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SHORT COMMUNICATION

Seizures following hippocampal kindling induce QT interval prolongation and increased susceptibility to arrhythmias in rats

Steven L. Bealer*, **Jason G. Little**

Department of Pharmacology and Toxicology, College of Pharmacy, University of Utah, Salt Lake City 84112, UT, United States

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Summary The prolonged seizures of status epilepticus produce chronic arrhythmogenic changes in cardiac function. This study was designed to determine if repeated, self-limiting seizures administered to kindled rats induce similar cardiac dysfunction. Multiple seizures administered to rats following hippocampal kindling resulted in cardiac QT interval prolongation and increased susceptibility to experimental arrhythmias. These data suggest that multiple, self-limiting seizures of intractable epilepsy may have cardiac effects that can contribute to sudden unexpected death in epilepsy (SUDEP).

Introduction

Sudden unexpected death in epilepsy (SUDEP) is a major cause of premature death in patients. Although the mechanisms contributing to SUDEP have not been definitively determined, a number of studies have suggested that respiratory distress may be a predominant, precipitating factor, which interacts with cardiac abnormalities to produce potentially lethal arrhythmias. For example, recent reports demonstrate that ictal hypoxemia resulting from depressed respiration is associated with cardiac repolarization abnormalities characterized by QT interval prolongation (Seyal et al., 2011). In addition, another study suggests that ictal hypoventilation contributes to arrhythmias in SUDEP (Bateman et al., 2010). Repolarization abnormalities, such as QT interval prolongation, could provide the cardiac substrate for ictal hypoxemia to induce arrhythmias resulting in SUDEP.

We have previously demonstrated that the protracted seizures of status epilepticus result in long lasting changes in cardiac function that increase susceptibility to arrhythmias, including QT interval prolongation (Bealer et al., 2010; Metcalf et al., 2009). However, the effects of multiple, self-limiting seizures, characteristic of epilepsy, on cardiac function have not been fully defined. The purpose of these experiments was to determine if administration of self-limiting seizures in rats following hippocampal kindling results in a cardiac repolarization abnormality, characterized by QT prolongation, and increased susceptibility to experimental arrhythmias.

* Corresponding author at: Department of Pharmacology and Toxicology, 20 South 2000 East Room 201, University of Utah, Salt Lake City, UT 84112-5820, United States. Tel.: +1 801 587 7706; fax: +801 585 5111.
E-mail address: steven.bealer@utah.edu (S.L. Bealer).

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Methods

The University of Utah Institutional Animal Care and Use Committee approved all procedures used in these experiments.

Animals

Commercially obtained (Charles River), male Sprague-Dawley rats (200—225 g) were housed individually with *ad libitum* access to food (Harlan 8640 Rat Chow) and water, and maintained at 22 °C on 12 h:12 h light:dark schedule.

Hippocampal kindling

Rats were anesthetized with Avertin (300 mg/kg), placed in a stereotaxic instrument, and implanted with a bipolar stimulating/recording electrode (9 mm length, 0.5 mm gap; Plastics1 Inc.) in the left or right ventral hippocampus (AP −3.6 mm, ML ± 4.9 mm, DV −5.0 mm, incisor bar +5.0; re: bregma and the dura). The electrode was secured to the skull with small screws and dental acrylic, and capped. Following a recovery period of 7—10 days, rats underwent rapid hippocampal kindling using previously described techniques (Lothman et al., 1985; Lothman and Williamson, 1993). These procedures lead to ''fully kindled'' animals that exhibited Stage 5 (Racine Scale) (Racine, 1972) behavioral seizures on subsequent stimulations.

After kindling, some kindled rats were administered 6—8 motor seizures (K-MS) on Monday, Wednesday, and Friday for two consecutive weeks. Seizures were monitored electrographically and EEG recorded. In addition, all electrographic seizures were associated with Stages 4 and 5 (Racine Scale) motor responses. There were no differences in intensity or duration of seizures between K-MS animals. A separate group of animals underwent kindling, but were not given any subsequent seizures (K-Cont). A final group of animals received all surgical procedures, but did not undergo kindling or seizures (Cont).

Heart rate, QT interval, and susceptibility to ventricular arrhythmias

Four to six days following the final day of seizure administration (K-MS) or the control period (K-Cont; Cont), all animals were prepared for evaluation of electrocardiograms (ECG) and susceptibility to experimentally induced cardiac arrhythmias. The rats were anesthetized (Avertin, 300 mg/kg) and catheters placed in a femoral vein using our previously described procedures (Bealer et al., 2010; Metcalf et al., 2009). 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 that were soldered to a microconnector, were sutured into the thoracic muscles to record ECG. The wires and catheter were led subcutaneously and exteriorized between the scapulae.

The following day, animals were anesthetized with urethane (1.2 g/kg), and 10—15 min ECG recordings were obtained to quantify QT interval and heart rate. The ECG leads were connected to a data acquisition system, and the signal was amplified (50×), filtered (1—1000 Hz) and digitized (PowerLab, ADInstruments, Colorado Springs, CO). The QT interval, which represents the total duration of ventricular electrical activity, was measured during 10—20 consecutive beats as the time (ms) between the start of the QRS-complex and the return of the T-wave to the isoelectric value. QTc, *i.e.* the QT interval corrected for heart rate was then calculated using Bazett's formula, $QTc = QT/RR^{1/2}$. This measure was calculated using previously described procedures for rodents (Chen et al., 2009; da Silva Costa et al., 2008; Volk et al., 2001), and QTc prolongation is a well-recognized indicator of risk for sudden cardiac death in humans (Chugh et al., 2009; Darbar et al., 1996; de Bruyne et al., 1998).

Vulnerability to experimentally induced ventricular arrhythmias was assessed by monitoring ECG activity during iv infusion of the arrhythmogenic agent aconitine, which is routinely used by us (Bealer et al., 2010; Metcalf et al., 2009) and others (Grippo et al., 2004; Li et al., 2007) to evaluate susceptibility to lethal arrhythmias in rats. Aconitine was infused intravenously from a remote syringe placed in a programmable pump at a constant dose of 5 μg/kg/min for 10 min. ECG was recorded continuously before and during the infusion. The times from the initiation of aconitine infusion to the onset of (1) the first premature ventricular contractions (PVC) (nonrecurring QRS with no P wave), (2) ventricular tachycardia (Vent. Tachy.) (series of ≥ 5 PVCs/P wave; and (3) ventricular fibrillation (Vent. Fib.) (no discernable rhythm, dissociation between QRS and P waves) were recorded.

Statistical analysis

Differences among multiple means were determined with one-way analysis of variance. A Newman—Keuls *a posteriori* test was used to determine differences between individual means following the ANOVA.

Results

K-MS animals were administered between 35 and 48 total seizures during the seizure period.

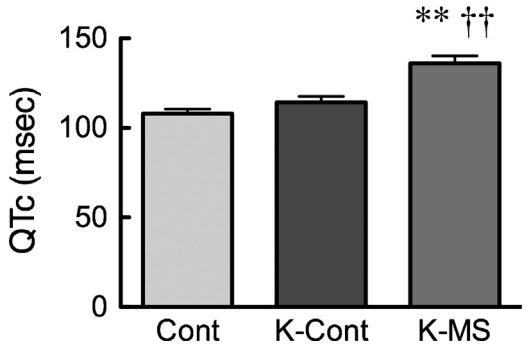

Figure 1 QTc intervals (ms) for control (Cont, $n = 5$), kindled control (K-Cont, $n = 6$) and kindled-motor seizure (K-MS, $n = 12$) rats. $^{**}p < 0.01$ compared to Cont; $^{††}p < 0.01$ compared to K-Cont.