Progressive development of cardiomyopathy following altered autonomic activity in status epilepticus

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Abstract

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 (320-350g) 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 hours post-seizure induction. By 48 hours 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 hours of seizure induction, and remained present 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.

New & Noteworthy

This study provides new insight into the significance of cardiomyopathy development following seizure. We clearly demonstrate that severe seizure provokes an immediate elevation in plasma noradrenaline, coupled with the formation of early and sustained myocardial fibrotic lesions and the development of dilated cardiomyopathy at 28 days following the seizure insult.
Introduction

Epilepsy has a prevalence of 1.4-3.3% and is associated with an increased mortality rate of two to three times that of the general population (75). Epileptiform activity has been increasingly associated with changes in autonomic function, particularly symptoms of sympathetic activation such as tachycardia, tachypnea, hypertension, pupil dilation, diaphoresis and facial 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 b.p.m.) 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 AV 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 heart rate variability (HRV), a non-invasive 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 while generalized tonic-clonic seizures are primarily associated with sympathetic dominance (15; 16; 22; 44).

In 7 to 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
brady-arrhythmias, as well as hypoventilation and pulmonary edema (72). As SUDEP is the most common non-accidental 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 non-anaesthetized rat model of status epilepticus. In contrast to earlier studies (3; 4; 38; 46; 47; 62), this investigation used implantable transmitters in conscious (non-anesthetized) 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 significance of cardiomyopathy development 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; 320-350g) were obtained from the University of Otago Animal Resource Unit. The animals were housed on a 12 hour 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 the study. Experiments were performed in accordance with the regulations of the University of Otago’s Committee on Ethics in the Care and Use of Laboratory Animals and the “Use of Laboratory Animals (NIH Publication No. 85-23, 1996)”.

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 Figure 1. Simultaneous EEG/ECG and behavioral activity were recorded for 3 hours immediately following seizure-induction and for 60 min at 24 and 48 hours, and at 7 days post-KA. Autonomic balance was assessed at 48 hours 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 amphoprim (0.2 ml, 60 mg/ml, b.i.d., sc) and carprofen (5 mg/kg, sc) prior to surgery and every 24 hours post-operatively for 3 days. Anesthesia was elicited using ketamine (75 mg/kg, sc), domitor (medetomidine 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 previously described (57). All animals were also implanted with a 26G intrahippocampal drug cannula (Coherent Scientific, Australia) 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 previously described (73). Animals were housed individually post-surgery and left to recover for 7 days before seizure induction.

**Seizure Induction.**

Rat behaviors were observed in a custom-made mirrored Perspex chamber (1 m x 0.5 m x 0.5 m; Aburn Glass, New Zealand) in which each rat was allowed to acclimatize for 30 min prior to study initiation. EEG and ECG were sampled at 2000 Hz, with receiver filters set to 0.1 Hz high pass and 1000 Hz low pass using a Powerlab 2/25 signal conditioner and LabChart v.6 Pro software (ADInstruments, Sydney, Australia). Seizures were induced by an intrahippocampal infusion of KA (2 nmol, 1 μl given over 1 minute), using an automated Beehive syringe driver (Bioanalytical Systems, West Lafayette, Indiana, USA) and glass Hamilton syringe (Hamilton, Reno, USA). 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 seconds for 3 hours with discrete changes in behavioral state additionally reported as they occurred. Behaviors were recorded by trained observers blinded to the treatment, using a 5-point scale as previously described (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 minute 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 was analyzed using the LabChart v.6 Pro ECG Analysis software module in order 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 two minutes in one minute blocks over the 3 hour observation period. The corrected QT (QTc) interval was calculated in order to adjust for rate by applying the Mitchell algorithm to the QT interval recordings, where

\[
\text{QTc} = \frac{\text{QT}}{\sqrt[2]{\text{RR}/100}}
\]

This algorithm is designed to correct for the higher HR and altered QRS-T wave morphology in rodents. HRV was analyzed every 30 minutes over a 5 minute period using the LabChart v.6 Pro HRV Analysis module software. Frequency domain analysis was used to separate the RR intervals respectively 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 \((n=6/\text{group})\), arterial systolic blood pressure (BP) was recorded prior to seizure induction and periodically post-KA, using a non-invasive tail pressure cuff monitor (ADInstruments, Dunedin, New Zealand). Four repeat BP recordings were recorded in the same animal using LabChart v.6 and mean systolic BP determined.

**Vagal-Sympathetic Effect.**

Vagal-sympathetic effect (VSE) was calculated from the ratio of intrinsic HR (iHR) to baseline HR as previously described (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 in the presence of the beta-blocker atenolol (5 mg/kg, sc) and the muscarinic receptor antagonist ipratropium (5 mg/kg, sc) (9). Pre-seizure measurements were performed 2-3 days prior to seizure induction. Baseline HR was recorded in the 30 minutes preceding either atenolol or ipratropium administration. HR was recorded for 30 minutes in the presence of the first antagonist, at which time the second antagonist was administered, and recordings continued for a further 30 minutes. Animals were randomly assigned to treatment groups to ensure that half received ipratropium first and half received atenolol first. The experiment was repeated at 48 hours or at 7 days following seizure induction \((n=6/\text{time})\).
point). Reported baseline HR represents the mean HR over the 30 minute recording period prior to drug treatment. The iHR was taken as the mean HR over 30 minutes following combined antagonist treatment. VSE (iHR/baseline HR) values greater than 1, were used to indicate an increase in parasympathetic dominance. Conversely, VSE values less than 1 have been used as an indicator of an elevated sympathetic control.

**Arrhythmia Risk.**

Following the 7 day behavioral study, rats \((n=6/\text{group})\) were administered a bolus injection of the pro-arrhythmogenic agent aconitine \((0.5 \text{ mg/kg, sc})\) in order to assess vulnerability to arrhythmias \((46)\). The latency from aconitine administration to presentation of ECG arrhythmias were recorded in each animal. These included first premature ventricular contraction \((\text{PVC}; \text{ premature QRS with no P wave}), \text{bigeminy (two or more PVC), ventricular tachycardia (a series of four PVCs for each P wave and corresponding QRS complex) and ventricular fibrillation (no discernible rhythm)}\) \((21)\). Rats were sacrificed at presentation of ventricular fibrillation as required by the University of Otago Animal Ethics Committee.

**Noradrenaline and Troponin I Levels.**

Plasma was extracted from tail vein blood samples taken at specified time points and frozen \((-80^\circ \text{C})\) until analysis. Plasma noradrenaline levels \((n=6/\text{group})\) were determined using a rat noradrenaline enzyme-linked immunosorbent assay \((\text{ELISA; Labor Diagnostika Nord, Germany, BA E-5200})\) and quantified according to the manufacturer’s instructions. Troponin I levels were determined \((n=6/\text{group})\) at 24 hours using a high sensitivity rat cardiac troponin I ELISA kit \((\text{Life Diagnostics, USA, 2010-2-HSP})\).

**Echocardiography.**

Seven days following KA or saline administration rats \((n=6/\text{group})\) 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 \((\text{GE Healthcare})\). 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×) measures of left ventricular dimensions were performed and the mean values calculated for 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 hours and 7 days), rats were anaesthetized with halothane and the heart 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 pressure) and maintained in 10% neutral buffered formalin (24 hours). Transverse apical ventricular tissue blocks (3, 6 and 9 mm depth from apex) were paraffin-embedded and 5 μm thick tissue sections prepared for staining with Martius scarlet blue (MSB) for assessment of fibrosis, apoptosis (ApopTag) and macrophage infiltration (CD68). DNA strand breaks were assessed enzymatically using an ApopTag® Peroxidase kit (Millipore, Germany) 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 minutes at 95°C). Sections were incubated with mouse anti-CD68 monoclonal IgG antibody (1:100; ab31630, Abcam, UK) and HRP-labelled goat anti-mouse polyclonal IgG antibody (1:250; 90 minutes; Pierce, USA). 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, Vista, USA). For the MSB-stained sections, the number of positive (blue) or negative (white) pixels were quantified across all three layers using ImageScope software (Aperio Technologies, Vista, USA), to quantify the extent of fibrosis. ImageScope Nuclear analysis module v.9 software was used to determine the number of ApopTag®-positive 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™ v.6 (GraphPad, San Diego, USA). Behavioral data were analyzed using a Kruskal-Wallis test with Bonferroni *post-hoc*. EEG, ECG, BP and HRV variables were analyzed using a 2-way repeated measures ANOVA with Bonferroni *post-hoc* analysis. Vagal sympathetic effect, echocardiography and noradrenaline levels were analyzed with a 1-way repeated measures ANOVA comparing to baseline using Dunnet’s *post-hoc* analysis. Troponin I levels were analysed using a paired t-test. Statistical significance was determined as $P<0.05$. Data presented as mean ± SEM.
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 elevated (mean seizure behavior of 2.9 ± 0.1 compared to 0.1 ± 0.03 in the controls, \( P<0.05 \); Figure 2A) over the initial 180 minute period, and animals were still displaying seizure behaviors at the 24 hour time point (0.9 ± 0.14; \( P<0.05 \)). KA dosed animals had an elevated cumulative behavioral score of 530 ± 19, with 326 ± 64 wet dog shakes recorded during the 180 minute period (compared to 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 minute study (\( P<0.05 \) compared to 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 b.p.m., 43 ± 3 ms and 98 ± 3 mmHg, respectively). KA infusion produced a sustained 20% elevation in mean HR which persisted during the 180 minute study (\( P<0.05 \) compared to baseline). This increase was associated with prolongation of the QTc interval by 10-15% over 76-178 minutes (Figure 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 hour post-KA which was maintained until the 48 hour time point (Figure 3A and 3B). Seizures were also associated with a 9.6-fold increase in plasma troponin I levels at 24 hours (296 pg/ml compared to 31 pg/ml pre-seizure; \( P<0.05 \)). There were no significant changes in the standard deviation of normal to normal RR intervals (SDNN) following saline or KA administration (data not shown). LF was significantly increased 24 hours to 7 days following seizure induction which was associated with a reduction in HF power (Figure 3C and D). There was a shift in the LF/HF ratio (by 1.5-2.1-fold) in favor of sympathetic dominance at 1 hour and at 24 hours to 7 days (Figure 3E). Ipratropium administration in the pre-seizure recording period produced an increase in HR by 103 ± 8 b.p.m. above baseline while atenolol decreased HR by 40 ± 8 b.p.m. (Figure 4A, B and C). In contrast, the response to ipratropium at 48 hours post-seizure was significantly
attenuated compared to the pre-treatment level (54 ± 15 b.p.m. above baseline). This was also associated with an increased sensitivity to atenolol, seen as a β-blocker induced drop in HR of 79 ± 9 b.p.m. During the pre-seizure recording period, combined blockade with ipratropium/atenolol revealed an intrinsic HR of 362 ± 3 b.p.m. (pre-treatment HR of 348 ± 9 b.p.m.; Figure 4D) and a VSE of 1.04 ± 0.02. These values were significantly reduced at the 48 hour and 7 day post-seizure recordings period, where the VSE dropped to 0.87 ± 0.02 and 0.89 ± 0.02, respectively (Figure 4E). Prior to seizure induction, baseline plasma noradrenaline levels were at 0.28 ± 0.12 ng/ml (Figure 5). KA administration resulted in a significant 8.9-fold (1.2 ± 0.2 ng/ml) increase in plasma noradrenaline levels at 3 hours following seizure induction, peaking at 48 hours (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 minutes, with PVC, bigeminy and eventually ventricular fibrillation observed at 32 ± 4.9, 34 ± 3.2 and 46 ± 4.3 minutes, respectively (Figure 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 to controls ($P<0.05$). Seizure rats also had a reduced latency to potentially fatal arrhythmias, such as ventricular tachycardia and ventricular fibrillation, compared to 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 to 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, respectively. 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 (Figure 7) of the hearts at 48 hours post-KA showed significant evidence of increased collagen deposition (4.8% of the entire left ventricular area; Figure 7B and Figure 8A) which was retained at 7 and 28 days. Hearts also showed evidence of reversible ischemic damage at 48 hours and 7 days as demonstrated by myocyte vacuolization (Figure 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, Figure 7D and Figure 8B) within the interstitial and perivascular tissues. Seizure activity was also associated with a 15-fold increase in ApopTag-positive cell labeling at 48 hours following seizure insult. This elevated degree of labeling was significantly reduced by 7 and 28 days (Figure 7F and Figure 8C).
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 delivery routes using 1 µl volumes of excitotoxin do not result in detectable systemic levels in plasma or ventricular tissues (73). Intrahippocampal KA delivery in this 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 hours. The sympathovagal balance changed in favor of sympathetic dominance at 48 hours and 7 days following seizure induction. Furthermore, seizure activity resulted in the development of cardiac micro-lesions 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. 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 (47). 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. also recently reported a similar VSE following audiogenic seizures, although disappointingly no timeline was provided (9). In clinical studies, altered autonomic activity and HRV have been commonly reported in epilepsy patients (1; 63; 71). Clinical data reports a shift in HRV
towards 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 non-invasive 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 hours 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 hours. 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 hour and 7 days recording, suggesting the possibility of injury to nodal pace maker tissue. The maintenance of a normal basal HR at 48 hours 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 which may contribute to sudden cardiac death in epileptics.

As discussed in our previous study (73), seizures originating in hippocampus as well as from the fronto-temporal cortex, insular cortex and amygdala can spread into key CNS cardiovascular control centers including the ventrolateral thalamus, paraventricular nucleus, medial parabrachial nuclei, locus coerules, ventrolateral pons, nucleus tractus solitarius and nucleus ambiguous and finally the dorsal motor nucleus of the vagus nerve. The immediate tachycardic and hypertensive response observed following intrahippocampal KA delivery in this study is therefore most likely a consequence of a glutamatergic activation of sympathetic
nuclei (Figure 9). Studies have demonstrated that activation of glutamatergic nuclei in close
proximity to the hippocampus mediates increases in HR and BP, while the rhombencephalon
(hindbrain) regions are associated with parasympathetic activity (Figure 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
centres 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 have also confirmed that
administration of 50 µM KA (data not published) or the KA analogue domoic acid (73)
directly to an isolated perfused heart produced no effect on HR or left ventricular
hemodynamics. Systemic KA delivery may therefore be assumed to affect cardiac function
through an indirect mechanism or mechanisms.

No extensive echocardiographic investigations have been published examining the effect of
seizure on cardiac function. A 2008 study by Sakamoto et al. 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 (62). 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). Dib and co-authors previously 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 (13). 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 may contribute to the high mortality rate in
epilepsy patients.
The cardiac pathology in the present study demonstrates similar features 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 post-mortem (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 previously been shown 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 co-administered with the non-selective 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 hours. Collagen deposition and inflammatory cell infiltration were observed as early as 48 hours with no reversal at 7 days. The current study showed that an increase in myocardial fibrosis 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 hours, which was attenuated by clonidine administration. The current improved model utilising intrahippocampal excitotoxin administration confirms that the cardiac damage observed following KA is a consequence of seizure rather 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 micro-lesions 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 supports the need for cardio-protectants as adjuncts to antiepileptic therapies.
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Conflict of Interest: None declared
References


Tables

Table 1. Echocardiographic analysis of left ventricular (LV) dimensions and function at 7 and 28 days following seizure induction (intrahippocampal KA) versus control (intrahippocampal saline).

Figure Legends

Figure 1. Distribution of rats into the various treatment groups, experimental protocols and time course studies. Echo: echocardiography, KA: kainic acid, VSE: vagal sympathetic effect study.

Figure 2. The effect of seizure induction versus control on: maximum behavioral score (A), heart rate (beats per minute, b.p.m.; B) and the QTc interval (C) in rats. Data are presented at 2 minute intervals over the initial 180 min post seizure induction. Data is represented as the mean ± SEM. *P<0.05 compared to control.

Figure 3. The effect of seizure induction versus control on: heart rate (A), systolic blood pressure (B) and heart rate variability (C-E). Heart rate variability expressed as low frequency (LF, 0.04-0.5 Hz, C) and high frequency (HF, 0.5-3 Hz, D) analysis expressed as normalized unit (nu) and low frequency/high frequency ratio (LF/HF; E). Data is represented by the mean ± SEM. *P<0.05 compared to control.

Figure 4. The effect of seizure induction versus control on autonomic function. A) Diagram showing the pharmacological inhibition protocol (adapted from 9; Damasceno et al., 2013) used to determine cardiac sympathetic and para-sympathetic tone. B) Change in heart rate (HR) during the 30 min following administration of either ipratropium (vagal effect) or (C) atenolol (sympathetic effect). D) Histogram showing intrinsic (drug denervated) HR. E) Graph demonstrates the vagal sympathetic effect determined as the ratio of baseline and intrinsic heart rate (VSE; iHR/HR ratio). Data is represented by the mean ± SEM. *P<0.05 compared to control. #P<0.05 compared to pre-seizure.

Figure 5. Elevation of rat plasma noradrenaline levels over 7 days following KA-induced seizure. Data is represented by the mean ± SEM. #P<0.05 compared to baseline.

Figure 6. The effect of seizure versus control on latency to typical aconitine-induced arrhythmias with representative ECG traces shown. Data is represented by the mean ± SEM. *P<0.05 compared to control.
Figure 7. Representative micrographs of the left ventricular myocardium at 7 days following saline vehicle (control) or KA (seizure) intrahippocampal administration. Figures A, C and E showing normal collagen distribution and tissue morphology, with minimal macrophage infiltration and apoptosis respectively in control rat myocardium. Myocardium from seizure animals showed (B) diffuse trichrome blue stained collagen deposition and myocyte vacuolisation (*), (D) DAB stained CD68 positive macrophage infiltration, and (F) DAB stained ApopTag positive cells.

Figure 8. Fibrosis (A), CD68 positive cells (B) and ApopTag cells (C) were quantified across three midplane left ventricular sections at 48 hours, 7 and 28 days following intrahippocampal saline or KA administration. Data is represented by the mean ± SEM. *P<0.05 compared to control, ΦP<0.05 compared to 48 hours.

Figure 9. Schematic illustration of a sagittal cross section of rat brain demonstrating the consequence of seizure induction following hippocampal glutamate agonist administration on sympathetic (red) and parasympathetic (green) effects in the various brain regions. Prefro. Cortex: Prefrontal cortex; CPu: Caudate putamen of the striatum, LSep: Lateral septum; PVN: Paraventricular nucleus; Amy: Amygdala; DMH: dorsal medial hypothalamus; PAG: Periaqueductal gray; PBN: Parabrachial nucleus; NTS: nucleus of the solitary tract; 10N: vagal nerve; NAm: Nucleus ambiguus; RVLM: rostral ventrolateral medulla; DVN: dorsal vagal nucleus. CVLM: caudal ventrolateral medulla. Rat brain structure adapted from (53; Paxinos & Watson, 2007).
Table 1. Echocardiographic analysis of left ventricular (LV) dimensions and function at 7 and 28 days following seizure induction (intrahippocampal KA) versus control (intrahippocampal saline).

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Kainic Acid</th>
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<tr>
<td></td>
<td>7 days</td>
<td>28 days</td>
</tr>
<tr>
<td>LVIDd (mm)</td>
<td>5.49 ± 0.40</td>
<td>5.63 ± 0.55</td>
</tr>
<tr>
<td>LVIDs (mm)</td>
<td>2.41 ± 0.28</td>
<td>2.48 ± 0.38</td>
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<tr>
<td>LVPWd (mm)</td>
<td>3.12 ± 0.15</td>
<td>3.05 ± 0.21</td>
</tr>
<tr>
<td>LVPWs (mm)</td>
<td>3.89 ± 0.11</td>
<td>3.82 ± 0.14</td>
</tr>
<tr>
<td>EF (%)</td>
<td>70.52 ± 1.33</td>
<td>70.52 ± 1.78</td>
</tr>
<tr>
<td>FS (%)</td>
<td>56.76 ± 2.02</td>
<td>56.53 ± 2.7</td>
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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 (mean ± SEM). *P<0.05 compared to the respective time matched control.