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Laryngospasm, central and obstructive apnea during seizures: Defining pathophysiology for sudden death in a rat model

K. Nakase[a], R. Kollmar[b,c], J. Lazar[d], H. Arjomandi[c], K. Sundaram[c], J. Silverman[c], R. Orman[a], J. Weedon[e], D. Stefanov[e], E. Savoca[b,c], L. Tordjman[a], K. Stiles[b], M. Ihsan[d], A. Nunez[d], L. Guzman[g], M. Stewart[a,f,*]

[a] Department of Physiology & Pharmacology, State University of New York, Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, New York, 11203, United States
[b] Department of Cell Biology, State University of New York, Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, New York, 11203, United States
[c] Department of Otolaryngology, State University of New York, Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, New York, 11203, United States
[d] Department of Medicine (Division of Cardiology), State University of New York, Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, New York, 11203, United States
[e] Department of Statistical Design & Analysis, State University of New York, Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, New York, 11203, United States
[f] Department of Neurology, State University of New York, Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, New York, 11203, United States
[g] Research Initiative for Scientific Enhancement (RISE) Program, City University of New York, Medgar Evers College, 1638 Bedford Avenue, Brooklyn, New York, 11225, United States

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ABSTRACT

Seizure spread into the autonomic nervous system can result in life-threatening cardiovascular and respiratory dysfunction. Here we report on a less-studied consequence of such autonomic derangements—the possibility of laryngospasm and upper-airway occlusion.

We used parenteral kainic acid to induce recurring seizures in urethane-anesthetized Sprague Dawley rats. EEG recordings and combinations of cardiopulmonary monitoring, including video laryngoscopy, were performed during multi-unit recordings of recurrent laryngeal nerve (RLN) activity or head-out plethysmography with or without endotracheal intubation. Controlled occlusions of a tracheal tube were used to study the kinetics of cardiac and respiratory changes after sudden obstruction.

Seizure activity caused significant firing increases in the RLN that were associated with abnormal, high-frequency movements of the vocal folds. Partial airway obstruction from laryngospasm was evident in plethysmograms and was prevented by intubation. Complete glottic closure (confirmed by laryngoscopy) occurred in a subset of non-intubated animals in association with the largest increases in RLN activity, and cessation of airflow was followed in all obstructed animals within tens of seconds by ST-segment elevation, bradycardia, and death. Periods of central apnea occurred in both intubated and non-intubated rats during seizures for periods up to 33 s and were associated with modestly increased RLN activity, minimal cardiac derangements, and an open airway on laryngoscopy. In controlled complete airway occlusions, respiratory effort to inspire progressively increased, then ceased, usually in less than 1 min. Respiratory arrest was associated with left ventricular dilatation and eventual asystole, an elevation of systemic blood pressure, and complete glottic closure.

Severe laryngospasm contributed to the seizure- and hypoxemia-induced conditions that resulted in sudden death in our rat model, and we suggest that this mechanism could contribute to sudden death in epilepsy.

1. Introduction

Seizure spread into the autonomic nervous system is thought to play an important role in sudden unexpected death in epilepsy (SUDEP; (Bermeo-Ovalle et al., 2015; Devinsky, 2011; Lathers et al., 2008; Sakamoto et al., 2008; Shorvon and Tomson, 2011; Stewart, 2011; Surges and Sander, 2012; Tolstykh and Cavazos,

* Correspondence to: SUNY, Downstate Medical Center School of Graduate Studies, 450 Clarkson Avenue, MSC 41, Brooklyn, NY 11203, United States.
E-mail address: mark.stewart@downstate.edu (M. Stewart).

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2013)). Approximately 1% of the US population lives with epilepsy; depending on how one defines sudden death, 2%–17% of deaths in these patients are labeled SUDEP (e.g. (Nei and Hays, 2010)). Among adults with epilepsy, mortality rates are 2–3 times greater than among their non-epileptic counterparts (Langan, 2000; Thurman et al., 2014), and SUDEP is the single most common cause of death (Lathers et al., 1998; Wannamaker, 1985).

Seizures are known to produce significant respiratory changes (reviewed in (Massey et al., 2014; Sowers et al., 2013)). Ictal apnea (Blum, 2009) is implicated in oxygen desaturation during seizures (Bateman et al., 2008; Seyal et al., 2010). Indeed, animal research established the importance of ictal hypoxemia in seizure-induced death, as studies in sheep have shown that ictal hypoventilation leads to severe bradycardia and death (Johnston et al., 1995, 1997). Similar findings have been noted in rats (Sakamoto et al., 2008; Stewart, 2011), cats (Schraeder and Lathers, 1983), and mice (Faingold et al., 2010; Uteshev et al., 2010). The physiological mechanisms, however, that link seizures to respiratory dysfunction have not been fully resolved.

One possible cause of ictal respiratory distress is laryngospasm, a tonic adduction of the vocal folds that partially or fully obstructs the upper airway. Laryngospasm has been observed during seizures or postictally, evidenced by stridor and a narrowed airway when attempting to place an endotracheal tube (Tavee and Morris, 2008) or intensive inspiratory effort with severe air hunger (Amir et al., 1983). Cats and piglets experienced hypoventilation and glottal obstruction during chemically-induced seizures (Leaming et al., 1999; Terndrup et al., 1995a, 1995b). That pulmonary edema is the most common single finding at autopsy in SUDEP cases is also indirect evidence of laryngospasm (Antoniuk et al., 2001; Morentin and Alcaraz, 2002; Salmo and Connolly, 2002). Pulmonary edema can occur when "pulling" against a closed airway – the inspiratory effort increases pulmonary capillary pressure (Ead, 2003; Murray-Calderon and Connolly, 1997; Umbrain and Camu, 1993). Seizures could cause ictal laryngospasms by spreading via autonomic medullary motor regions to the laryngeal branches of the vagus nerve, the efferent innervation of the vocal folds.

To test the hypothesis that ictal laryngospasm can cause death, we have extended our urethane/kainate rat model (reviewed in (Naggar and Stewart, 2015; Stewart, 2011)) to permit detailed study of laryngeal physiology during seizure activity. This model is unique in its advantages for invasive monitoring during seizure activity. We are able to obtain recordings from the recurrent laryngeal nerve, the principal motor output to the larynx (Bartlett, 2011; Brancatisano et al., 1991; Kuna et al., 1991, 1988, 1990), along with simultaneous laryngoscopy (Mor et al., 2014) to define the patterns of RLN activity during seizures, the impact of seizure activity on laryngeal function, and the impact of laryngeal dysfunction on breathing. These data highlight the complexity of laryngospasm during seizures, and how changes in laryngeal function can contribute to death.

2. Materials and methods

All procedures were approved by an Animal Care and Use Committee and conducted in accordance with the United States Public Health Service's Policy on Humane Care and Use of Laboratory Animals. Adult male Sprague-Dawley albino rats (180–340 g; Harlan, Chicago, IL) were housed in AAALAC-accredited facilities and maintained on a 12 h light:dark cycle with a temperature of 23° C and humidity of 55%, monitored daily, and had unrestricted access to water and food.

Urethane (1.5 g/kg ip) was used for anesthesia. Temperature was monitored with a rectal thermometer and maintained with an isothermal heating pad (Deltaphase, Braintree Scientific, Braintree, MA).

2.1. Monitoring/Recordings

EEG RECORDINGS. To place epidural EEG electrodes, the scalp over dorsal surface of skull was incised, the skin retracted, and the periosteum scraped from skull surface. Burr holes were drilled for placement of stainless steel screw electrodes bilaterally over dorsal CA3 (5.8 mm anterior to lambda, 3.5 mm lateral to midline; (Paxinos and Watson, 1998)), and over cerebellum as a recording reference. Signals were amplified, filtered to pass 1 Hz to 1 kHz, and digitized at 2–20 kHz. Seizure activity was recognized as an average peak-to-peak amplitude that was ≥3 times the baseline peak-to-peak amplitude, typically associated with frequent spiking. Seizure onset and offset times were estimated from the EEG raw data and the rate of change of the amplitude measure.

RECURRENT LARYNGEAL NERVE (RLN) RECORDINGs. After shaving the ventral neck region, an incision exposed the anterior neck muscles. These were bluntly divided to reveal the larynx, thyroid gland, and trachea. The RLN was identified on the lateral aspect of the trachea (Plate 157 in (Greene, 1968)). A platinum-iridium parallel bipolar electrode recorded multi-unit activity from the left or right RLN. Signals were amplified (A-M Systems 1800 or 1700, Sequim WA), filtered to pass 1 Hz to 5 or 10 kHz, and digitized at 10 or 20 kHz (Spike2, CED, Cambridge, UK). For measures of relative activity, stored recordings were digitally high-pass filtered (270 Hz within Spike 2), full-wave rectified and integrated over periods of 1–5 s (results expressed per second). We used the integration method to quantify activity levels in place of a rate measure because of the variability of spike shapes in multi-unit recordings. It is less sensitive than counting spikes, so the magnitude of seizure-induced changes measured by our integration method is likely to be an underestimate.

ECG RECORDINGS. Limb-lead ECG was recorded using copper strips coated with conductive gel wrapped around limbs for limb-lead ECGs. Signals were amplified and filtered to pass 1 Hz to 1 kHz and digitized at 2, 10, or 20 kHz. The presence of abnormally shaped QRS complexes indicated ectopic beats. Rate was calculated from the number of beats per unit time. Rhythm was assessed by reviewing P waves and associated QRS complexes for variations in wave shape, beat-to-beat intervals, and atrial-ventricular coupling.

BLOOD PRESSURE. Continuous blood pressure (BP) recordings were made from an arterial catheter (polyethylene tubing, cut square, 0.5 mm inside diameter, 0.8 mm outside diameter) in the right carotid artery that was connected to a blood pressure transducer (CyQ, Columbus Instruments, Columbus, OH). Signals were digitized along with other signals recorded at the same time.

PULSE OXIMETRY. Blood oxygen saturation was measured by using a clip sensor on the thigh (TDR-43C, Med Associates, St Albans, VT) and monitored by using a pulse oximeter (CANL-425SV-A, Med Assoc.). The continuous output of the devices was digitized (≥5 kHz) with other signals and reflected beat-to-beat variation in blood flow at the sensor. Mean saturation values were recorded at 10 s intervals.

ECHOCARDIOGRAPHY. Transthoracic echocardiography was performed using Phillips 5500 echocardiography machine (Phillips, Andover, MA, USA) equipped with a 15-MHz transducer. Animals were imaged in the optimal 2D-guided M-mode parasternal short axis view with machine and gain settings adjusted for best image quality. Parameters were measured from recorded video for 3 cardiac cycles at a sweep speed of 100 mm/s. Left ventricular end systolic dimension (LVESD) and left ventricular end diastolic dimension (LVEDD) were measured. The ejection fraction (EF) was calculated, assuming conical volumes, from the formula: $EF = 100 \times [(LVEDD^2 - LVESD^2)/LVEDD^2]$ (Mascareno et al., 2012; Naggar et al., 2012).

PLETHYSMOGRAPHY. Tidal-breathing flow-volume loops were recorded using head-out plethysmography (Renninger, 2006).