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Autonomic and Cellular Mechanisms Mediating Detrimental Cardiac Effects of Status Epilepticus

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Abstract

Prolonged seizure activity (status epilepticus; SE) can result in increased susceptibility to lethal ventricular arrhythmias for an extended period of time following seizure termination. SE is accompanied by acute, intense activation of the sympathetic nervous system (SymNS) and results in myocyte myofilament damage, arrhythmogenic alterations in cardiac electrical activity, and increased susceptibility to ventricular arrhythmias. However, the mechanisms mediating the changes in cardiac function, and the specific arrhythmogenic substrate produced during SE are unknown. To determine if detrimental cardiac effects of SE are mediated by SymNS stimulation of the heart, we examined the effects of B-adrenergic blockade (atenolol) during seizure activity on blood pressure, heart rate, myocyte myofilament injury (cardiac troponin I, cTnI), electrocardiographic activity, and susceptibility to arrhythmias. Furthermore, we determined if SE was associated with altered expression of the Kv4.x potassium channels, which are critical for action potential repolarization and thereby contribute significantly to normal cardiac electrical activity. Lithium-pilocarpine induced SE was associated with acute tachycardia, hypertension, and cardiomyocyte damage. Arrhythmogenic alterations in cardiac electrical activity accompanied by increased susceptibility to experimentally induced arrhythmias were evident during the first 2 wks following SE. Both were prevented by atenolol treatment during seizures. Furthermore, one and two weeks after SE, myocyte ion channel remodeling, characterized by a decreased expression of cardiac Kv4.2 potassium channels, was evident. These data suggest that the cardiac effects of prolonged and intense SymNS activation during SE induce myofilament damage and downregulation of Kv4.2 channels, which alter cardiac electrical activity and increase susceptibility to lethal arrhythmias.

Keywords

seizures; sudden cardiac death; Kv4.x potassium channels; troponin; QTc; QTcd

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1. Introduction

Potentially lethal seizure activity lasting longer than 30 min, defined as status epilepticus (SE) (Lowenstein and Alldredge, 1998), can result from metabolic or electrolyte disturbances, head trauma, CNS infections, chronic alcohol abuse, and exposure to organophosphate poisons or nerve gases (Lowenstein and Alldredge, 1998). Although death can occur during SE (Logroscino et al., 2005), most mortality follows seizure activity by 1 to 30 days (DeLorenzo et al., 1992, Towne et al., 1994). A number of studies indicate that lethal cardiac arrhythmias contribute to death following SE (Boggs et al., 1993, Boggs et al., 1998, Painter et al., 1993, Schraeder and Lathers, 1983).

Intense, uncontrolled seizure activity produces cardiac dysfunction and myocardial damage in both patients (Legriel et al., 2008, Painter et al., 1993, Shimizu et al., 2008), and animal experiencing seizures induced by lethal nerve agents (Singer et al., 1987, Tryphonas et al., 1996). Furthermore, we recently reported that the protracted seizures routinely observed in a chemoconvulsant model of SE result in subtle myocardial myocyte damage and produce chronic arrhythmogenic electrocardiographic (ECG) activity with increased susceptibility to experimentally-induced ventricular arrhythmias (Metcalf et al., 2009). The precise mechanisms mediating SE-induced myocyte damage, altered ECG activity, and a long-lasting increase in susceptibility to arrhythmias are not known.

Intense activation of the sympathetic nervous system (SymNS) can result in cardiac contractile dysfunction, characterized by minimal and diffuse myocyte damage and increased susceptibility to ventricular arrhythmias (Ishiguro and Morgan, 2001, Shimizu et al., 2008). Since SE produces large increases in SymNS activity (Sakamoto et al., 2008, Shimizu et al., 2008), we propose that excessive stimulation of cardiac SymNS receptors during seizures produces the detrimental cardiac effects of SE.

Furthermore, the cellular effects of SE in cardiomyocytes that produce chronic arrhythmogenic changes in the electrical activity of the heart and the resulting increase in susceptibility to arrhythmias and sudden death have not been defined. One potential mechanism is seizure-induced myocyte ion channel remodeling. In the heart, Kv4.x channels are critical for repolarization of cardiomyocytes (Birnbaum et al., 2004). Decreased expression of these channels is reported in several cardiomyopathies characterized by an increased risk of sudden cardiac death including myocardial infarction (Kaprielian et al., 1999), ventricular hypertrophy (Kassiri et al., 2002), and diabetes (Xu et al., 1996). Previous studies demonstrate that Kv4.2 channels are decreased in neuronal tissue following SE (Birnbaum et al., 2004, Lugo et al., 2008, Su et al., 2008). However, changes in Kv4.x channels in cardiac tissue following SE have not been investigated. We have previously shown that Kv4.2 knockout mice have a decrease in seizure threshold and experience sudden unexplained death following chemoconvulsant stimulation (Barnwell et al., 2009). Thus, we propose that one candidate mechanism underlying the arrhythmogenic electrical activity and increased susceptibility to arrhythmias following SE is cardiac ion channel remodeling, and in particular Kv4.x channel downregulation.

Therefore, the purposes of the present experiments were to determine the effects of B-adrenergic blockade during SE on myocardial damage, development of subsequent arrhythmogenic electrical activity, and increased susceptibility to ventricular arrhythmias. Furthermore, SE-associated changes in Kv4.x channels were evaluated to determine if cardiomyocyte ion channel remodeling is a candidate locus for alterations in cardiac electrical activity and increased susceptibility to arrhythmias following SE.

2. Methods

2.1 Animals

Male Sprague-Dawley rats (225–250 g) were purchased from a commercial supplier (Charles River, Wilmington, MA). Animals had *ad libitum* access to standard laboratory rat chow and water, and were housed in animal quarters maintained at 23 °C and 12h: 12h light: dark cycle. All experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Utah.

2.2 Induction of SE

SE was induced by administration of lithium and pilocarpine (Pilo) using previously described procedures (Glein et al., 2001, Kulkarni and George, 1995). Briefly, rats were initially administered 127 mg/kg lithium chloride (Sigma, St. Louis, MO) ip, and the next day, given methyl-scopolamine (2 mg/kg; Sigma, St. Louis, MO, ip) followed 30 min later by either pilocarpine (30 mg/kg, Sigma, St. Louis, MO, ip) to induce SE, or an equivalent volume of saline vehicle (Cont). Seizure activity typically began within 20 min of pilocarpine administration, and was quantified using a modified Racine scale (Racine, 1972). The first sustained grade III or greater seizure was defined as the onset of SE. Following 90 min of continuous seizure activity, animals were administered 400 mg/kg valproic acid (Sigma, St. Louis, MO) ip. Cont rats were also given valproic acid at an equivalent time point.

All animals were then returned to their home cages, and food and water ingestion were monitored for several days. Rats undergoing SE which exhibited hypophagia and/or hypodipsia were supplemented with daily injections of 3 ml lactated Ringer's solution (ip) and offered water-softened palatable breakfast cereal (Froot Loops) in addition to normal rat chow.

2.3 Vascular Catheterization

On the day prior to induction of SE or Cont procedures, some animals were prepared for evaluation of blood pressure (BP) and heart rate (HR) during seizures, obtaining blood samples for analysis of cardiac troponin I, and administration of the B-1 adrenergic antagonist Atenolol (AT) or vehicle (saline). These rats were anesthetized (Avertin, 300 mg/kg, ip) and implanted with catheters (heparin-gilled, 50 U/ml) in a femoral artery and femoral vein (Cont, n=11; SE, n=15). Other animals which were to undergo cardiac testing 12–14 days following seizures or Cont procedures were implanted with a femoral vein catheter only, for administration of AT or saline vehicle during the seizure period (Cont, n=14; SE, n=15). These rats were allowed to recover and were left undisturbed until prepared for cardiac testing. Catheters were constructed of 40 mm PE-10 polyethylene tubing cemented into PE-50. The catheter tips were advanced to approximately the level of the renal arteries, and the distal ends were led subcutaneously to exit between the scapulae.

2.4 Blood Pressure and Heart Rate

Pulsatile BP was measured directly from the femoral artery catheter that was 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.

2.5 B-Adrenergic Blockade

Animals undergoing SE or Cont treatments were administered the B1-adrenergic antagonist AT (iv, 1 mg/kg) or vehicle prior to the initiation of seizure activity. Additional AT was

given at 30–45 min intervals, or as needed in SE rats, when HR exceeded pre-seizure values by >10 bpm. AT was selected since it does not easily cross the blood brain barrier.

2.6 Plasma Concentration of Cardiac Troponin I

Cardiac troponin I (cTnI) is a sensitive and specific blood borne indicator of myocardial damage, released into the circulation in proportion to myocyte injury in both man (Antman, 2002, Sarko and Pollack, 2002) and experimental animals (Kurata et al., 2007, O'Brien et al., 2006). In these studies, the concentration of cTnI was evaluated in plasma (100 ul) obtained from the arterial catheter 60 min following the onset of SE, and at an identical time in Cont animals. Plasma cTnI levels were measured by ELISA assay using a commercially available kit designed for rat (Life Diagnostics, Inc. West Chester, PA).

2.7 QTc, QT Dispersion and Susceptibility to Ventricular Arrhythmias

For cardiac testing, SE and Cont rats were prepared with venous catheters and electrodes to record electrocardiograms (ECG) for evaluation of cardiac electrical activity and susceptibility to experimentally induced arrhythmias 12 to 14 days following seizures. Animals were anesthetized and implanted with a femoral vein catheter. In addition, two incisions (≈ 10 mm long) made in the skin of the upper right and lower left quadrants of the chest and the exposed (≈ 5 mm) tips of insulated silver wire were sutured to the thoracic muscles. The wires were connected to the data acquisition system (PowerLab, ADInstruments, Colorado Springs, CO), and the ECG signals were amplified (50x), filtered (1–1000 Hz), digitized, and recorded.

On the day following these procedures, animals were anesthetized with urethane (1.2 g/kg) and a 10 min ECG recording was obtained for measurement of the QT and RR intervals. The QT interval is the total duration of ventricular electrical activity, measured from the start of the QRS-complex to the return of the T-wave to the isoelectric value. The RR interval is the time between successive heartbeats. These variables were averaged across 10–20 consecutive heartbeats. The QT interval was subsequently corrected for heart rate (QTc) using Bazett's formula, $QTc = QT / (RR)^{1/2}$. QTc dispersion (Qtcd) is the difference between maximum and minimum QTc intervals, and is calculated by subtracting the minimum QTc from the maximum QTc for each animal. These measures were determined using previously described procedures for rodents (Chen et al., 2009, Volk et al., 2001), and are well recognized indicators of risk for sudden cardiac death in humans (Chugh et al., 2009, Darbar et al., 1996).

After baseline ECG was recorded for QT and RR intervals, we assessed vulnerability to experimentally induced ventricular arrhythmias by determining the latency to onset of arrhythmias evoked by iv infusion of the arrhythmogenic agent aconitine. Aconitine infusion was administered from a syringe placed in a programmable pump at a constant dose of 5 ug/kg/min for 7 min. ECG was recorded continuously before, during, and for 5 min following the infusion. The times from the initiation of iv aconitine and; 1) the first premature ventricular contractions (1st PVC; nonrecurring QRS with no P wave), 2) ventricular tachycardia (VT; series of 5 PVCs with no intermittent P wave; and 3) ventricular fibrillation (VF; no discernable rhythm, dissociation between QRS and P waves) were recorded. These procedures are commonly used to evaluate susceptibility to cardiac arrhythmias in rodents (Grippio et al., 2004, Shu et al., 2004).

2.8 Expression of Cardiac Kv4.x Potassium Channels

The protein levels of cardiac Kv4.2 and Kv4.3 channels were evaluated in hearts obtained from Cont and SE animals 1 wk (Cont, n=5; SE, n=8) and 2 wk (Cont, n=8; SE, n=9) following treatment.

2.8.1 Western blotting—Hearts were frozen in liquid nitrogen and powdered using a mortar and pestle. Heart powder was then homogenized in 10 ml of 4mM HEPES buffer (pH 7.0) containing protease inhibitor cocktail (Roche, San Francisco, CA). Samples were then centrifuged at 4 °C for 10 minutes at 2000 g. Subsequently, supernatants were centrifuged at 4 °C for 1 hour at 100,000 g. Resulting membranes were resuspended in 4mM HEPES buffer and protein concentration was determined using the Bradford Protein Assay relative to a Bovine serum albumin standard curve (Bio Rad, Hercules, CA). Equal amounts of protein were diluted in Laemmli loading buffer and separated through SDS-PAGE. Twenty micrograms of protein sample from each heart were separated in 10 % polyacrylamide gels, and transferred to Hybond-P polyvinyl difluoride membranes (PVDF; GE). To block non-specific signal, membranes were incubated in 5% non-fat milk diluted 1 × Tris Buffered Saline with 0.1% Tween (TBS-T) for 1 hour at room temperature. Membranes were then incubated overnight at 4 °C with the primary antibodies diluted in 2.5 % non-fat-milk in 1× - TBS. Primary antibodies used: mouse anti -Kv4.2 and -Kv4.3 (Neuromab, Davis, CA) and rabbit anti-GapDH (Santa Cruz Biotechnology, Santa Cruz, CA). Following incubation in primary antibodies membranes were washed in 1× TBS-T (3 × 5 min). Membranes were then incubated with horseradish peroxidase linked secondary antibodies: anti-rabbit IgG or anti-mouse IgG (Cell Signaling, Danvers, MA). After 3× 5 min washes in 1× TBS-T, membranes were incubated with ECL and apposed to film.

2.8.2 Data analysis for Western Blots—Optical densities of immunoreactive bands were analyzed using the Image J software (NIH, Bethesda, MD). Kv4.2 or Kv4.3 protein levels from each individual heart sample were normalized to the GapDH levels for the same sample. Once data were collected, groups were normalized to control values and shown as percent control and standard errors. Significance was set to < 0.05 and was determined by *t*-test analyses using the GraphPad Prism (La Jolla, CA) software.

2.9 Evaluation of CNS damage induced by SE

As an additional measure of seizure intensity, we evaluated CNS damage by fluoro-jade B immunohistochemistry in the dentate gyrus obtained from separate groups of animals undergoing SE or Cont procedures and receiving either saline or AT. Twenty-four hrs following treatment, these rats were anesthetized (Avertin 300 mg/Kg, ip.) and transcardially perfused with 300mL of 0.1M neutral phosphate buffered 10% formalin. The brains removed and post-fixed overnight in 10% formalin + 20% sucrose. For evaluation of neuronal damage, 6–8 horizontal tissue sections (40 um) were collected through the hippocampi, mounted to slides, and allowed to air dry for 30 min. The sections were then stained with Fluoro-Jade B according to the manufacturer's directions (Chemicon International, Billerica, MA). Fluoro-Jade B treated sections were visualized under a confocal microscope equipped with a FITC filter, and the amount of neurodegeneration in each animal was quantified by calculating the average number of FluoroJade B positive neurons in all hippocampal sections.

2.10 Statistical Analysis

All data are presented as means ± SEM. Differences between two means were evaluated with student *t*- tests, and differences among multiple means determined following 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. A *p*<0.05 was considered significant.

3. Results

3.1 Blood Pressure (BP), Heart Rate (HR) During SE

Fig. 1 shows maximum BP and HR in Cont and SE animals during the 90 min seizure period. There were no differences in either BP or HR between Cont, Cont + Aten, SE, or SE + Aten animals prior to administration of Pilo or control procedures. However, SE rats treated with vehicle demonstrated a significant tachycardia and hypertensive response during seizures. Furthermore, treatment with AT during seizures prevented the increase in HR, but did not lower the seizure-related BP response. These data demonstrate that the tachycardia associated with SE is mediated by increased B-adrenergic stimulation.

3.2 Plasma Concentration of Cardiac Troponin I (cTnI)

SE induced significant damage to cardiac myocytes within 60 min of the initiation of seizure activity, as denoted by the increase in plasma concentration of cTnI (Fig. 2). However, SE animals treated with AT during seizures (SE+AT) had plasma cTnI concentrations that were comparable to Cont and Cont+AT rats, demonstrating that seizure-related cardiac damage was prevented by AT. These data are consistent with the proposal that inhibition of the seizure-related tachycardia during SE with AT protects the cardiomyocytes from seizure-induced damage.

3.3 QTc, QT Dispersion and Susceptibility to Ventricular Arrhythmias

Prolonged QTc and increased Qtcd are indicators of increased risk of cardiac arrhythmias and sudden death. As can be seen in Fig. 3, QTc and Qtcd are both increased following untreated SE in animals tested 12–14 days following seizures. However, AT administration to maintain HR at pre-seizure (control) values prevented the increase in both indicators of cardiac risk resulting from SE, while AT did not alter either measure of cardiac electrical activity in Cont rats. These data suggest that AT can be cardioprotective following SE.

Figure 4 shows the latencies to arrhythmias induced by aconitine infusion 12–14 days following SE or control procedures. As previously demonstrated (Metcalf et al., 2009), animals undergoing SE and treated with saline (AT vehicle) exhibited increased susceptibility to experimentally-induced arrhythmias, as denoted by significantly smaller latencies to the 1st PVC, VT, and VF during aconitine infusion. However, the onsets of these arrhythmias in SE rats treated with AT during seizures were similar to those observed in Cont animals and Cont rats treated with AT (Fig. 4). These data demonstrate that B-adrenergic blockade during SE prevented increased susceptibility to experimentally induced arrhythmias following seizure activity.

3.4 Protein levels of Kv4.x Potassium Channels in Cardiac Tissue

Figure 5 shows western blot analyses of Kv4.2 and Kv4.3 potassium channels in cardiac tissue 1 wk and 2 wk following seizure activity or control procedures. Protein levels for Kv4.2 channels were significantly decreased by more than 60% both 1 wk and 2 wk following SE, compared to control animals. In contrast, protein levels for Kv4.3 channels are unchanged following seizures.

3.5 Neuronal Damage

Figure 6, Panel A shows confocal photomicrographs of representative hippocampal sections obtained from animals undergoing SE or Cont procedures and treated with AT or vehicle. As indicated in the representative sections, summary data (Fig. 6, Panel B) demonstrated that there were no positively stained neurons in hippocampi obtained from Cont and Cont +AT animals, indicating no neuronal damage in these groups. However, both SE and SE

+AT animals exhibited significant and equivalent numbers of damaged neurons in the hippocampus. These data indicate that seizure related-brain damage, and thus seizure intensity, was not altered by treatment with AT.

4. Discussion

These studies have demonstrated that preventing tachycardia during SE with the peripheral B1-adrenergic antagonist AT is cardioprotective. Specifically, when HR is maintained at pre-seizure levels by progressive administration of AT during seizures, cardiomyocyte damage, the subsequent development of arrhythmogenic electrical activity, and the associated increase in susceptibility to experimentally induced arrhythmias normally observed in SE rats (Metcalf et al., 2009) are prevented. Furthermore, these studies demonstrate that SE causes down regulation of the Kv4.2 potassium channel that is critical for normal repolarization of myocytes. Taken together, these data are consistent with the proposal that intense cardiac B-adrenergic receptor stimulation produced by SymNS activation during SE causes immediate damage to cardiac myocytes, as well as longer lasting alterations in cardiac electrical activity and increased susceptibility to cardiac arrhythmias. Furthermore, these arrhythmogenic effects are associated with myocardial ion channel remodeling, specifically at the level of the potassium channel, Kv4.2.

A number of previous studies demonstrated that SE produces intense activation of the SymNS in both patients and in animal models (Sakamoto et al., 2008, Shimizu et al., 2008), increasing both HR and BP during the seizure (Goodman et al., 1990, Johnston et al., 1997, Sakamoto et al., 2008). In patients, SE induces a reversible decrement in cardiac function (Legriel et al., 2008), and increased risk of lethal arrhythmias for a period following seizure activity (Gao et al., 1995, Sato et al., 1999). We previously found that SE also induces myocyte damage, arrhythmogenic changes in the ECG, and increased susceptibility to experimentally induced arrhythmias (Metcalf et al., 2009). These data suggest that the prolonged seizures of SE induce cardiac dysfunction and increases risk of lethal arrhythmias due to intense SymNS cardiac stimulation. Results from the present study support this proposal, as peripheral B1-adrenergic blockade prevented acute myocyte damage, chronic alterations in cardiac electrical activity, and susceptibility to arrhythmias.

Furthermore, results from the present experiments found that SE produces a significant decrease in the protein levels of Kv4.2 channels, which encode for one of the two subunits assembling the rapidly activating and rapidly inactivating transient outward potassium current ($I_{to,f}$). Severe reduction in channel function caused by the dominant negative W362F mutation, leads to prolonged QT interval, enhanced dispersion of repolarization and refractoriness (London et al., 2007). Our observations suggest that a similar mechanism may occur with a significant notch reduction that occurs in phase 1 of the cardiac action potential, causing a prolonged depolarizing wave leading to increased QT interval and QT dispersion. Further, reduction of Kv4.2 protein levels may also affect the transmural gradient between epicardium and endocardium, leading to an imbalance in homogeneity of cardiac depolarization.

The ion channel remodeling, secondary to enhanced SymNS activation, mimics what occurs in ion channel dysfunction caused by primary mutations in the genes encoding for the ion channel subunits or for channel interacting proteins (ChIP) (Schimpf et al., 2009, Ueda et al., 2008, Vatta et al., 2006, Wu et al., 2008). In diseases such as long QT syndrome (LQTS), loss of function of potassium channels occur due to genetic mutations or acquired inactivation subsequent to blood alkalosis, antibiotics or other drug-induced inactivation. These ion channel defects caused by primary gene defects in ion channels or associated proteins define the underlying diseases as a channelopathy (Schimpf et al., 2009). Therefore,

our results suggest that the decrease in Kv4.2 channel protein levels is a candidate mechanism leading to myocardial stunning, increased QTc and QTd, and susceptibility to arrhythmias following SE (Metcalf et al., 2009).

The extended period of ventricular repolarization, which results in prolonged QTc and QTd, is a physiological correlate of diminished Kv4.2 protein (London et al., 2007). In our studies the prolonged QTc and QTd were normalized in rats treated with AT during status epilepticus. Consequently, we predict that B1-adrenergic blockade would prevent Kv4.2 remodeling in this model. However, we did not evaluate the effects of AT treatment on myocardial Kv4.2 protein levels in these studies.

The precise mechanism by which intense SymNS activation induces arrhythmogenic changes in cardiac function and cardiac myocyte remodeling is not known. It has been proposed that tachycardic ischemia resulting from positive inotropic and chronotropic responses to catecholamine stimulation, produces myocyte damage, which has detrimental effects on cardiac function (Bolli, 1990). SE is characterized by increased HR and dP/dt (Goodman et al., 1990, Johnston et al., 1997, Metcalf et al., 2009, Sakamoto et al., 2008). Furthermore, seizure activity induces increased BP, thus increasing cardiac afterload. The additional stress of cardiac afterload on the heart may contribute to neurogenic myocardial stunning (Kloner and Jennings, 2001). In the present experiments, preventing the increase in HR during SE by blockade of B-adrenergic receptors eliminated evidence of neurogenic cardiomyocyte damage, alterations in cardiac electrical activity and increased susceptibility to arrhythmias, without significantly reducing the pressor response. These data suggest that increased afterload alone does not contribute to neurogenic myocardial stunning. However, the precise contribution of tachycardia alone without increased afterload is not known.

There are probably multiple mechanisms mediating CNS neuronal damage, which occurs during prolonged seizures. Hypoxia resulting from reduced cerebral blood flow, secondary to decreased BP associated with extended seizures, may contribute to CNS damage (Lowenstein and Alldredge, 1998, Tomlinson et al., 1993). Consequently, it is possible that preventing the SE-related tachycardia could alter the pressor response, since cardiac output does not increase, thus increasing cerebral hypoxia and neuronal damage. However, in the present studies, maintaining HR, and presumably cardiac output, at pre-seizure levels with AT did not significantly alter the pressor response. Furthermore, AT-treated rats undergoing SE did not demonstrate significantly greater hippocampal damage than untreated animals. These data indicate that the brain is not at increased risk of additional neuronal damage due to diminished perfusion pressure during the cardioprotective pharmacotherapy used in these experiments.

Some centrally acting B-adrenoceptor antagonists can decrease seizure threshold (Nakamura et al., 2008) and/or enhance the anticonvulsant potency of anti-epileptic medications (De Sarro et al., 2002, Luchowska et al., 2001, Luchowska et al., 2002). However, AT, which does not readily penetrate the blood brain barrier, does not alter seizure activity or effectiveness of anticonvulsant agents (De Sarro et al., 2002, Luchowska et al., 2002). Furthermore, we observed no differences in seizure onset, intensity, or duration of SE animals treated with AT in the present studies. Finally, direct evaluation of hippocampal tissue stained with fluoro-jade B revealed similar amounts of neuronal damage in rats undergoing SE treated with vehicle or AT. Consequently, it does not appear likely that the cardioprotective effects of AT resulted from diminished seizure intensity induced by the B-adrenoceptor antagonist.

SE in patients can result in mortality from lethal ventricular arrhythmias (Boggs et al., 1998). We have previously shown that in addition to functional deficits in cardiac

performance reported in epilepsy, SE produces cardiomyocyte damage resulting in a long lasting alteration of cardiac electrical activity and increased susceptibility to experimentally induced arrhythmias in an animal model (Metcalf et al., 2009). The present studies extend these earlier reports by demonstrating that maintaining HR at pre-seizure levels with B1-adrenergic blockade can eliminate these cardiac effects. These results are consistent with the proposal that detrimental cardiac effects and susceptibility to sudden cardiac death after SE result from acute tachycardic ischemia induced by excessive catecholamine stimulation during SE. Furthermore, SE is associated with decreased protein levels of Kv4.2 channels, which are necessary for post-contraction repolarization in myocytes, and probably account for the increase in QT interval and dispersion, as well as the subsequent increase in risk of lethal arrhythmias. These data suggest the possibility that SE-induced alterations in this potassium channel may contribute to the molecular mechanisms of cardiac mortality in the period following SE. These data support the concept that cardioprotective pharmacotherapy starting as soon as possible following SE termination, measurement of cTnI, and continued monitoring of ECG and cardiac function in the period following seizures may reduce subsequent mortality due to sudden cardiac death.

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Figure 1.

Maximum mean arterial blood pressure (MABP; top panel) and heart rate (beats/minute; bpm; bottom panel) observed during the seizures or a comparable time in control animals (Cont; n=7), control rats administered atenolol (Cont+AT; n=4), vehicle treated animals undergoing status epilepticus (SE; n=10), and status epilepticus animals treated with atenolol (SE+AT; n=5). †, p<0.01 compared to Cont and Cont +AT; **, p<0.01 compared to all other groups.

Figure 2.

Concentrations of troponin I in plasma obtained 60 min following the onset of seizures (SE; n=6) and SE animals treated with atenolol (SE+AT; n=6) rats, and at comparable time points in control animals treated with vehicle (Cont; n=4) and atenolol (Cont+AT; n=4) animals.

**, $p < 0.01$ compared to all other groups.

Figure 3. QTc duration (top panel) and QTc dispersion (QTcd; bottom panel) observed 12–14 days following treatment in control animals (Cont; n=7), control rats administered atenolol (Cont +AT; n=7), vehicle treated animals undergoing SE (n=8), and SE animals treated with atenolol (SE+AT; n=7). **, p<0.01 compared to all other groups.

Figure 4.

Latency between the initiation of aconitine infusion and the 1st premature ventricular contraction (1st PVC), ventricular tachycardia (VT), and ventricular fibrillation (VF) measured 12–14 days following treatment in control animals (Cont; n=4), control rats administered atenolol (Cont+AT; n=7), vehicle treated animals undergoing SE (n=6), and SE animals treated with atenolol (SE+AT; n=5). **p<0.01 compared to all other groups.

Figure 5.

Western blot analyses of Kv4.2 and Kv4.3 potassium channel proteins in cardiac tissue obtained 1 week (Cont, n=5; SE, n=8) and 2 weeks (Cont, n=8; SE, n=9) following seizure SE or control (Cont) procedures. [†], p<0.01 compared to Cont.

Figure 6.

Panel A. Confocal photomicrographs showing positive fluorojade-B stained neurons in representative sections of hippocampus obtained from control rats (Cont) and animals undergoing SE and treated with either vehicle (Veh) or atenolol (AT). Panel B. Mean number of fluorojade-B positive neurons per section of hippocampus from control animals (Cont; n=4), control rats administered atenolol (Cont+AT; n=4), vehicle-treated animals undergoing SE (n=5), and SE animals treated with atenolol (SE+AT; n=5). †, $p < 0.01$ compared to Cont and Cont+AT.