Epilepsy-induced electrocardiographic alterations following cardiac ischemia and reperfusion in rats

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Abstract

The present study evaluated electrocardiographic alterations in rats with epilepsy submitted to an acute myocardial infarction (AMI) model induced by cardiac ischemia and reperfusion. Rats were randomly divided into two groups: control (n=12) and epilepsy (n=14). It was found that rats with epilepsy presented a significant reduction in atrioventricular block incidence following the ischemia and reperfusion procedure. In addition, significant alterations were observed in electrocardiogram intervals during the stabilization, ischemia, and reperfusion periods of rats with epilepsy compared to control rats. It was noted that rats with epilepsy presented a significant increase in the QRS interval during the stabilization period in relation to control rats (P<0.01). During the ischemia period, there was an increase in the QRS interval (P<0.05) and a reduction in the P wave and QT intervals (P<0.05 for both) in rats with epilepsy compared to control rats. During the reperfusion period, a significant reduction in the QT interval (P<0.01) was verified in the epilepsy group in relation to the control group. Our results indicate that rats submitted to an epilepsy model induced by pilocarpine presented electrical conductivity alterations of cardiac tissue, mainly during an AMI episode.

Key words: Epilepsy; Heart; Acute myocardial infarction; Ischemia; Reperfusion

Introduction

Epilepsy is the most common chronic neurological disorder, and people with epilepsy are two to three times more likely to die prematurely when compared with people without epilepsy (1-3). The most common epilepsy-related category of death is sudden unexpected death in epilepsy (SUDEP) (3,4), a leading cause of mortality in patients with epilepsy. Although the cause of SUDEP is still unknown, one of the main mechanisms of SUDEP is autonomic dysregulation (i.e., cardiac abnormalities during and between seizures) (5-7). Suspected mechanisms include cardiac arrhythmia, central and/or obstructive apnea, neurogenic pulmonary edema, and primary cessation of brain activity (3,8-11). In fact, clinical seizures are often accompanied by intense autonomic changes, and there is evidence that sympathetic activity is increased even interictally in patients with temporal lobe epilepsy (10,12,13). Ictal tachycardia is the more frequent arrhythmia (14,15), but ictal bradycardias, high-grade atrioventricular block, and asystole are also observed (10,16,17). Curiously, ictal bradycardia also appears to be associated with a higher risk of SUDEP (10,18). Several case reports have described asystole or bradycardia during epileptic seizures and have linked these conditions to a higher risk of SUDEP (19,20). Trying to explain these...
cardiac conduction abnormalities, P-Codrea Tigaran et al. (21) performed a prospective setting case-control study of all SUDEP cases in Denmark and verified that the SUDEP cases displayed significant fibrosis of the myocardium. The authors raised a hypothesis that this fibrosis could be the consequence of myocardial ischemia as a direct result of repetitive epileptic seizures, which, associated with the ictal sympathetic storm, may lead to lethal arrhythmias. Other studies also identified that epilepsy might be a risk and an adverse prognostic factor for acute myocardial infarction (AMI) (22,23).

Considering that the mechanisms underlying SUDEP remain uncertain and that AMI risk and prognosis in epilepsy have been poorly explored, it would be useful to obtain, in an animal model, additional information about cardiac alterations associated with epilepsy under AMI conditions. Thus, we evaluated in vivo ventricular arrhythmias (VA), atrioventricular block (AVB), and mortality in rats with epilepsy submitted to an AMI model induced by cardiac ischemia and reperfusion.

### Material and Methods

#### Induction of epilepsy

Twenty-six male Wistar rats (280±30 g) were used in this study. All experimental protocols described below were approved by the Ethics Committee of the Universidade Federal de São Paulo (81130/11). The colony room was maintained at 21±2°C with a 12:12-h light/dark schedule, and the animals were given food and water ad libitum throughout the experiments. Rats were randomly divided into 2 groups: control (n=12) and epilepsy (n=14). Rats from the epilepsy group were submitted to a temporal lobe epilepsy model induced by a single administration of pilocarpine hydrochloride (350 mg/kg, ip; Sigma, USA) (24). Scopolamine methylnitrate (1 mg/kg, sc; Sigma) was administered 30 min before pilocarpine injection to limit peripheral cholinergic effects (24). The systemic administration of potent muscarinic agonist pilocarpine in rats promotes sequential behavioral and electrographic changes that build up progressively into a limbic status epilepticus (SE), which lasts 24 h. During the SE, two rats died. Following this acute period of the epilepsy model, the surviving animals (n=12) were continuously monitored over 24 h for detection of spontaneous recurrent seizures, using a video system. After the first spontaneous recurrent seizure had been detected, animals were monitored over the following 120 days by two researchers in the experimental room for observation periods ranging from 2 to 4 h. During this period, the only observation was whether seizures were present in the rats (to ensure that they were epileptic rats).

#### Ischemia and reperfusion protocol

After 120 days, animals from the control and epilepsy groups were submitted to an AMI model induced by cardiac ischemia and reperfusion similar to that previously described (25). All rats were anesthetized with urethane (1.2 g/kg, ip) and fixed in the supine position. After intubation with a catheter (Jelco 14G, USA), rats were mechanically ventilated with room air with a stroke volume of approximately 10 ml/kg (~82 breaths min) using a ventilator (InsiNett EFF 312, Brazil). Under clean dissection, indwelling polyethylene catheters were inserted into a common carotid artery. The body temperature was maintained at 37.5°C with a heated operating platform and appropriate heating lamps, and was monitored routinely via a rectal thermometer. The electrocardiogram (ECG) was recorded using a biopotential amplifier by means of needle electrodes placed subcutaneously on the limbs. The chest was opened with a left thoracotomy and the heart was gently exteriorized using pressure on the abdomen. A ligature (4/0 braided silk suture attached to a 10-mm micropoint reverse-cutting needle, Ethicon K-890H, USA) was placed around the left anterior descending coronary artery, approximately 2 mm from its origin. A polyethylene tube was threaded over the suture and the heart was replaced in the chest cavity with the ligature ends exteriorized. After a stabilization period of 15 min, the ligature was tied, characterizing the ischemic period, and, following a period of coronary occlusion (10 min), the reperfusion was obtained by cutting the suture (Figure 1). Successful ligation of the coronary artery was validated by ECG alterations (increase in R wave and ST segment) and reduction in arterial pressure (26,27).

#### Monitoring, recording, and ECG waveform analysis

The ECG of the rats was monitored and recorded from the beginning of the stabilization period using a commercial acquisition system (AqDados 7.02; Lynx Tecnologia Ltda., Brazil). The recorded raw data were evaluated using the commercial software included in the acquisition system (AqDAnalysis 7, Lynx Tecnologia Ltda.; Figure 1). Heart rate as well as incidence of reperfusion-induced VA, AVB, and lethality were measured. To simplify the presentation of the results, ventricular fibrillation, torsades de pointes, and ventricular tachycardia parameters were considered only as VA. ECG measurements were evaluated according to Lambeth conventions (28,29). P duration was manually measured as the time from the beginning of the upstroke of the P wave until its return to the isoelectric baseline. QRS duration was measured from the beginning of the Q wave to the peak amplitude of the downward deflection of the S wave. PR interval was measured from the beginning of the Q wave to the peak amplitude of the downward deflection of the S wave. RR interval was measured as the time between consecutive R wave peaks. QT interval was measured from the beginning of the Q wave until the T wave returned to the isoelectric baseline (30).

#### Data analysis

In control and epilepsy groups, the incidences of VA,
AVB, and lethality were compared with Fisher’s exact test. Statistical analyses of ECG data were carried out by analysis of variance followed by Bonferroni’s post hoc test. Values were considered significant when $P<0.05$. Statistical analyses were performed with the Prism 5.0 software (GraphPad, USA), and data are reported as means±SE.

Results

Both control and epilepsy groups showed no arrhythmias during stabilization. The arrhythmias were observed and measured during ischemia and reperfusion (Table 1). No significant difference was detected in the incidence of VA and lethality between studied groups [VA incidence = 83.3% in the control group (10/12) and 75% in the epilepsy group (9/12) $P = 1.00$; lethality = 66.7% in the control group (8/12) and 58.3% in the epilepsy group (7/12) $P = 1.00$]. However, the incidence of AVB was significantly lower in the epilepsy group [25% (3/12)] than in the control group [83.3% (10/12) $P = 0.012$; Table 1].

In the ECG waveform analysis, it was noted that rats with epilepsy presented a significant increase in the QRS interval during the stabilization period in relation to control rats ($P<0.01$; Figure 2). During the ischemia period, a significant increase in the QRS interval ($P<0.05$) and reduction in P wave and QT intervals ($P<0.05$ in both) were found in rats from the epilepsy group compared to rats from the control group. During the reperfusion period, a significant reduction in the QT interval ($P<0.01$) was verified in the epilepsy group in relation to the control group.

Discussion

The present study evaluated electrocardiographic alterations in rats with epilepsy submitted to an AMI model induced by cardiac ischemia and reperfusion. Rats with epilepsy presented a significant reduction in AVB incidence following the ischemia and reperfusion procedure. In addition, significant alterations were observed in ECG intervals during the stabilization and ischemia and reperfusion periods of rats with epilepsy compared to control rats. Our results indicated that the rats submitted to an epilepsy model induced by pilocarpine presented electrical conductivity alterations of the cardiac tissue, mainly during an AMI episode.

During the stabilization period, no abnormality in P wave or PR, RR, and QT intervals was observed in rats with epilepsy. However, a significant increase in the interval of the QRS complex was noted in these animals, which is indicative of conduction abnormalities. This result showed that the period of ventricular depolarization is

| Groups     | VA    | AVB   | Lethality |
|------------|-------|-------|-----------|
| Control    | 10/12 | 10/12 | 8/12      |
| Epilepsy   | 9/12  | 3/12* | 7/12      |

* $P<0.05$, compared to the control group (Fisher’s exact test).
already altered in the epilepsy group following stabilization. This alteration could be related to different factors, such as pathological fibrosis of the myocardium, morphological abnormalities of the cardiac conduction system, or an inappropriate autonomous nervous system response (3). Indeed, some studies have suggested that SUDEP cases in humans displayed significant fibrosis of the myocardium, which is known to be the substrate leading to discontinuous propagation and spatial dispersion of cardiac conduction (31,32). Although no pathological analyses have been performed in the present study, our experimental finding is in agreement with reports showing a widening QRS complex as a common cardiac abnormality in patients with epilepsy (33).

During the ischemia and reperfusion periods, significant alterations in PR and RR intervals between studied groups were not observed. However, changes in the incidence of AVB and in the P, QRS, and QT intervals were found during the periods of ischemia and/or reperfusion in animals with epilepsy. These results show that the period of depolarization is different between animals with epilepsy and controls in conditions of tissue injury. We cannot explain the exact mechanism of the decrease in AVB incidence in epileptic rats. However, it might be related, at least in part, to the KV 1.1 potassium channel, the deficiency of which leads to aberrant parasympathetic neurotransmission and consists of a candidate mechanism for AVB in epileptic animals (12). In the control group, increases in the duration of cardiac atrial or ventricular electrical activities (P wave and QT interval) occur during periods of ischemia and reperfusion compared to the stabilization period. In contrast, these enlargements, which could lead to atrial fibrillation or ventricular arrhythmias, such as torsades de pointes, were not observed in the epilepsy group during ischemia and reperfusion, leading to a significant difference between groups. This result is interesting and intriguing. It is possible that this particular ECG alteration in rats with epilepsy is related to a compensatory cardiac mechanism. Another possible explanation could be attributed to the seizure effects on gene expression. The molecular mechanism for the malignant brain-heart relationship has remained elusive until the recent discovery of arrhythmogenic mutations. Mutations in potassium (KCNH2, KCNQ1, KCNJ2) and calcium channel subunit genes have been identified to cause short QT syndrome (SQTS) (34), which is a rare, sporadic, or autosomal dominant disorder characterized by markedly accelerated cardiac repolarization and is manifested by a dramatically shortened QT interval, atrial and ventricular arrhythmias, and sudden cardiac death. Although it has been recognized for some time that prolongation of cardiac repolarization is a substrate for arrhythmias, the link between accelerated repolarization and arrhythmia has only recently been established (34).

Considering that variations in ventricular action potential can lead to increased or decreased QT interval and arrhythmias, it has been established that QT dispersion is a significant predictor of cardiovascular mortality (5,35). In our study in an animal model, a clear correlation between QT enlargement and high incidence of VA and deaths was found in the control group during ischemia and reperfusion. Although the high incidence of mortality in both epilepsy and control groups was noted, the lower QT interval did not modify the mortality incidence in the epilepsy group during ischemia and reperfusion, demonstrating that epilepsy did...
not represent a high risk of lethal arrhythmias in this model. Nevertheless, it is important to point out that the period of injury has been greatly extended (10 min of ischemia) in our experiments in order to observe significant differences in lethality between the groups. It is probable that a less aggressive stimulus could reveal more subtle differences. Despite this, conductivity alterations of the cardiac tissue were detected in rats with epilepsy following an ischemia and reperfusion protocol. Our results suggest that epilepsy can result in electrical conductivity alterations of the cardiac tissue, mainly during an AMI episode.

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