Video Article

Methods for ECG Evaluation of Indicators of Cardiac Risk, and Susceptibility to Aconitine-induced Arrhythmias in Rats Following Status Epilepticus

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Abstract

Lethal cardiac arrhythmias contribute to mortality in a number of pathological conditions. Several parameters obtained from a non-invasive, easily obtained electrocardiogram (ECG) are established, well-validated prognostic indicators of cardiac risk in patients suffering from a number of cardiomyopathies. Increased heart rate, decreased heart rate variability (HRV), and increased duration and variability of cardiac ventricular electrical activity (QT interval) are all indicative of enhanced cardiac risk¹⁻⁴. In animal models, it is valuable to compare these ECG-derived variables and susceptibility to experimentally induced arrhythmias. Intravenous infusion of the arrhythmogenic agent aconitine has been widely used to evaluate susceptibility to arrhythmias in a range of experimental conditions, including animal models of depression⁵ and hypertension⁶, following exercise⁷ and exposure to air pollutants⁸, as well as determination of the antiarrhythmic efficacy of pharmacological agents^{9,10}.

It should be noted that QT dispersion in humans is a measure of QT interval variation across the full set of leads from a standard 12-lead ECG. Consequently, the measure of QT dispersion from the 2-lead ECG in the rat described in this protocol is different than that calculated from human ECG records. This represents a limitation in the translation of the data obtained from rodents to human clinical medicine.

Status epilepticus (SE) is a single seizure or series of continuously recurring seizures lasting more than 30 min^{11,12}, and results in mortality in 20% of cases¹³. Many individuals survive the SE, but die within 30 days^{14,15}. The mechanism(s) of this delayed mortality is not fully understood. It has been suggested that lethal ventricular arrhythmias contribute to many of these deaths¹⁴⁻¹⁷. In addition to SE, patients experiencing spontaneously recurring seizures, i.e. epilepsy, are at risk of premature sudden and unexpected death associated with epilepsy (SUDEP)¹⁸. As with SE, the precise mechanisms mediating SUDEP are not known. It has been proposed that ventricular abnormalities and resulting arrhythmias make a significant contribution¹⁸⁻²².

To investigate the mechanisms of seizure-related cardiac death, and the efficacy of cardioprotective therapies, it is necessary to obtain both ECG-derived indicators of risk and evaluate susceptibility to cardiac arrhythmias in animal models of seizure disorders²³⁻²⁵. Here we describe methods for implanting ECG electrodes in the Sprague-Dawley laboratory rat (*Rattus norvegicus*), following SE, collection and analysis of ECG recordings, and induction of arrhythmias during iv infusion of aconitine.

These procedures can be used to directly determine the relationships between ECG-derived measures of cardiac electrical activity and susceptibility to ventricular arrhythmias in rat models of seizure disorders, or any pathology associated with increased risk of sudden cardiac death.

Protocol

1. Materials to Construct

1. A jugular vein catheter is constructed from a piece (100 mm) of PE-50 polyethylene tubing, beveled at one end, and then filled with heparin saline (50 U heparin/mL saline).
2. The ECG recording electrodes are constructed from two 100 mm lengths of insulated silver wire (30AWG). One end of both wires is stripped and soldered to a microconnector, and the insulation is twisted to form a third wire that is used as ground. Five-mm of insulation is stripped from the distal end of the wires and discarded.

2. Implanting Jugular Vein Catheter

1. Anesthetize the animal by administration of Urethane, ip (1.2 g/kg).
2. Routinely check (10 min intervals) depth of anesthesia by evaluating the pedal withdrawal response and/or eye reflexes. If needed, supplement anesthesia.
3. After the animal is at the appropriate plane of anesthesia, shave the right side of the neck from the clavicle to the chin.
4. Make a longitudinal incision (10-15 mm) in the skin above the carotid artery and open the incision.
5. Using blunt dissection techniques, spread the underlying muscle to locate and isolate the right jugular vein.
6. Place two pieces of surgical silk (#2) under the jugular vein, and position the sutures at the rostral and caudal most portions of the incision. Tie the rostral ligature to discontinue flow through the vein.
7. Retract the caudal ligature to lift the vessel off the underlying tissue and make a small cut. Then insert the beveled end of the heparin-filled (50 U/ml saline) PE-50 polyethylene catheter (100 mm in length), and advance the tip approximately 8 mm to the level of the right atrium.
8. In order to secure the catheter, tie the caudal ligature around both the vessel and catheter. Finally, close the wound with either wound clips or suture.
9. During the implantation of the catheter, the exposed end is either clamped with a hemostat or plugged to prevent back flow of blood prior to attachment to an infusion pump.

3. Implanting CG Electrodes

1. Place the animal on its back and shave the chest.
2. For placement of the electrodes, make two longitudinal incisions (≈ 10 mm), one in the upper right and one in the lower left quadrants of the chest, and free the overlying skin to expose the thoracic muscles.
3. Suture one of the exposed 5-mm tips of the silver-wire electrodes into the thoracic muscles in each of the exposed areas, and place the ground electrode subcutaneously.
4. Close the incisions with wound clips and plug the microconnector into the recording device.
5. Wrap the animal loosely in a towel to maintain body temperature during the procedure.

4. Recording ECG

1. Recordings are made using a Powerlab Data Acquisition System and Macintosh computer or similar hardware/software (amplified, 50X; filtered, 1-1000 Hz).
2. Following a 20-30 min equilibration period, record ECG for 20 min.

5. Inducing Ventricular Arrhythmias

1. Attach the jugular vein catheter to a remote syringe placed in a programmable infusion pump.
2. Initiate the aconitine infusion at a rate to deliver a constant dose of 5 ug/kg/min for 7 minutes.
3. Following ventricular fibrillation or the end of the infusion period, euthanize the animal using an approved procedure.

6. Analysis of ECG

1. The QT interval represents the total duration of ventricular electrical activity. Fig. 1 shows a model ECG recording and the duration of the QT interval. Calculate the mean time between the initiation of the Q-wave and termination of the T-wave for all recorded heartbeats over a five-minute interval obtained during the 20 min recording period.
2. To correct the QT interval for differences in heart rate (QTc), use Bazett's formula, $QTc = QT / (RR)^{1/2}$, where RR is the mean interval between heartbeats. The RR interval is calculated as the duration between successive Q-waves obtained during the analyzed period.
3. The QT dispersion (QTd) is calculated by subtracting the minimum QT from the maximum QT recorded for each animal.

7. Analysis of Heart Rate Variability

1. Software programs (i.e. LabChart 7, ADInstruments, Inc.) that analyze variability in the RR intervals obtained during the period of analysis are used for automated calculation of both temporal and/or spectral variance in heart rate.

8. Analysis of Susceptibility to Ventricular Arrhythmias

1. Calculate the latency from the initiation of aconitine infusion to the first premature ventricular contraction (PVC, Fig. 2, Panel 1), ventricular tachycardia (VT, Fig. 2, Panel 2) and ventricular fibrillation (VF, Fig. 2 Panel 3).

9. Representative Results

Fig. 3 illustrates one measure of HRV in the spectral domain (root mean squared of the standard deviation of the RR interval, RMSSD) in control rats and in animals that underwent SE one week prior to testing. This measure of HRV, which represents changes in the parasympathetic control of cardiac function²⁶, is significantly decreased in animals following status epilepticus. Fig. 4 shows that both corrected QT interval (QTc, Panel A) and QT dispersion (QTd, Panel B) are significantly increased two weeks following SE²⁷. Taken together, these data predict that SE increases risk of ventricular arrhythmias. This prediction is confirmed by the findings that the periods of aconitine infusion necessary to induce the experimental arrhythmias (PVC, VT, VF) observed in these studies were significantly smaller in animals following SE (Fig. 5)²⁷.

Figure 1. Model ECG recording of a normal heartbeat showing P, QRS, and T waves. The QT interval is calculated as the time between the dashed lines.

Figure 2. **Panel 1)** Premature ventricular contractions (PVCs) are isolated contractions of the ventricles (B) that occur between normal heartbeats (A). PVC recordings are characterized by the absence of P and T waves. **Panel 2** Ventricular tachycardia (VT) is a series of multiple ventricular contractions (C) that occur with no intervening normal heartbeats (A). **Panel 3** Ventricular fibrillation (VF) is the lack of discernable rhythm and no association between QRS and P waves (D).

Figure 3. The root mean squared of the standard deviation (RMSSD; msec) of the intervals between heartbeats observed in control rats and in animals undergoing status epilepticus (SE) 1 wk prior to testing. ** $p < 0.01$ compared to Control.

Figure 4. Mean corrected QT interval (Panel A) and QT dispersion (Panel B) calculated from ECG recordings obtained from control animals (Cont) and from rats undergoing status epilepticus (SE) two weeks prior to testing. * $p < 0.05$ compared to Cont. (Metcalf, et al., Am. J. Physiol. Heart and Circ, 2009, Am Physiol Soc, used with permission from ²⁷).

Figure 5. Latencies (sec) between the initiation of aconitine infusion and the 1st premature ventricular contraction (PVC), ventricular tachycardia (VT), and ventricular fibrillation (VF) in control animals (Cont) and rats undergoing status epilepticus (SE) two weeks prior to testing. * $p < 0.05$, ** $p < 0.01$ compared to Cont. (Metcalf, et al., Am. J. Physiol. Heart and Circ, 2009, Am Physiol Soc, used with permission from ²⁷).

Discussion

Two aspects of the described procedures are of critical importance. Firstly, the rate of aconitine administration to the heart must be equivalent across animals. This requires consistent placement of the jugular vein catheter tips relative to the heart, and careful adjustment of the infusion rate. The rate of aconitine delivery to the hearts must be equal in order to appropriately evaluate the onset of ventricular arrhythmias relative to altered susceptibility. If aconitine delivery varies, then the latency to arrhythmias may be due to differences in drug concentration and not alterations in cardiac function. Secondly, preparation and placement of the ECG recording electrodes must yield artifact-free recordings with clearly discernable P, QRS, and T waves. While heart rate, HRV, and occurrence of ventricular arrhythmias can be determined from QRS waves alone, QTc and QTd must be calculated from recordings containing clear Q-wave onset, and T-wave termination.

One obvious limitation of these techniques is that they are conducted in anesthetized animals. However, this is necessary for two reasons. 1) Since the ECG electrodes are implanted in skeletal muscle tissue, they are subject to artifacts produced during movement in conscious rats. These non-cardiac signals frequently obscure the ECG activity necessary for appropriate analysis of heart function. 2) Aconitine induced arrhythmias raise a potential ethical issue when evoked in conscious animals.

These procedures allow quantification of several well-accepted prognostic indicators of SCD, along with direct analysis of susceptibility to ventricular arrhythmias in the same animal. These techniques are valuable for determining relative cardiac risk, as well as efficacy of cardioprotective therapies, in seizure disorders, and any pathology associated with lethal ventricular arrhythmias that can be modeled in the rodent.

Disclosures

We have nothing to disclose.

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