Status epilepticus induces cardiac myofilament damage and increased susceptibility to arrhythmias in rats

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Metcalf CS, Poelzing S, Little JG, Bealer SL. Status epilepticus induces cardiac myofilament damage and increased susceptibility to arrhythmias in rats. Am J Physiol Heart Circ Physiol 297: H2120–H2127, 2009. First published October 9, 2009; doi:10.1152/ajpheart.00724.2009.—Status epilepticus (SE) is a seizure or series of seizures that persist for >30 min and often result in mortality. Death rarely occurs during or immediately following seizure activity, but usually within 30 days. Although ventricular arrhythmias have been implicated in SE-related mortality, the effects of this prolonged seizure activity on the cardiac function and susceptibility to arrhythmias have not been directly investigated. We evaluated myocardial damage, alterations in cardiac electrical activity, and susceptibility to experimentally induced arrhythmias produced by SE in rats. SE resulted in seizure-related increases in blood pressure, heart rate, and the first derivative of pressure, as well as modest, diffuse myocyte damage assessed by terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling staining. Ten to twelve days following seizures, electrocardiographic recordings showed arrhythmic alterations in cardiac electrical activity, denoted by prolonged QT interval corrected for heart rate and QT dispersion. Finally, SE increased susceptibility to experimentally induced (intravenous aconitine) cardiac arrhythmias. These data suggest that SE produces tachycardic ischemia following the activation of the sympathetic nervous system, resulting in cardiac myofilament damage, arrhythmogenic alterations in cardiac electrical activity, and increased susceptibility to ventricular arrhythmias.

seizures; cardiomyocyte; QT interval corrected for heart rate; QT dispersion; troponin I

STATUS EPILEPTICUS (SE) is defined as a single seizure or multiple recurrent seizures lasting over 30 min (64) and can result from a number of causes including stroke, hypoxia, metabolic derangements, head trauma, and fever (64). This medical emergency has a mortality rate ranging between 20 and 30% (43, 44, 51) and accounts for as many as 55,000 deaths annually (21). More than 90% of SE-induced mortality does not occur during the seizures or within the initial 24 h (20, 22, 60) but within the 30 days following seizure activity (22, 60). The mechanism of this extended period of risk is not completely understood.

The potential mechanisms contributing to mortality in the weeks following SE include lethal cardiac arrhythmias (5, 14, 29, 44). Boggs et al. (5) described two distinct patterns of hemodynamic changes observed during the 24 h before death, both ending in lethal arrhythmias in patients during the weeks following SE. One pattern was characterized by a gradual decline in blood pressure (BP) and heart rate (HR) before death, in which autopsies revealed overt myocardial injury. The remaining patients showed no change in cardiovascular parameters immediately before death and no obvious cardiac damage. These investigators suggested that this second group of patients sustained very subtle cardiac injury resulting in latent arrhythmogenicity, which eventually culminated in a latent cardiac event (5). Consistent with this proposal, histological evaluations of cardiac tissue from some patients who die following SE show significant, overt cardiac pathology, whereas others die with no gross evidence of cardiac pathology (5, 45, 49). These findings suggest that significant cardiac damage following SE is not necessary for a subsequent generation of lethal arrhythmias.

A limited number of animal studies have examined the effects of severe, uncontrolled SE on cardiac tissue. For example, 2 h of uncontrolled SE in neonatal pigs (68) and the severe seizure activity associated with nerve gas exposure result in significant cardiac damage in rats (46, 57, 61) and macaques (9). However, despite the incidence of sudden cardiac death in patients following SE, the effects of more moderate, controlled seizures on cardiac tissue, as well as subsequent susceptibility to arrhythmias, have not been directly investigated.

The present experiments were designed to more thoroughly evaluate cardiovascular responses and cardiac damage during SE and subsequent susceptibility to lethal arrhythmias following recovery from the seizure activity. We measured gross cardiac structural alterations [hematoxylin-eosin and terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) stain] and myocyte myofilament damage [cardiac troponin I (cTnI)] produced during SE and electrocardiographic activity [QT interval corrected for HR (QTc) and QT dispersion (QTD)] and susceptibility to experimentally induced arrhythmias (aconitine) 10–12 days following seizures.

METHODS

Animals. Male Sprague-Dawley rats weighing between 175 and 225 g were obtained from a commercial supplier (Charles River, Wilmington, MA) and housed two to three per cage in Plexiglas cages before treatment and individually following SE. Animals were allowed access to standard laboratory rat chow and water ad libitum. Room temperature was maintained at 23°C, and room lights were on for 12 h/day. All experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Utah.

Induction of SE. These experiments used a well-documented and thoroughly characterized model of SE, in which seizures are induced with sequential administrations of lithium and pilocarpine (Li-Pilo) (27, 31, 39, 58). On the day before the induction of seizures, rats received lithium chloride (127 mg/kg ip; Sigma, St. Louis, MO).
Approximately 18–20 h following lithium administration, rats were given methylscopolamine (2 mg/kg ip, Sigma) 30 min before Pilo (30 mg/kg ip, Sigma). Control animals (Cont) were treated similarly, except they were administered 0.9% saline instead of Pilo. A modified Racine scale (50) was used to characterize behavioral seizure activity, and seizures typically began within ~20 min following injection of Pilo. The onset of SE was defined as the first grade III or greater seizure that progressed to similar repeated or prolonged behavioral seizures. SE was allowed to continue for 90 min before the administration of valproic acid (400 mg/kg ip, Sigma), which terminated behavioral seizure activity.

Typically, Li-Pilo-induced SE causes temporary aphagia and adip sia. Consequently, food and water ingestion were monitored for several days following SE, and animals that exhibited decreased food and water consumption were supplemented daily with lactated Ringer solution (3 ml ip) and offered softened palatable breakfast cereal (Froot Loops) in addition to normal rat chow.

Vascular catheterization. Anesthetized (Avertin, 300 mg/kg ip) animals were prepared with polyethylene (PE) catheters (heparin filled, 50 U/ml) in a femoral artery and femoral vein, which were led subcutaneously to exit between the scapulae. The catheters consisted of a 40 mm length of PE-10 cemented in PE-50, and the tips advanced to approximately the level of the renal arteries.

BP, HR, and first derivative of pressure. Pulsatile BP was monitored directly from a femoral artery catheter attached to a pressure transducer and recorded by a PowerLab Data Acquisition System and Macintosh computer. Mean BP and HR were calculated by the computer from the pressure pulse signal and recorded continuously. The first derivative of pressure (dP/dt) was analyzed directly from 10 min of pressure pulse signals following 45–60 min of seizure activity.

Histological evaluation of structural damage in cardiac tissue. Cardiac structural damage was evaluated using previously described techniques (47, 63). Briefly, following the experiment, some animals were transcardially perfused with 0.9% NaCl (saline). The vena cavae were cut near the atrium, and the aorta was clamped (~20 mm above the heart). Saline (60 ml) was infused through the aorta. The hearts were then excised by cutting the major vessels and then blotted and weighed. The hearts were then flash frozen by submersion in isopentane surrounded by dry ice and stored frozen until sectioned for processing. Before being sectioned, the hearts were placed in 10% neutral-buffered formalin for 24 h. Whole hearts were then blocked, embedded in paraffin, and transversely sliced. Some sections from each animal were stained with hematoxylin-eosin and evaluated using light microscopy with a Nikon Diaphot inverted light microscope (Nikon). Five unique regions of interest were photographed from the ventricles of each heart. Images were classified as exhibiting or not exhibiting gross structural damage.

Other sections from each animal were processed for in situ terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling assay (TUNEL assay) as previously described (26). TUNEL-positive areas were also analyzed with the Nikon Diaphot inverted fluorescent microscope from five unique regions. Hearts were classified as TUNEL positive if any image exhibited TUNEL-positive staining. Hearts with no observable TUNEL positive staining in any image were classified as TUNEL negative.

The presence of hematoxylin-eosin or TUNEL staining was evaluated by an observer blinded to the experimental condition of the animal.

Plasma concentration of cTnI. cTnI is a well-established, highly sensitive and specific blood-borne indicator of myocardial degeneration, which is released from the myocardium into the circulation in proportion to myocardial injury in both humans (2, 53) and laboratory animals, including rats (40, 48). Blood samples for the analysis of cTnI were obtained from animals implanted with arterial catheters on the day before the induction of SE or Cont procedures. The concentration of cTnI was evaluated in plasma (100 μl) collected from the arterial catheter 60 min following the onset of SE in Li-Pilo-treated rats and at a comparable time point in Cont animals. Plasma cTnI levels were measured by ELISA assay using a commercially available kit designed for rats (Life Diagnostics, West Chester, PA).

QTc, QTd, and susceptibility to ventricular arrhythmias. Twelve to fourteen days following SE or Cont procedures, separate groups of
Li-Pilo and Cont animals were prepared for evaluation of electrocardiograms (ECG) and susceptibility to experimentally induced cardiac arrhythmias by implanting a venous catheter and electrodes for measurement of ECG. The rats were anesthetized (Avertin, 300 mg/kg), and catheters were placed in the femoral vein using the procedures described previously. In addition, two incisions (≏10 mm) were made through the skin in the upper right and lower left quadrants of the chest. The exposed (≏5 mm) tips of insulated silver wire were sutured into the thoracic muscles to record ECG. The wires, which were soldered to a microconnector, were connected to a data acquisition system, amplified (50×), filtered (1–1,000 Hz), and digitized (PowerLab, ADInstruments, Colorado Springs, CO).

On the following day, the animals were anesthetized with urethane (1.2 g/kg), and 10–15 min ECG recordings were obtained to quantify QT and RR intervals. The QT interval, which represents the total duration of ventricular electrical activity, was measured from 10–20 consecutive beats as the time (in ms) between the start of the QRS complex to the return of the T wave to the isoelectric value. The QTc was then calculated using Bazett’s formula, QTc = QT/RR1/2. QTd, defined as the difference between maximum and minimum QT intervals, was determined by subtracting the minimum QT interval from the maximum QT interval for each animal without correcting for HR. Both measures were calculated using previously described procedures for rodents (13, 17, 62) and are well-recognized indicators of risk for sudden cardiac death in humans (16, 18, 19).

After obtaining data for QT interval and dispersion calculations, the vulnerability to experimentally induced ventricular arrhythmias was assessed by monitoring ECG activity during intravenous infusion of the arrhythmogenic agent aconitine, which is routinely used to evaluate susceptibility to lethal arrhythmias in rats (28, 35, 56, 59). Aconitine was infused from a remote syringe placed in a programmable pump at a constant dose of 5 μg·kg⁻¹·min⁻¹ for 7 min. ECG was recorded continuously before, during, and 5 min following the infusion. The times from the initiation of aconitine infusion to the onset of 1) the first premature ventricular contractions (first PVC; nonrecurring QRS with no P wave), 2) ventricular tachycardia (series of 5 PVCs/P wave), and 3) ventricular fibrillation (no discernable rhythm, dissociation between QRS and P waves) were recorded. Aconitine infusion was terminated if ventricular fibrillation occurred before the end of the 7-min infusion period.

**Statistical analysis.** All data are presented as means ± SE. Differences between two means were determined using Student’s t-tests. Differences among multiple means were determined with one-way or multiple-factor analysis of variance for repeated measures. A Newman-Keuls a posteriori test was used to determine differences between individual means following ANOVA.

**RESULTS**

**BP, HR, and dP/dt during SE.** Figure 1 shows BP (Fig. 1A; Cont, n = 4, and SE, n = 5) and HR (Fig. 1B; Cont, n = 4, and

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**Fig. 2.** A: representative images of hematoxylin-eosin (H&E)-stained myocardial sections obtained from a Cont animal (left) and a rat following SE (post-SE; right). B, left: a typical terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL)-negative image of a stained section from a Cont-treated rat. B, middle and right: typical TUNEL-positive images of stained myocardium obtained from animals following SE (post-SE) and showing diffuse (middle) or strong (right) staining. C: summary data demonstrating that post-SE rats exhibit a significantly higher percentage of TUNEL-positive myocardium relative to Cont myocardium. Numbers in bars represent the number of animals in each group exhibiting TUNEL-positive staining/total number of animals evaluated. *P < 0.01 compared with Cont.
SE, $n = 5$) before (pretreatment) and 60 min following the onset of SE or Cont procedures. Both BP and HR significantly increased during the seizure period compared with both pre-treatment levels and values observed in Cont rats. Furthermore, $dP/dt$, measured from pulse pressure recordings 45–60 min following the onset of SE or Cont procedures (Fig. 1C; Cont, $n = 7$, and SE, $n = 7$) was significantly greater in SE animals than in Cont rats. These data are consistent with the proposal that SE results in sympathetic nervous system (SymNS) activation which increases BP, HR, and cardiac contractility during the seizure period.

**Evaluation of structural damage in cardiac tissue.** Figure 2A shows representative images of hematoxylin-eosin-stained ventricular myocardium from SE and Cont rats. All images were obtained from the anterior left ventricular subepicardium in transversely sliced hearts. Contraction band necrosis, deposition of fibrotic tissue, or other gross structural changes (positive hematoxylin-eosin stain) were not observed in any imaged sections obtained from Cont rats ($n = 10$) or SE animals ($n = 9$). These data are consistent with clinical reports of patients who die from sudden death following SE and exhibit no gross structural cardiac damage (5).

In distinction, positive TUNEL staining was present in both Cont rats and SE animals. Although images from Cont animals were predominantly TUNEL negative (representative section Fig. 2B), sections obtained from two of nine Cont rats showed some TUNEL-positive staining. However, the number of hearts from SE rats demonstrating TUNEL-positive staining (8 of 10) was significantly higher than Cont animals (Fig. 2C). It is important to note that TUNEL-positive staining in postictal rats ranged from diffuse and localized in small regions of the myocardium (Fig. 2B, middle) to strong TUNEL staining distributed more widely throughout the section (Fig. 2B, right). There was no relationship between TUNEL-positive staining and any particular regions of the ventricular myocardium.

**Plasma concentration of cTnI.** cTnI was measured in plasma obtained from Cont ($n = 6$) and SE rats ($n = 8$) at a time point equivalent to 60 min following the onset of seizures in SE animals (Fig. 3). Whereas cTnI was essentially undetectable in plasma obtained from Cont rats, animals experiencing SE had significant levels of plasma cTnI. These data demonstrate that seizures during SE produced myocyte myofilament injury.

**QTc, QTd, and susceptibility to ventricular arrhythmias.** Figure 4 illustrates summary data describing QTc (Fig. 4A) and QTd (Fig. 4B) in animals 10–12 days following Cont ($n = 5$) or SE ($n = 6$). Seizures resulted in long-lasting, significant increases in QTc compared with those in Cont animals. Furthermore, QTd was also significantly greater in animals that had undergone SE. These findings demonstrate that following SE, the electrical activity of the heart is altered in a manner consistent with an increased risk of ventricular arrhythmias and sudden death.

Susceptibility to aconitine-induced arrhythmias was significantly enhanced in animals 10–12 days following SE relative to Cont rats. Figure 5 shows time (in s) from the onset of aconitine infusion to the first PVC, ventricular tachycardia, and ventricular fibrillation in SE and Cont rats (Cont, $n = 6$; and SE, $n = 6$). Mean latencies for the induction of these measured arrhythmias were significantly shorter in animals that had previously experienced SE. At the time of testing, these rats were healthy and completely recovered from the initial, acute effects of seizures. These data demonstrate that SE increases the risk of lethal arrhythmias for an extended period following seizure activity and are consistent with the changes observed in QTc and QTd.

**DISCUSSION.**

These experiments demonstrate that seizure activity in an animal model of SE increases BP, HR, and $dP/dt$, consistent with the activation of the SymNS, and induces cardiac myofilament damage. Furthermore, 10–12 days following seizures, the electrical activity of the heart is altered and the susceptibility to lethal ventricular arrhythmias is increased, although there is little or no gross structural and/or histological evidence of cardiac damage. These data support the proposal that SE can induce neurogenically mediated, subtle cardiac myofilament damage due to tachycardic ischemia, which produces electrical changes in cardiac function and increases susceptibility to lethal ventricular arrhythmias, in the absence of gross structural damage, including contraction band necrosis.

The findings reported in previous clinical studies are consistent with this proposal. SE in humans is associated with increased SimNS activity (3, 55, 65), and in recent studies, neurogenically stunned myocardium has been reported in patients following sustained seizures (42, 55). Similar, neurally mediated cardiac effects are observed following subarachnoid hemorrhage (10, 24, 41), brain tumors (15), and Guillian-Barré syndrome (4). This cardiomyopathy is characterized by a reversible decrement in ventricular function, modest increases in cTnI and/or troponin T (1, 55, 67), contraction band necrosis (55), and QT prolongation (23, 67). Although the long-term prognosis for stunned myocardium is positive (7, 36), there is a transient period of increased susceptibility to sudden cardiac death (25, 54). The present experiments found that SE produces some cardiac effects similar to those reported during myo-
Cardiac stunning produced by other pathologies, including elevated cTnI, prolonged QT interval, and increased susceptibility to sudden death. Although we did not observe contraction band necrosis and have not assessed contractility, these results suggest that SE produces a similar stress-related cardiomyopathy which may contribute to cardiac death following intense seizure activity.

Neurogenic myocardial stunning results from increased SymNS activity and catecholamine secretion (30, 41). A number of previous experiments have demonstrated that SE activates the SymNS and increases catecholamine release in both humans (55, 65) and in laboratory animals (38, 52), increasing both HR and BP (52). The present experiments found similar responses in the Li-Pilo model of SE and found increased dP/dt associated with seizure activity. These results support the possibility that increased chronotropic and inotropic effects on the heart associated with SE induce tachycardic ischemia, resulting in cardiac injury. Furthermore, the hypertensive response observed in these studies increases afterload, which has been suggested to contribute to myocardial stunning (37, 54). Taken together, these data demonstrate that convulsive SE produces cardiac and vascular responses previously shown to induce transient cardiomyopathy, increasing susceptibility to ventricular arrhythmias.

Although the activation of the SymNS and the increased catecholamine release are well documented during SE (38, 52, 55, 65), the specific sympathoexcitatory brain sites mediating this effect are unknown. It has been proposed that seizure-induced excitation of the rostral brain areas projecting to the autonomic centers in the brain stem are responsible for the cardiorespiratory effects of seizure activity. Indeed, it has been demonstrated that seizures activate areas in the medulla, such as the nucleus tractus solitarius, the dorsal motor nucleus of the vagus, and the ventrolateral medulla, (32, 33), independent of the associated hypertension (34). These brain areas are critical in regulating peripheral SymNS activity, and stimulation would result in sympathoexcitation (11, 12). Consequently, it appears that an intense activation of the sympathoexcitatory centers throughout the brain during SE produces increased SymNS activity and catecholamine release, inducing myocyte damage and altered ECG.

The detrimental effects on cardiac tissue that produce mortality following SE have not been definitively determined. A pathological evaluation of cardiac tissue from patients demonstrates that gross anatomical and/or structural damage in cardiac tissue is present in some, but not all, patients and animals following death after SE (5, 45, 49). Furthermore, in patients with cardiac damage, it is unclear whether the damage was produced by SE or was present before the seizure activity. These findings led to the proposal that SE can produce more subtle cardiac damage, leading to latent arrhythmias and sudden cardiac death (5). Similarly, previous studies using animal models of SE have not clearly defined the relationship between SE and cardiac damage. Clear anatomical alterations in cardiac tissue have been reported following uncontrolled seizures in neonatal pigs (68) and severe seizures characteristic of exposure to nerve agents in rats (46, 57, 61) and macaques (9). However,
these studies produced no consensus regarding the relationship between seizure activity and cardiac damage. The results from the present experiments suggest that SE can result in life-threatening increases in susceptibility to lethal arrhythmias following subtle myofilament damage, with little or no gross anatomical or structural cardiac damage. The diffuse TUNEL-positive staining following SE may explain the elevations in cTnI observed in both this study and patients that otherwise do not exhibit gross cardiac structural damage, including contraction band necrosis.

Furthermore, the effects of SE on electrical activity of the heart and the susceptibility to ventricular arrhythmias that persist after termination of the seizure have not been previously reported. Prolonged QTc and QTd are both predictors of an increased risk of ventricular arrhythmias and sudden cardiac death in humans and animals (13, 16–19, 62) and result from stress cardiomyopathy (23, 67). In the present study, an evaluation of the ECG from anesthetized rats 10–12 days following SE demonstrated that both electrical measures of ventricular function were increased, indicating an increased risk of ventricular arrhythmias. In addition, a direct measurement of susceptibility to experimentally induced arrhythmias using intravenous acetylcholine infusion demonstrated that SE-related changes in electrical activity of the heart were indeed associated with an increased risk of arrhythmias. It should be noted that animals exhibiting prolonged QTc and QTd and increased susceptibility to arrhythmias 10–12 days following SE were not receiving anticonvulsant agents, indicating that the observed effects were due to seizure activity. These data suggest an extended period of cardiac risk following SE in rats and are consistent with clinical reports that death can occur days to weeks following SE (20, 22, 60), apparently due to lethal arrhythmias (5, 14, 29, 44). The increased risk of lethal arrhythmias is characteristic of myocardial stunning (6–8), which occurs following subarachnoid hemorrhage (10, 24), brain tumors (15), cerebral infarction (66), and Guillain-Barré syndrome (4). The results from the present experiments demonstrate that SE induces several cardiac effects which are qualitatively similar to those observed in stunned myocardium. In the present study produced no consensus regarding the relationship between seizure activity and cardiac damage. Determining whether β-adrenergic blockade during and/or immediately following SE is cardioprotective and whether subsequent antiarrhythmic therapy reduces mortality following the postseizure period of risk is an area of research that could be beneficial in reducing SE-induced mortality.

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DISCLOSURES

No conflicts of interest are declared by the author(s).

REFERENCES

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CARDIAC EFFECTS OF STATUS EPILEPTICUS


