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Beta adrenergic blockade prevents cardiac dysfunction following status epilepticus in rats

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Summary Status epilepticus (SE) can result in temporary cardiac dysfunction in patients, characterized by reduced ejection fraction, decreased ventricular contractility, and alterations in electrical activity of the heart. Although reversible, the cardiac effects of seizures are acutely life threatening, and may contribute to the delayed mortality following SE. The precise mechanisms mediating acute cardiac dysfunctions are not known. These studies evaluated effects of self-sustaining limbic SE in rats on cardiac performance 24 h following seizures, and determined if sympathetic nervous system activation during seizures contributed to cardiac dysfunction. Rats subjected to SE received either vehicle (saline) or the B1 adrenergic antagonist atenolol (AT) prior to and during 90 min of seizure activity. Control rats were similarly treated, except they did not undergo seizures. Twenty-four hours after SE, animals were anesthetized and catheterized for measurement of cardiac performance variables. Animals undergoing SE demonstrated significantly reduced cardiac output, decreased ventricular contractility and relaxation, increased blood pressure, and prolonged QT interval. However, heart rate was not altered. Treatment with AT prevented all changes in cardiac performance due to SE, and attenuated the increase in QT interval. These data demonstrate that SE in the rat results in cardiac dysfunction 24 h following seizures, mediated by the sympathetic nervous system.

Introduction

Status epilepticus (SE) is any protracted or recurrent seizure without recovery of consciousness lasting at least 30 min (DeLorenzo et al., 1992; Hauser, 1990), and results in approximately 55,000 deaths per year (DeLorenzo et al., 1992). Unfortunately, the mechanism(s) that increase mortality risk following SE are unknown.

In both patients and animal models, accumulating evidence suggests a strong correlation between an increased occurrence of cardiac abnormities following SE and mortality (Chin et al., 2004; Longroscino et al., 2002). Clinical studies confirm that patients exhibit cardiac dysregulation including arrhythmias, conduction abnormalities, subtle myocyte damage, and acute, left ventricular dysfunction within 24 h of SE (Boggs et al., 1993; Legriel et al., 2008b; Lemke et al., 2008; Manno et al., 2005). Although normal cardiac function eventually returns in patients with no

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additional insults, the period of diminished performance probably contributes to post-seizure mortality (Boggs et al., 1993).

The precise mechanisms that produce the myocardial dysregulation following SE have not been determined. However, a sudden and prolonged activation of the sympathetic nervous system (SymNS) and increased secretion of catecholamines that stimulate B-adrenergic receptors on cardiac muscle produces similar cardiac effects in other pathological conditions such as subarachnoid hemorrhage, cerebral infarction, and brain tumors (Bolli, 1992; Chuang and Chao, 2000; Jain et al., 2004; Wang et al., 1997). These reversible deficits in cardiac function are termed ''myocardial stunning'' in patients. Since SE is associated with increased SymNS activity (Sakamoto et al., 2008; Shimizu et al., 2008; Simon, 1985), B-adrenoreceptor activation during SE may contribute to detrimental cardiac effects in patients. This proposal is consistent with reports that some longer lasting cardiac effects of SE are prevented by B1-adrenergic blockade at the time of seizures (Bealer et al., 2010).

Cardiac dysfunction, characterized by decreased cardiac output, occurs in animal models of SE during seizure activity (Kreisman et al., 1993; Young et al., 1985). However, lasting alterations in ventricular performance following cessation of seizures have not been evaluated in animal models of SE. Therefore, the present experiments were designed to determine if; (1) left ventricular dysfunction is present in rats 24 h following cessation of SE, and (2) administration of a B-1 antagonist during seizures prevents these adverse cardiac effects. Twenty-four hours after SE, cardiac function in rats was determined by measuring cardiac output, recording left ventricular pulse pressure, mean blood pressure, and electrocardiographic (ECG) activity. These recordings were further evaluated for heart rate, QT interval corrected for heart rate (QTc), and the first derivative of the maximum and minimum left ventricular pressure change over time (dP/dt max and min) which are measures of ventricular contractility and relaxation, respectively.

Methods

Animals

Male Sprague-Dawley rats (175—225 g) had *ad libitum* access to standard laboratory rat chow and water, and were housed in animal quarters maintained at 23 °C and a 12 h light:dark cycle. Rats were purchased from a commercial supplier (Charles River, Wilmington, MA). The Institutional Animal Care and Use Committee at the University of Utah approved all experimental procedures.

Self sustaining limbic status epilepticus protocol

SE bipolar electrode implantation

Rats were implanted with a bipolar electrode in the left amygdala, which served as both a means of electrical stimulation to induce SE and to record electroencephalographs (EEG). To implant electrodes, rats were anesthetized (Avertin, 300 mg/kg, ip), their heads shaved and then placed into a stereotaxic frame. A 2—3 cm midline incision was made to expose the skull, and a small burr hole was drilled directly dorsal to the amygdala. Adjacent to the hole, three small plastic anchor screws were inserted into the skull. A bipolar stimulating electrode (9 mm length with 1 mm gap; Plastics1 Inc.) was stereotaxically implanted in the lateral nucleus of the left amygdala (−3.6 posterior to bregma, 5.0 lateral to the midline, −6.5 ventral to the dura) through the hole and fixed into place with dental acrylic. Once the dental acrylic had hardened the skin was closed with wound clips and an antibiotic ointment was applied. Finally, a cap (Plastics 1 Inc.) was placed on the electrode to keep it clean. After all surgeries, animals were administered Baytril (antibiotic) and Rimadyl and returned to their home cages. Rats were allowed to recover for a minimum 12—14 days before SE was induced.

SE induction

To induce SE and record EEG, rats were connected to stimulating (IsoMax-100 Biphasic Stimulus Isolator) and EEG recording equipment (Powerlab 2-20; ADInstruments). In animals undergoing SE, an electronic switch (Grass Instruments) was used to alternate between stimulation and EEG recording modes. Stimulations consisted of 100 ms trains of 1 ms biphasic square wave pulses at 600 μA, delivered at 60 Hz every 0.5 s for 40 min. Electrographic seizures were evaluated from the EEG. Motor seizure activity was visually monitored during the stimulation period and quantified using the Racine scale (Racine, 1972). Motor seizures typically began intermittently within the first few minutes of stimulation, and progressed in severity and duration until the seizures became persistent and self-sustaining. After 40 min the stimulation was stopped, successful SE induction was ascertained by monitoring spontaneous motor seizure behavior and recurrent, spontaneous spike wave activity in the EEG (Nissinen et al., 2000). Following 90 min of continuous SE, animals were administered valproic acid (400 mg/kg ip; Sigma, St. Louis, MO) to terminate seizure activity. All animals were then returned to their home cages where food and water ingestion were monitored. Rats undergoing seizures were supplemented with an injection of 3 ml lactated Ringer's solution (ip) and offered water-softened palatable breakfast cereal (Froot Loops) in addition to normal rat chow. Control (Cont) animals received identical treatment at concurrent time points, but did not receive electrical stimulation of the amygdala.

Evaluation of seizure severity by EEG activity

EEG activity was evaluated by counting the number of spontaneous spike wave occurrences during 5 min observation periods obtained at 5, 30, and 90 min after the amygdala stimulation was discontinued in seizing animals.

B-1 adrenergic blockade with AT

Rats undergoing SE were administered saline (SE) or AT (1 mg/kg; SE + AT) via tail vein injection immediately prior to the initiation of seizure activity. Additional AT was given at 30—35 min intervals to maintain baseline heart rate during seizures. This procedure maintains heart rate at pre-seizure levels during SE (Bealer et al., 2010). Cont rats received saline injections at equivalent intervals. Our previous work demonstrated that AT treatment in Cont animals has no effect on any variable measured in these experiments (Bealer et al., 2010). Consequently, we did not include Cont animals treated with AT in the present studies.

Cardiovascular measures

ECG recording protocol

ECG electrodes were implanted 24 h after SE and at an equivalent time point in Cont rats. Leads were constructed from two insulated silver wires with one end of both wires soldered to a connector. To implant these electrodes, rats were anesthetized (urethane 1.2 g/kg, ip), and incisions (≈10 mm) were made in shaved areas on the upper right and lower left quadrants of the chest. The exposed tips (≈5 mm) of the wires were inserted through the incisions and