.9 ± 97.8 ng/mL (range 13–386) (figure 2A). The postictal serum 5-HT levels compared to interictal levels were higher in the non-PCCA group (p < 0.001) than in PCCA (p = 0.22) using a nonparametric Wilcoxon paired sample t test.

When PCCA and non-PCCA groups were compared, the mean changes (postictal minus interictal) in serum 5-HT were higher in non-PCCA (73.5 ± 59.0 ng/mL) than in PCCA (31.9 ± 67.5 ng/mL) (figure 2B) using a nonparametric Mann-Whitney U test (p = 0.03).

5-HT levels with PCCA and ICA in GCS

ICA preceded PCCA in 3/8 GCS (37.5%), as shown in table 2. In the non-PCCA group, there were 6/17 GCS (35.3%) that had ICA (table 2). In 2 seizures in the non-PCCA group, ICA could not be reliably commented upon and therefore were excluded from analysis. Mean ICA duration was 10.3 ± 6.7 seconds for patients with PCCA and 14.5 ± 5.4 seconds for the non-PCCA group (p = 0.34). There was no significant difference in comparing postictal serum 5-HT levels or the difference in postictal to interictal levels between the 2 groups of GCS with and without ICA.

A multivariate linear regression analysis was performed to assess the extent to which change in postictal to interictal 5-HT levels reflected the effect of GCS and its relation to PCCA and ICA. Other variables included in the analysis were age, sex, age at epilepsy onset, and time to postictal blood draw. There was no significant association between changes in serum 5-HT levels and age, sex, age at epilepsy onset, and time to postictal blood draw for all 3 groups. The prediction significance for the whole generalized convulsive seizures group was p = 0.235, subgroup of generalized seizures with only PCCA was p = 0.615, and subgroup of generalized seizures with only ICA was p = 0.710.

5-HT levels and HR in GCS

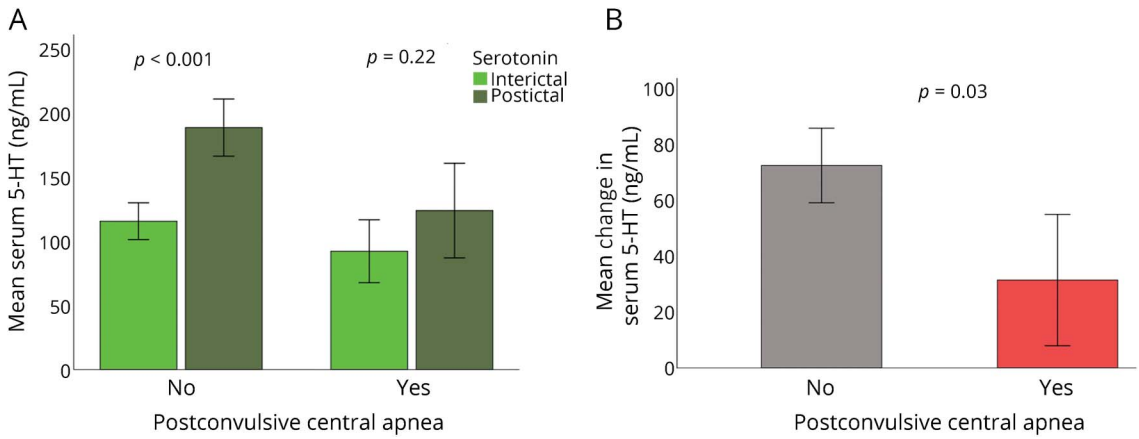
HR at baseline, generalized clinical onset of seizure, and the maximum HR within 3 minutes postictally was measured for all patients with GCS with and without PCCA, as shown in table 2. The change in serum 5-HT levels (postictal minus

Table 2 Clinical characteristics of seizures with and without postconvulsive central apnea (PCCA)

	PCCA (n = 8)	No PCCA (n = 19)	*p* Value
Demographics			
Sex			
Male	1 (12.5)	11 (58.0)	0.05
Female	7 (87.5)	8 (42.0)	
Age, y	38.0 ± 13.1	43.4 ± 17.3	0.43
BMI	29.9 ± 7.7	27.7 ± 7.4	0.49
History			
Age at epilepsy onset, y	16.0 ± 5.9	27.1 ± 21.0	0.16
Epilepsy duration, y	22.0 ± 17.0	16.3 ± 11.3	0.31
Respiratory comorbidities	1 (12.5)	2 (11.0)	
Cardiac comorbidities	1 (12.5)	1 (5.0)	
Epileptogenic zone			
Generalized	2 (25.0)	5 (26.3)	
Temporal	1 (12.5)	9 (47.4)	
Frontal	4 (50.0)	2 (10.5)	
Unknown	1 (12.5)	3 (15.8)	
Seizure semiology			
Generalized onset motor tonic-clonic	4 (50.0)	7 (36.8)	
Focal to bilateral tonic-clonic	4 (50.0)	12 (63.2)	
Seizure measures			
Clinical seizure duration, s	49.1 ± 17.8	55.4 ± 18.4	0.42
PCCA duration, s	7.1 ± 2.0		
SpO_2 nadir, %	64.3 ± 14.0	59.2 ± 13.1 (n = 15)	0.39
Presence of hypoxemia	6 (75.0)	11 (58) (n = 18)	
Hypoxemia duration	110.6 ± 46.3 (n = 5)	128.4 ± 77.2 (n = 11)	0.64
Tonic phase duration, s	8.7 ± 5.6 (n = 6)	9.8 ± 4.7 (n = 11)	0.67
Jittery phase duration, s	12.9 ± 11.5 (n = 6)	11.7 ± 7.8 (n = 13)	0.79
Clonic phase duration, s	31.4 ± 11.1 (n = 8)	41.7 ± 19.5 (n = 19)	0.18
Sleep state			
Awake	4 (50)	7 (37)	
Asleep	4 (50)	12 (63)	
Baseline HR	78.0 ± 31.0	70.1 ± 13.5	0.78
HR at generalized clinical onset	96.5 ± 29.7	94.3 ± 29.8	0.86
Maximum heart rate within 3 Minutes	154.0 ± 29.5	145.4 ± 15.4	0.31
Presence of PGES	7 (87.5) n = 8	6 (46.2) n = 13	
PGES duration, s	32.7 ± 23.1 (n = 7)	34.3 ± 14.8 (n = 6)	0.88
ICA incidence	3 (37.5) (n = 8)	6 (35.3) (n = 17)	

Abbreviations: BMI = body mass index; HR = heart rate; ICA = ictal central apnea; PGES = postictal generalized EEG suppression; SpO_2 = peripheral capillary oxygen saturation. Values are given as n (%) or mean ± SD.

Figure 2 Serum 5-HT levels and postconvulsive central apnea (PCCA) after generalized convulsive seizures



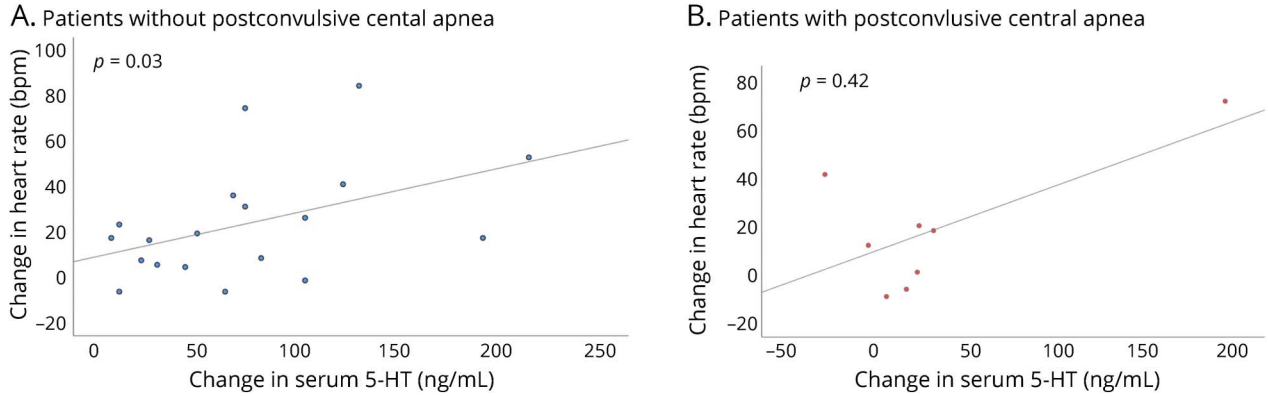
(A) Elevated levels of postictal 5-HT in generalized convulsive seizures (GCS). The mean serum interictal 5-HT levels are shown in light green bars and postictal 5-HT levels (ng/mL) are shown in dark green bars for the 2 seizure groups: PCCA (n = 8) and non-PCCA (n = 19). The levels of postictal serum 5-HT in the absence of PCCA were higher when compared to interictal levels ($p < 0.001$), but not when PCCA occurred ($p = 0.22$). (B) Elevated serum 5-HT levels in the absence of PCCA. The change in 5-HT (postictal minus interictal) was plotted for seizures without PCCA (in gray) and with PCCA (in red). The change in serum 5-HT (postictal minus interictal levels) was moderate when the 2 groups were compared ($p = 0.03$).

interictal) was plotted against change in HR (HR at generalized clinical onset of seizure minus HR at baseline) (figure 3). In patients without PCCA, an increase in 5-HT was associated with an increase in HR (figure 3A; $p = 0.03$), but not in patients with PCCA (figure 3B; $p = 0.42$). The change in serum 5-HT level was less than 50 ng/mL for the entire PCCA group, except for one patient at ~200 ng/mL of 5-HT (figure 3B).

Discussion

We previously reported that serum 5-HT levels in GCS were significantly higher postictally than in the interictal state compared to focal seizures.[15] However, given the critical role of 5-HT in breathing modulation and arousal,[9,10] its influence on human peri-ictal breathing features is unknown. Here, we sought to determine the relationship between 5-HT levels and seizure-related breathing dysfunction and underlying risk factors associated with SUDEP. In this study, limited to a small number of patients (n = 49), we found that (1) serum 5-HT levels were significantly higher postictally compared to the interictal state for patients who did not develop ICA, (2) serum 5-HT levels were significantly higher postictally compared to interictal levels in patients with GCS who did not develop PCCA compared to those who developed PCCA, and (3) higher serum 5-HT levels were positively associated with increased HR changes in patients without PCCA. We were able to show that patients with GCS can be separated

Figure 3 Association of heart rate (HR) with serum 5-HT



(A) The differences in serum 5-HT levels (postictal minus interictal) were plotted against the change in HR (difference in HR at clinical onset of seizure minus baseline) for seizures without postconvulsive central apnea (PCCA) (n = 19) shown as blue dots. Increased change in serum 5-HT was associated with an increase in heart rate ($p = 0.03$). (B) Similar analysis revealed no association between serum 5-HT levels and heart rate for patients with PCCA (n = 8) and shown as red dots ($p = 0.42$).

into a potentially high SUDEP risk group (low postictal 5-HT levels, presence of PCCA, and low HR). These findings suggest that serum 5-HT levels may reflect changes in physiology that play a role in the development and potentially the severity of peri-ictal apneic phenomena and HR responses in epileptic seizures.

5-HT is a monoaminergic neurotransmitter synthesized through the actions of 2 different tryptophan hydroxylase isoforms encoded by the genes *TPH1* and *TPH2*. These are expressed in intestinal enterochromaffin cells and serotonergic neurons in the brainstem, respectively. Most 5-HT (>95%) is found outside the CNS. Some of the peripheral 5-HT synthesized by enterochromaffin cells is taken up by platelets, which express the 5-HT transporter (SERT). The release of 5-HT from enterochromaffin cells is typically in response to acetylcholine, raised intraluminal pressure, sympathetic nerve stimulation, and low intestinal pH.[16] The origin of almost all brain 5-HT is a small group of neurons in the medullary raphe nuclei and midbrain.[17] Raphe 5-HT neurons project throughout the neuraxis and are vital for many brain functions including mood, sleep/arousal, thermoregulation, autonomic control, and breathing.[17,18]

Peripheral (serum) 5-HT does not readily cross the blood–brain barrier (BBB), which is made up of a single layer of endothelial cells connected by tight junctions, and acts as a barrier that prevents exchange of water-soluble substances between the brain and blood.[19] During intense seizure activity, such as status epilepticus, BBB permeability increases, allowing potential exchange of 5-HT between the CNS and the peripheral circulation.[20] BBB dysfunction as a consequence of epileptic seizures was also recently reported using a newly established quantitative MRI protocol.[21] Thus, peripherally generated 5-HT may pass into the brain due to seizure-induced disruption of the BBB and add to the stimulatory effects of synaptically released 5-HT on respiration and arousal.[19] The significance of this BBB breakdown and the extent to which it allows 5-HT transfer remain unclear, but if breakdown does occur the increase in serum (5-HT) would be expected to stimulate breathing and arousal.

Patients who undergo SUDEP or near-SUDEP are known to have profound dysregulation of breathing after generalized convulsive seizures.[6,22,23] Serotonergic raphe neurons in the brainstem, which play a major role in respiratory chemoreception[9] and arousal,[10] are suspected to play a role in SUDEP and SIDS.[24,25] 5-HT_{2C} receptor knockout mice and DBA1/DBA2 mouse models of seizures and SUDEP exhibit apneic fatalities in the postictal state if not resuscitated[26,27] and death is preventable with serotonin reuptake inhibitor (SRI) pretreatment in these mice.[28] In human seizures, SRI treatment has been found to correlate with reduced severity of ictal SpO_2 decreases,[29] further emphasizing the positive role of 5-HT in seizure-related respiratory phenomena. ICA is usually brief, self-limited, and benign, and usually occurs as a semiologic phenomenon in temporal lobe epilepsy,[4] as confirmed in our data. Our findings suggest that it is not associated with known SUDEP risk factors such as early age at onset of epilepsy and duration of epilepsy. However, instances of prolonged ICA of >60 seconds with severe decreases in SpO_2 may confer SUDEP risk. We found that patients who exhibited significantly increased postictal serum 5-HT compared to the interictal state were less likely to have ICA. However, whether these increases mitigate SUDEP risk due to prolonged ICA is uncertain because in this small sample, we were unable to show any significant correlation between serum 5-HT levels and ICA duration. Further studies are required to examine this aspect.

PCCA may have greater SUDEP relevance than ICA and has been associated with near-SUDEP cases as well as SUDEP, the latter in a case with prospective follow-up.[5] PCCA occurs much less frequently than ICA; the latter is a preconvulsive phenomenon in focal epilepsy, likely driven by seizure discharges in cortical sites that modulate breathing[4,30,31]; and is phenomenologically distinct from PCCA. PCCA, which usually occurs in the absence of concurrent electrographic seizure discharge, may be driven by brainstem mechanisms akin to a pontomedullary Todd paresis–like phenomenon, rather than cortical phenomena. Significant differences between postictal and interictal serum 5-HT levels were noted in patients without PCCA, potentially conferring protection from this phenomenon in these patients. Patients with PCCA were unable to produce similar significant postictal increases in 5-HT. On the basis of our findings, we speculate that large postictal increases in serum 5-HT (figure 2B) may play a role in modulation of respiration in these patients, as evidenced by the above. Alternatively, the increase in serum 5-HT that we measured may be a surrogate for an increase in brain 5-HT levels that may depend on similar physiologic mechanisms, rather than serum 5-HT directly stimulating breathing. Fatal postictal apnea/bradycardia is well-described in animal[32,33] and human[2,34] SUDEP. Its mechanisms may differ in different types of SUDEP, but in Dravet syndrome mice it is due to a direct effect of hypoxia on cardiac muscle.[32] In our study, in patients without PCCA, an increase in 5-HT was associated with an increase in HR, but not in patients with PCCA. No association was seen between changes in 5-HT levels with age, sex, epilepsy duration, and time to postictal blood draw when analyzed for the whole group of GCS, the PCCA-only group, or the ICA-only group, possibly reflecting the small sample size of the study. Based on 5-HT levels, SpO_2 changes, and PCCA presence, separation of patients into high and low SUDEP risk groups may be feasible, and larger scale studies to this effect are warranted.

Neuropathologic examination of SUDEP brainstems[12] shows evidence of structural injury in serotonergic neurons of the medullary raphe, and somatostatin and neurokinin-1 receptor neurons in the ventrolateral medulla, where the pre-Bötzinger complex[35] nucleus is located. The latter is an important site for inspiratory rhythm generation and a key target for the respiratory stimulation effect of the periaqueductal gray,

which is strongly implicated as critical in animal and human SUDEP.[14,36] These findings provide potential anatomical substrates that mediate the breathing and cardiac dysfunction seen in PCCA and near-SUDEP/SUDEP.

Several issues remain unexplained. It is not clear if the apparent postictal rise in serum 5-HT level reflects a difference in serum 5-HT that occurs some time prior to seizure (preictal), which would be independent of the "interictal serum 5-HT" used in our study. It is possible that changes in interictal/preictal serum 5-HT would even promote occurrence of seizures through various mechanisms (including change in vigilance), explaining why the apparent postictal serum 5-HT levels would be increased compared to "interictal serum 5-HT." How serum 5-HT levels reflect neuronal 5-HT activity is unknown, despite the possibility that a permeable BBB in the seizure state allows serum 5-HT egress into the CNS.[20,37] Future studies on this issue are needed. The postictal increase in serum 5-HT levels in patients with GCS can be explained by the observation that serum 5-HT elevations occur in both humans and horses after moderate to intensive exercise[16,38]; physical exertion triggered by GCS may be akin to muscular activity encountered during exercise and may have a similar effect on 5-HT levels. However, the finding that patients with PCCA do not exhibit larger postictal 5-HT increases is not consistent with this mechanism. Apneic patients tended to slightly delay postictal blood draws, although not significantly so. Significant elevation in serum 5-HT (which has a half-life of 3 days)[39,40] compared to baseline is recorded as long as 35 minutes after intensive exercise in humans,[38] and differences between PCCA and non-PCCA groups remained even when the outlying patients were excluded from analysis. It is possible that patients without PCCA possess greater platelet reserves of 5-HT, possibly reflecting increased gut synthesis. In this context, consideration of differences in gut microbiota in the epilepsy population may be pertinent, since enterochromaffin cells of the intestine are the major source of serum 5-HT synthesis, and gut microbiota are known to regulate host 5-HT biosynthesis as well as affect platelet function.[41]

Our study, with its small sample size of 49 patients, has several limitations, and our inferences are based on a number of assumptions. Not least of these is the timing of blood draws. Our study design was pragmatic, and thus to lessen cost, interictal samples were taken at an assumed baseline state after seizures had occurred, rather than before. We also did not take serial postictal samples at designated time points, again due to cost and logistical reasons. Nonetheless, our findings indicate that low levels of postictal serum 5-HT are associated with potentially deleterious breathing phenomena and warrant further large-scale study.

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Continued

Appendix *(continued)*

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