Progressive development of cardiomyopathy following altered autonomic activity in status epilepticus

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Read MI, McCann DM, Millen RN, Harrison JC, Kerr DS, Sammut IA. Progressive development of cardiomyopathy following altered autonomic activity in status epilepticus. Am J Physiol Heart Circ Physiol 309: H1554–H1564, 2015. First published September 4, 2015; doi:10.1152/ajpheart.00256.2015.—Seizures are associated with altered autonomic activity, which has been implicated in the development of cardiac dysfunction and structural damage. This study aimed to investigate the involvement of the autonomic nervous system in seizure-induced cardiomyopathy. Male Sprague-Dawley rats (320–350 g) were implanted with EEG/ECG electrodes to allow simultaneous telemetric recordings during seizures induced by intrahippocampal (2 nmol, 1 µl/min) kainic acid and monitored for 7 days. Seizure activity occurred in conjunction with increased heart rate (20%), blood pressure (25%), and QTc prolongation (15%). This increased sympathetic activity was confirmed by the presence of raised plasma noradrenaline levels at 3 h post-seizure induction. By 48 h post-seizure induction, sympathovagal balance was shifted in favor of sympathetic dominance, as indicated by both heart rate variability (LF/HF ratio of 3.5 ± 1.0) and pharmacological autonomic blockade. Functional cardiac deficits were evident at 7 and 28 days, as demonstrated by echocardiography showing a decreased ejection fraction (14% compared with control, P < 0.05) and dilated cardiomyopathy present at 28 days following seizure induction. Histological changes, including cardiomyocyte vacuolization, cardiac fibrosis, and inflammatory cell infiltration, were evident within 48 h of seizure induction and remained present for up to 28 days. These structural changes most probably contributed to an increased susceptibility to aconitine-induced arrhythmias. This study confirms that prolonged seizure activity results in acute and chronic alterations in cardiovascular control, leading to a deterioration in cardiac structure and function. This study further supports the need for modulation of sympathetic activity as a promising therapeutic approach in seizure-induced cardiomyopathy.

flushing (27). These changes can lead to seizure-induced cardiovascular dysfunction, pulmonary edema, and postictal depression of autonomic respiratory reflexes (12). Within the different seizure classifications, status epilepticus has the highest mortality risk, with death generally occurring within 30 days of the initial convulsant activity (39). Death often occurs in the absence of seizures and is believed to be due to an imbalance in autonomic function, resulting in altered cardiac control, electrical instability, and increased risk of lethal cardiac arrhythmias (46, 51).

Tachycardia [heart rate (HR) >100 beats/min] is reported in 33–100% of seizures and has the potential to cause long-term myocardial damage and fatal arrhythmias (27, 52, 68). Ventricular tachyarrhythmias account for 80% of sudden cardiac deaths and are generally caused by increased sympathetic tone and structural damage (55). Electrocardiogram (ECG) abnormalities are common in refractory and temporal lobe epilepsy (TLE), with up to 40–60% of patients developing at least one ictal rhythm or repolarization abnormality (12, 50, 52, 68). These changes include the development of atrial fibrillation, supraventricular tachycardia, ventricular premature depolarization, branch block, and first-degree atroventricular block (12, 50). Most changes are benign; however, potentially serious abnormalities (such as ST depression and T wave inversion) have been reported to occur in 10% of seizures (52). A prolonged QT interval is an established risk factor for life-threatening Torsade de Pointes tachycardia and ventricular arrhythmias (64). Even a transient increase in QT, as observed during epileptic discharge, can predispose a patient to ventricular fibrillation (69). Alterations in HR variability (HRV), a noninvasive index of autonomic activity, have been reported in individuals with epilepsy (16). HRV analysis has demonstrated both sympathetic (1, 10, 15) and parasympathetic (43, 54, 71) dominance in patients with TLE, whereas generalized tonic-clonic seizures are associated primarily with sympathetic dominance (15, 16, 22, 44).

In 7–17% of epileptics, the cause of death is sudden and has led to the diagnostic term sudden unexpected death in epilepsy (SUDEP) (52, 66–68). SUDEP is proposed to involve ictal changes in cardiorespiratory function, contributing to the development of tachy- and bradyarrhythmias as well as hyperventilation and pulmonary edema (72). Because SUDEP is the most common nonaccidental cause of death in epilepsy (67), understanding the underlying pathophysiology of these autonomic and cardiovascular changes is critical to the advancement of seizure research and treatment.

This study examined the effect of kainic acid (KA) administration on cardiac function in a nonanesthetized rat model of status epilepticus. In contrast to earlier studies (3, 4, 38, 46, 47, 62), this investigation used implantable transmitters in con-
scious (nonanesthetized) animals to simultaneously assess cardiac function and encephalographic activity during and following seizure activity. Using an adaptation of a previously validated protocol of excitotoxic seizure induction (73), intrahippocampal KA administration was employed to produce seizures while excluding any direct systemic effects of the excitotoxin. HRV analysis with pharmacological autonomic blockade was applied to this model to assess the involvement of the sympathetic system in the development of seizure-induced cardiomyopathy, and an aconitine stress test provided evidence of seizure-induced ventricular electrical remodeling. This study offers new insight into the development of dilated cardiomyopathy during and following seizure. We demonstrate that severe seizure provokes an immediate elevation in plasma noradrenaline levels, coupled with the formation of early and sustained myocardial fibrotic lesions and the development of dilated cardiomyopathy 28 days following the seizure insult.

METHODS

Materials. All reagents were purchased from BDH (Palmerston North, New Zealand) and Sigma-Aldrich (Auckland, New Zealand). Prescription remedies were obtained from the University of Otago's Drug Control Officer at the University of Otago Animal Welfare Office (Dunedin, New Zealand). KA was purchased from Tocris (Bristol, UK) and dissolved in sterile saline (0.9% NaCl).

Animals. Sprague-Dawley rats (54 males, 320–350 g) were obtained from the University of Otago Animal Resource Unit. The animals were housed on a 12:12-h light-dark cycle at 22°C with access to food and water ad libitum and left to acclimatize for 5 days prior to surgery. Rats were accustomed to handling prior to the start of early and sustained myocardial fibrotic lesions and the development of dilated cardiomyopathy 28 days following the seizure insult.

Experimental protocol. All animals had an intrahippocampal cannula for drug infusion implanted into the right hippocampus to allow for direct administration of KA while excluding significant systemic diffusion (73). Combined electroencephalograph (EEG) and ECG recordings were obtained in a subset of animals (n = 42), using implantable radiotelemeters (Telemetry Research, Auckland, New Zealand) in conscious animals to avoid any confounding effects of anesthetics on cardiovascular responses (76). At 7 days following implantation of the telemetric devices, animals were randomized into control (saline) or KA-administered groups, as described in Fig. 1. Simultaneous EEG/ECG and behavioral activities were recorded for 3 h immediately following seizure induction, for 60 min at 24 and 48 h, and at 7 days post-KA. Autonomic balance was assessed at 48 h and 7 days using HRV analysis and pharmacological autonomic denervation. Topical lignocaine was applied to the tail of each rat to reduce stress when tail vein blood samples were taken over the course of the study.

Surgical implantation of telemetric transmitters. Animals were administered amphotoprim (0.2 ml, 60 mg/ml bid sc) and carprofen (5 mg/kg sc) prior to surgery and every 24 h postoperatively for 3 days. Anesthesia was elicited using ketamine (75 mg/kg sc), domitor (me-detomidine hydrochloride; 0.5 mg/kg sc), and atropine (0.05 mg/kg sc). Body temperature was maintained at 37°C throughout the surgery. Transmitter implantation and electrode positioning procedures for EEG and ECG monitoring were performed as described previously (57). All animals were also implanted with a 26-G intrahippocampal drug cannula (Coherent Scientific) secured into the right hippocampus (5.2 mm posterior of Bregma and 5 mm right of the midline at a depth of 5.2 mm), as described previously (73). Animals were housed individually postsurgery and left to recover for 7 days before seizure induction.

Seizure induction. Rat behaviors were observed in a custom-made mirrored Perspex chamber (1 × 0.5 × 0.5 m; Aburn Glass) in which each rat was allowed to acclimatize for 30 min prior to study initiation. EEG and ECG were sampled at 2,000 Hz, with receiver filters set to 0.1 Hz high pass and 1,000 Hz low pass using a Powerlab 2/25 signal conditioner and LabChart version 6 Pro software (ADInstruments, Sydney, Australia). Seizures were induced by an intrahippocampal infusion of KA (2 nmol, 1 μl given over 1 min), using an automated Beehive syringe driver (Bioanalytical Systems, West Lafayette, IN) and glass Hamilton syringe (Hamilton, Reno, NV). Control animals were administered an equivalent volume of sterile saline. Following treatment, rats were immediately returned to the chamber for behavioral observation and telemetric recordings. Behavioral activity was assessed by a blinded observer and recorded every 15 s for 3 h, with discrete changes in behavioral state additionally

[Diagram showing experimental protocol]

Fig. 1. Distribution of rats into the various treatment groups, experimental protocols, and time course studies. Echo, echocardiography; KA, kainic acid; VSE, vagal-sympathetic effect.
reported as they occurred. Behaviors were recorded by trained observers blinded to the treatment, using a 5-point scale as described previously (57). Behavior levels were defined as normal behaviors (level 0), discomfort behaviors (level 1), seizure behaviors confined to the head (level 2), seizure behaviors associated with limbs or trunk, such as wet dog shakes (level 3), generalized seizure behaviors (level 4), and clonic-tonic convulsions (level 5). The cumulative behavioral score was determined as the sum of the maximum score every minute over the 180-min recording period. The total number of wet dog shakes and level 4 behaviors were quantified over the 180-min recording period.

ECG and blood pressure analysis. ECG data were analyzed using the LabChart version 6 Pro ECG Analysis software module to assess HR and QT intervals. The end of the T wave was determined when the wave returned to the isoelectric line. Data were analyzed every 2 min in 1-min blocks over the 3-h observation period. The corrected QT (QTc) interval was calculated to adjust for the rate by applying the Mitchell algorithm to the QT interval recordings, where QTc = QT(RR/100)^0.5 (48). This algorithm was designed to correct for the higher HR and altered QRS-T wave morphology in rodents. HRV was analyzed every 30 min over a 5-min period using the LabChart version 6 Pro HRV Analysis module software. Frequency domain analysis was used to separate the RR intervals into a low-frequency (LF; 0.04–0.5 Hz) band reflective of both sympathetic and parasympathetic modulation and a high-frequency (HF; 0.5–3 Hz) band controlled almost exclusively by parasympathetic and respiratory effects (6). These frequency bands were determined by baseline spectral analysis conducted in all rats prior to seizure induction. Autonomic balance was determined from the ratio of LF/HF. In a subset of animals (n = 6/group), arterial systemic blood pressure (BP) was recorded prior to seizure induction and periodically post-KA using a noninvasive tail pressure cuff monitor (ADInstruments, Dunedin, New Zealand). Four repeat BP recordings were recorded in the same animal using LabChart version 6, and mean systemic BP was determined.

Vagal-sympathetic effect. Vagal-sympathetic effect (VSE) was calculated from the ratio of intrinsic HR (iHR) to baseline HR, as described previously (40, 47). iHR is defined as HR in the absence of neural influences and represents the basal activity of the cardiac pacemaker (40). iHR was determined by the presence of the blocker atenolol (5 mg/kg sc) and the muscarinic receptor antagonist ipratropium (5 mg/kg sc) (9). Preseizure measurements were performed 2–3 days prior to seizure induction. Baseline HR was recorded during the 30 min preceding either atenolol or ipratropium administration. HR was recorded for 30 min in the presence of the first antagonist, at which time the second antagonist was administered, and recordings continued for a further 30 min. Animals were randomly assigned to treatment groups to ensure that one half received ipratropium first and the other half received atenolol first. The experiment was repeated at 48 h and 7 days following KA or saline administration. For the MSB-stained sections, the number of positive (blue) or negative (white) pixels was quantified across all three layers using ImageScope software (Aperio Technologies) to quantify the extent of fibrosis. ImageScope nuclear analysis module version 9 software was used to determine the number of ApopTag- and CD68-positive cells, and these values were expressed as a ratio of the total stained tissue area (cells/mm²).

Statistics. Statistical analysis was performed using Prism version 6 (GraphPad, San Diego, CA). Behavioral data were analyzed using a Kruskal-Wallis test with Bonferroni post hoc analysis. EEG, ECG, BP, and HRV variables were analyzed using a two-way repeated-measures ANOVA with Bonferroni post hoc analysis. VSE, echocardiography, and noradrenaline levels were analyzed with a one-way repeated-measures ANOVA compared with baseline using Dunnett’s post hoc analysis. Troponin I levels were analyzed using a paired t-test. Statistical significance was determined as P < 0.05. Data are presented as means ± SE.

RESULTS

Seizure activity and ECG analysis. Intrahippocampal infusion of saline vehicle produced no effect on rat behaviors or EEG activity. KA administration resulted in the immediate development of seizure activity, which remained significantly

Noradrenaline and troponin I levels. Plasma was extracted from tail vein blood samples taken at specified time points and frozen (−80°C) until analysis. Plasma noradrenaline levels (n = 6/group) were determined using a rat noradrenaline enzyme-linked immunosorbent assay (ELISA; BA E-5200; Labor Diagnostika Nord) and quantified according to the manufacturer’s instructions. Troponin I levels were determined (n = 6/group) at 24 h using a high-sensitivity rat cardiac troponin I ELISA kit (2012-06SP, Life Diagnostics).

Echocardiography. Seven days following KA or saline administration, rats (n = 6/group) were sedated with domitor. Transthoracic echocardiography was performed to determine left ventricular dimensional and functional parameters across the parasternal short-axis view using the Vivid E9 ultrasound system (GE Healthcare). Left ventricular end-systolic and end-diastolic diameters were measured at the level of the papillary muscles using two-dimensional guided M-mode imaging. Repeat (>20) measurements of left ventricular internal dimension during diastole (LVIDd) and systole (LVIDs), posterior wall thickness during diastole (LVPWd) and systole (LVPWs), ejection fraction (EF = stroke volume/end diastolic volume), and fractional shortening (FS = [LVIDd − LVIDs]/LVIDd) (62).

Histology and immunohistochemistry of myocardial injury. Following the termination of the HRV study (48 h and 7 days), rats were anesthetized with halothane, and the heart was excised (n = 6/group). Hearts were arrested in diastole by flushing with 20 ml of 0.9% saline (4°C) containing 20 mM of KCl. The tissue was perfusion-fixed (73.6 mmHg of pressure) and maintained in 10% neutral buffered formalin (24 h). Transverse apical ventricular tissue blocks (3, 6, and 9 mm depth from the apex) were paraffin-embedded and 5-µm-thick tissue sections prepared for staining with Martius scarlet blue (MSB) to detect the presence of free DNA 3′-OH termini. Sections were then dehydrated with ascending concentrations of ethanol and cover-slipped using Di-N-butylphthalate (DPX in xylene) mountant. Cardiac macrophage infiltration (CD68), DNA strand breaks were assessed enzymatically using an ApopTag Peroxidase kit (Millpore) with Gill’s hematoxylin counterstain to detect the presence of free DNA 3′-OH termini. Sections were then dehydrated with ascending concentrations of ethanol and cover-slipped using Di-N-butylphthalate (DPX in xylene) mountant. Cardiac macrophage infiltration (CD68) was assessed in antigen-retrieved sections using sodium citrate buffer (10 mM, pH 6, 20 min at 95°C). Sections were incubated with mouse anti-CD68 monoclonal IgG antibody (1:100, ab31630; Abcam) and horseradish peroxidase-labeled goat anti-mouse polyclonal IgG antibody (1:250, 90 min; Pierce). Antibody binding was visualized with 3,3′-diaminobenzidine (DAB) and counterstained with hematoxylin. Examination of the above stained sections was conducted using an Aperio Scanscope CS2 image digital scanning system (Aperio Technologies). For the MSB-stained sections, the number of positive (blue) or negative (white) pixels was quantified across all three layers using ImageScope software (Aperio Technologies) to quantify the extent of fibrosis. ImageScope nuclear analysis module version 9 software was used to determine the number of ApopTag- and CD68-positive cells, and these values were expressed as a ratio of the total stained tissue area (cells/mm²).

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elevated (mean seizure behavior of 2.9 ± 0.1 compared with 0.1 ± 0.03 in the controls, \( P < 0.05 \); Fig. 2A) over the initial 180-min period, and animals were still displaying seizure behaviors at the 24-h time point (0.9 ± 0.14, \( P < 0.05 \); Fig. 2B). KA-dosed animals had an elevated cumulative behavioral score of 530 ± 19, with 326 ± 64 wet dog shakes recorded during the 180-min period (compared with 27 ± 4 and 6 ± 2 in the controls, respectively, \( P < 0.05 \)). Seizure animals displayed frequent level 4 behaviors (138 ± 36) over the course of the 180-min study (\( P < 0.05 \) compared with control). These seizure behaviors were associated with significant increases in high-amplitude EEG spiking, confirming the occurrence of neuronal seizure activity following KA administration. Control animals receiving intrahippocampal saline showed no significant change in HR, QTc, or systolic BP from baseline levels (mean of 354 ± 10 beats/min, 43 ± 3 ms, and 98 ± 3 mmHg, respectively). KA infusion produced a sustained 20% elevation in mean HR that persisted during the 180-min study (\( P < 0.05 \) compared with baseline; Fig. 2B). This increase was associated with prolongation of the QTc interval by 10–15% over 76–178 min (Fig. 2C).

**Autonomic modulation of cardiovascular function.** The increased HR activity occurring with seizure coincided with a significant and sustained increase in BP (26 ± 5%) at 1 h post-KA that was maintained until the 48-h time point (Fig. 3, A and B). Seizures were also associated with a 9.6-fold increase in plasma troponin I levels at 24 h (296 pg/ml compared with 31 pg/ml preseizure, \( P < 0.05 \)). There were no significant changes in the standard deviation of normal to normal RR intervals following saline or KA administration (data not shown). LF was significantly increased 24 h to 7 days following seizure induction, which was associated with a reduction in HF power (Fig. 3, C and D). There was a shift in the LF/HF ratio (by 1.5- to 2.1-fold) in favor of sympathetic dominance at 1 h and at 24 h to 7 days (Fig. 3E). Ipratropium administration in the preseizure recording period produced an increase in HR by 103 ± 8 beats/min above baseline, whereas atenolol decreased HR by 40 ± 8 beats/min (Fig. 4, A–C). In contrast, the response to ipratropium at 48 h postseizure was significantly attenuated compared with the pretreatment level (54 ± 15 beats/min above baseline). This was also associated with an increased sensitivity to atenolol, seen as a \( \beta \)-blocker-induced drop in HR of 79 ± 9 beats/min. During the preseizure recording period, combined blockade with ipratropium/atenolol revealed an intrinsic HR of 362 ± 3 beats/min (pretreatment HR of 348 ± 9 beats/min; Fig. 4D) and a VSE of...
1.04 ± 0.02. These values were significantly reduced at the 48-h and 7-day post-seizure recordings period, where the VSE dropped to 0.87 ± 0.02 and 0.89 ± 0.02, respectively (Fig. 4E). Prior to seizure induction, baseline plasma noradrenaline levels were at 0.28 ± 0.12 ng/ml (Fig. 5). KA administration resulted in a significant 8.9-fold (1.2 ± 0.2 ng/ml) increase in plasma noradrenaline levels at 3 h following seizure induction, peaking at 48 h (2.5 ± 1.0 ng/ml, *P < 0.05) and returning to baseline levels by 7 days.

**Arrhythmia risk.** In control rats, aconitine administration resulted in the development of arrhythmias within 40 min, with PVC, bigeminy, and eventually ventricular fibrillation observed at 32 ± 4.9, 34 ± 3.2, and 46 ± 4.3 min, respectively (Fig. 6). Animals tested at 7 days post-seizure induction showed an increased susceptibility to benign arrhythmias, with a reduced latency to PVC and bigeminy by 55 and 29% compared with controls (*P < 0.05). Seizure rats also had a reduced latency to potentially fatal arrhythmias, such as ventricular tachycardia and ventricular fibrillation, compared with control animals (*P < 0.05).

**Echocardiogram.** Left ventricular function was assessed via echocardiogram in sedated rats (Table 1). Seizure activity produced no significant change in LVID at 7 days (Table 1). However, there was a significant decrease in LVPW thickness during systole by 21% compared with the control animals. In the seizure hearts, there was a significant reduction in ejection fraction by 14%, which was associated with a drop in fractional shortening by 19% at 7 days. By 28 days following seizure induction, the hearts showed evidence of dilated cardiomyopathy, as demonstrated by a significant increase in LVID (10 and 67%) and a reduction in posterior wall thickness (27 and 22%) during diastole and systole, respectively, which were associated with decreased ejection fraction (15%) and fractional shortening (23%) (Table 1).

**Histology and immunohistochemistry.** MSB staining (Fig. 7) of the hearts at 48 h post-KA showed significant evidence of increased collagen deposition (4.8% of the entire left ventricular area; Figs. 7B and 8A) that was retained at 7 and 28 days. Hearts also showed evidence of reversible ischemic damage at 48 h and 7 days, as demonstrated by myocyte vacuolization (Fig. 7B). Inflammatory cell infiltration after seizure was confirmed by increased immunohistological staining of CD68-positive macrophage cells (escalated by 3.4- to 5.2-fold; Figs. 7D and 8B) within the interstitial and perivascular tissues. Seizure activity was also associated with a 15-fold increase in ApopTag-positive cell labeling at 48 h following seizure insult.
This elevated degree of labeling was significantly reduced by 7 and 28 days (Figs. 7F and 8C).

DISCUSSION

This study clearly demonstrates that sustained seizure activity results in myocardial fibrotic lesions and deterioration of cardiac function due to elevated sympathetic modulation and adds new insight into the development of seizure-induced cardiomyopathy.

Previous work by our group has already established that intrahippocampal deliver routes using 1-μL volumes of excitotoxin do not result in detectable systemic levels in plasma or ventricular tissues (73). Intrahippocampal KA delivery in the current study produced recurrent seizure activity lasting for several hours, similar to previous studies (56). This seizure activity was associated with an early activation of the sympathetic system, as demonstrated by increased plasma noradrenaline levels, tachycardia, and elevated BP within 3 h. The sympathovagal balance changed in favor of sympathetic dominance at 48 h and 7 days following seizure induction. Furthermore, seizure activity resulted in the development of cardiac microlesions and fibrotic deposition, which were associated with a concurrent increased susceptibility to aconitine-induced arrhythmias.

In the current study, KA administration caused a progressive increase in EEG activity and seizure behaviors such as myoclonic jerks and foaming at the mouth. This high-level seizure activity was found to coincide with the development of detrimental cardiac alterations, including sustained tachycardia and QTc prolongation. This finding is supported by previous animal studies where generalized seizure activity is associated with cardiac dysfunction (46, 47, 70). In piglets, pentylenetetrazole-induced seizures coincided with alterations in HR, hypertension, increased ventilation, and decreased cardiac output (70). Systemic KA administration in rats has also been associated with increased HR and QTc prolongation as well as elevated sympathetic nerve activity and decreased vagal tone (24, 25, 57). In addition, Metcalf et al. (47) also looked at VSE in rats following status epilepticus and found that the VSE ratio (iHR/HR) dropped from 0.98 to 0.87 at 7 days, suggesting sympathetic dominance. A concern with this result was that the VSE was assessed following determination of baroreflex responsiveness in the presence of phenylephrine and nitroprusside. Administration of phenylephrine, an α1 agonist, will produce hypertension and subsequent reflex bradycardia, which may produce an altered VSE. Damasceno et al. (9) also recently reported a similar VSE following audiogenic seizures, although disappointingly, no timeline was provided. In clinical studies, altered autonomic activity and HRV have been reported commonly in epilepsy patients (1, 63, 71). Clinical data

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<th>Table 1. Echocardiographic analysis of left ventricular dimensions and function at 7 and 28 days following seizure induction (intrahippocampal kainic acid) versus control (intrahippocampal saline)</th>
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Values are means ± SE. LV internal dimension during diastole (LVIDd) and systole (LVIDs), LV posterior wall thickness during diastole (LVPWd) and systole (LVPWs), ejection fraction (EF), and fractional shortening (FS) were measured. *P < 0.05 compared with the respective time-matched control.
report a shift in HRV toward sympathetic dominance in patients with generalized tonic-clonic seizures (16), thereby supporting the clinical relevance of the data generated in the current study.

Heart rate variability analysis has been advocated in the literature as a noninvasive measure of autonomic function (15, 16, 22, 44) and was used to provide multiple temporal measures of cardiac autonomic function over the duration of the seizure response. Importantly, to the best of our knowledge, this is the first time HRV has been used to assess cardiac control in a rat model of seizure. It is critical that when using HRV, particularly in a conscious animal model, the optimal frequency bands are determined prior to disease induction, as HRV indices can vary depending on a number of factors, including species, age, and circadian rhythm. The current study demonstrated that intrahippocampal administration of KA produced increased sympathetic activity at 48 h and 7 days following seizure induction. Significantly, this finding was supported by the use of pharmacological autonomic blockade, where seizure activity produced a drop in parasympathetic responsiveness to ipratropium and an increased responsiveness to atenolol at 48 h. By effectively blocking cardiac innervation, this pharmacological inhibition protocol allowed for an estimation of intrinsic HR. A reduction in the intrinsic HR is associated with aging, myocardial ischemic damage, and diabetes (41). In this seizure model, intrinsic HR decreased at the 48-h and 7-day recordings, suggesting the possibility of injury to nodal pacemaker tissue. The maintenance of a normal basal HR at 48 h and 7 days after seizure induction in animals with reduced intrinsic HRs suggests that there is an adaptive shift in autonomic control in favor of sympathetic dominance. The use of these protocols in the present study demonstrates seizure-induced changes in cardiac autonomic function. Importantly, the study also showed that plasma noradrenaline levels were elevated during seizure, consistent with prior clinical evidence showing an association between seizure and increased catecholamine levels (5, 74). These results, combined with prior literature, confirm an increased sympathetic dominance during generalized seizure activity that may contribute to sudden cardiac death in epileptics.

Fig. 7. Representative micrographs of the left ventricular myocardium at 7 days following saline vehicle (control) or KA (seizure) intrahippocampal administration. A, C, and E: normal collagen distribution and tissue morphology, with minimal macrophage infiltration and apoptosis, respectively, in control rat myocardium. Myocardium from seizure animals showed diffuse trichrome blue-stained collagen deposition and myocyte vacuolization (*; B), DAB-stained CD68-positive macrophage infiltration (D), and 3,3′-diaminobenzidine (DAB) stained ApopTag-positive cells (F). MSB, Martius scarlet blue.
ity to the hippocampus mediates increases in HR and BP, whereas the rhombencephalon (hindbrain) regions are associated with parasympathetic activity (Fig. 9) (2, 7, 8, 11, 14, 19, 20, 26, 28, 29, 33, 34, 36, 42, 45, 58–61, 65, 77). The location of these key autonomic centers may also explain why systemic subcutaneous KA delivery in our previous publications (57) produced bradycardia, which was not seen following intrahippocampal KA infusion in the current study. Previous work in our laboratory has also confirmed that administration of 50 µM KA (data not published) or the KA analog domoic acid (73) directly to an isolated perfused heart produced no effect on HR or left ventricular hemodynamics. Therefore, systemic KA delivery may be assumed to affect cardiac function through an indirect mechanism or mechanisms.

No extensive echocardiographic investigations examining the effect of seizure on cardiac function have been published. A 2008 study by Sakamoto et al. (62) indicated that seizure induction by KA administration in a rat decreased ejection fraction and increased left ventricular diastolic diameter; however, the study did not deliver any information on incidence, extent, or time course of left ventricular dysfunction. Elevated sympathetic stimulation and catecholamine levels have been reported to produce left ventricular dilation, elevated end-diastolic pressure, decreased wall thickness, and reduced ejection fraction (30). Stress-induced cardiomyopathy (also called Takotsubo syndrome) occurs from a stressful event (emotional or physical) leading to enhanced sympathetic stimulation, thereby resulting in reversible apical ventricular ballooning and reduced ejection fraction (17). Stress-induced cardiomyopathy has been linked to epilepsy, with 1.0–3.2% of patients presenting with seizures at the time of hospital admission (37). Previously, Dib et al. (13) reported that stress-induced cardiomyopathy was associated with reduced ejection fraction in patients at the time of admission, although hemodynamic...
recovery was seen within 5–7 days. The pathology observed in the current study has some similarities with the acute phase of stress-induced cardiomyopathy, such as increased HR and BP, as well as elevated noradrenaline levels. Pertinently dilated cardiomyopathy with reduced contractility and ejection fraction could also occur in this situation as a consequence of sustained tachycardia, where left ventricular dimensions are increased in association with wall thinning and fibrosis (35). Clinically, this study has implications in epilepsy, as recurrent seizure activity may produce a progressive deterioration in cardiac function and contribute to the high mortality rate in epilepsy patients.

The cardiac pathology in the present study demonstrates features similar to those described in both animal and clinical studies of seizures (31, 46, 49). Structural myocardial changes have been reported in 33% of epileptic patients, with evidence of fibrosis, reversible myocyte vacuolization, leukocytic infiltration, and edema observed postmortem (31, 49). Structural cardiac damage following seizures may arise through tachycardia-induced ischemic damage, which is potentiated by direct catecholamine-induced cardiotoxicity, leading to cardiomyocyte apoptosis, necrosis, and fibrosis (18). Administration of adrenergic agonists in rats has been shown previously to induce myocardial inflammatory cell infiltration, apoptosis, and fibrosis (18). In addition to contributing to contractile dysfunction, fibrosis also impedes myocardial electrical conductivity (32). Increased susceptibility to aconitine-induced arrhythmias has previously been demonstrated in pilocarpine-induced status epilepticus in rats (46), with ventricular tachycardia occurring 40% faster than in control animals. However, this pilocarpine model of seizure has strong limitations when assessing cardiac function, as pilocarpine is coadministered with the nonselective muscarinic blocker methyl-scopolamine to reduce cholinergic peripheral side effects. Scopolamine may consequently amplify the cardiac dysfunction through prolonged elevation of HR.

The present study demonstrated that cardiac damage occurs during the early stages of seizure, with troponin I levels elevated at 24 h. Collagen deposition and inflammatory cell infiltration were observed as early as 48 h, with no reversal at 7 days. The current study showed that an increase in myocardial fibroplasia in seizure animals occurred in parallel with an increased susceptibility to aconitine-induced arrhythmias. The role of fibrosis in cardioelectrical remodeling and the increased cardiac sympathetic tone in seizure animals further supports the potential significance of cardiac arrhythmias in sudden cardiac death in epilepsy. This new study directly evaluating cardiac structure and arrhythmia susceptibility in the rodent model provides critical insight into the proposed mechanisms of seizure-induced cardiomyopathy.

Previous work in our laboratory using subcutaneous KA demonstrated that seizures resulted in alterations in HR and structural damage at 48 h, which were attenuated by clonidine administration. The current improved model utilizing intrahippocampal excitotoxin administration confirms that the cardiac damage observed following KA is a consequence of seizure rather than a direct excitotoxic effect of KA on the myocardium. This study was also extended to 7 and 28 days and clearly demonstrated a deterioration in cardiac function associated with chronic activation of the sympathetic system. This is the first study to conclusively show that seizure activity results in a decrease in left ventricular function, as demonstrated by a reduction in ejection fraction and fractional shortening. These detrimental effects are most likely a consequence of increased ventricular fibrotic deposition resulting in an increased risk of arrhythmias.

To conclude, this study confirms that seizures can result in cardiac dysfunction and damage subsequent to sympathetic hyperactivation. Sustained tachycardia can increase cardiac oxygen demand while reducing coronary blood supply during diastole (23). This oxygenation imbalance combined with catecholamine-induced toxicity and coronary vasospasms can result in microlesions and arrhythmogenic development as well as the deterioration in left ventricular function seen in this study. The results obtained in this study clearly show the importance of protecting the heart against sympathetic overdrive during the early stages of seizure and further support the need for cardioprotectants as adjuncts to antiepileptic therapies.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


