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ABSTRACT
Among the causes for sudden unexpected death (SUDEP) in epilepsy, the effects of antiepileptic drugs on the heart have been poorly explored. Based on this, the aim of our study was to evaluate the heart rate (in vivo and isolated ex vivo) and ventricular pressure (isolated ex vivo) of rats with and without epilepsy treated with carbamazepine. Four groups of adult, male Wistar rats (200-250 g) were studied: [A] control rats (n=8), received neither pilocarpine nor carbamazepine [B] carbamazepine-treated rats (n=8), received a daily dose of 120 mg/Kg, i.p. of carbamazepine for two weeks; [C] rats with epilepsy that received just saline solution (n=8); [D] rats with epilepsy that received a daily dose of 120 mg/Kg, i.p. of carbamazepine for two weeks (n=8). Our results showed significant increase in heart rate in animals with epilepsy (with and without the use of carbamazepine) when compared to the control groups in vivo. In contrast, we did not find differences during isolated ex vivo experiments comparing animals with and without epilepsy and despite the use of carbamazepine. Our results suggest that, in isolation, carbamazepine may not be a potential risk factor for sudden unexpected death in epilepsy.

Key words: epilepsy, heart, SUDEP, seizure, antiepileptic drugs, carbamazepine.

RESUMO
Entre as causas de morte súbita em epilepsia (SUDEP), os efeitos das drogas antiepilépticas no coração têm sido pouco explorados. Desta forma, o objetivo deste estudo foi avaliar a frequência cardíaca (in vivo e de forma isolada ex vivo) e a pressão ventricular (de forma isolada ex vivo) de ratos com e sem epilepsia tratados com carbamazepina. Quatro grupos de ratos Wistar machos adultos (peso 200 a 250 g) foram estudados: [A] ratos controle (n=8), não receberam pilocarpina ou carbamazepina; [B] ratos tratados com carbamazepina (n=8), receberam dose diária de carbamazepina de 120 mg/kg intraperitoneal, durante duas semanas (n=8); [C] ratos com epilepsia que receberam solução salina; [D] ratos com epilepsia que receberam dose diária de carbamazepina de 120 mg/kg intraperitoneal durante duas semanas. Nossos resultados evidenciaram uma diferença estatisticamente significativa na média da frequência cardíaca in vivo entre os animais com epilepsia (com e sem o uso de carbamazepina) quando comparados aos grupos controle in vivo. Em contraste, não observamos diferenças estatisticamente nos experimentos ex vivo quando comparados os animais com ou sem epilepsia, a despeito do uso da carbamazepina. Nossos resultados sugerem que, de forma isolada, a carbamazepina pode não ser um fator de risco potencial para a ocorrência de morte súbita em epilepsia.

Palavras-chave: epilepsia, coração, SUDEP, convulsão, drogas antiepilépticas, carbamazepina.
Epilepsy is the most common serious neurological condition. Epidemiological studies suggest that between 70 and 80% of people developing epilepsy will go into remission, while the remaining patients continue to have seizures and are refractory to treatment with the currently available therapies. The most common risk factors for epilepsy are cerebrovascular diseases, brain tumors, alcohol abuse, traumatic head injuries, cortical developmental malformations, genetic inheritance and central nervous system infections. In resource-poor countries, endemic infections, such as malaria and neurocysticercosis seem to be major risk factors.

Sudden unexpected death in epilepsy (SUDEP) is defined as sudden, unexpected, witnessed or unwitnessed, nontraumatic and nondrowning deaths in patients with epilepsy, with or without evidence of a seizure and excluding documented status epilepticus (SE), in which postmortem examination does not reveal a toxicological or anatomical cause of death. Comparisons of estimates of incidence for SUDEP are difficult as different definitions of SUDEP have been used, not all patients have postmortem examination, case ascertainment methods and source populations is varied. The incidence of SUDEP has been estimated as 3.5/1000 person-years in a lamotrigine clinical trial, 0.5-1.4/1000 person-years in people with treated epilepsy, 5.9/1000 person-years in outpatients with epilepsy at a tertiary referral centre, 9/1000 person-years in candidates for epilepsy surgery and 0.35/1000 person-years in a population-based study. The National General Practice Study of Epilepsy (NGPSE), a community-based study in the United Kingdom has seen the first case of SUDEP after 11,000 person-years of follow-up and the results of the Medical Research Council Antiepileptic Drug Withdrawal Study showed that SUDEP among patients with epilepsy in remission is a rare event. Information concerning risk factors for SUDEP is conflicting, but potential risk factors include: age, early onset of epilepsy, duration of epilepsy, uncontrolled seizures, mainly in the temporal lobe epilepsy, seizure frequency, seizure type, winter temperatures and antiepileptic drugs therapy (AEDs). The impact of AEDs on the risk of SUDEP has been evaluated from different angles: some studies have suggested more frequent AEDs changes or suboptimal AEDs dosage at the time of death in SUDEP patients, however, some studies failed to confirm these findings or detect a difference in compliance to AEDs therapy between SUDEP and non-SUDEP patients. Moreover, the potential arrhythmogenic side effect of carbamazepine has also been suspected to increase the risk of SUDEP, mainly when associated with plasma levels above 40 μmol/L, but this issue also remains controversial.

Considering the potential importance of carbamazepine (CBZ) on cardiovascular function that could culminate in SUDEP, the aim of our study was to analyze the heart rate and the ventricular pressure in vivo and isolated ex vivo of rats with epilepsy and treated with CBZ.

**METHOD**

**Animals**

Four groups of adult, male Wistar rats (200-250 g) were studied: [A] control rats (n=8), received neither picrotoxin nor CBZ; [B] CBZ-treated rats (n=8), received just a dose of 40 mg/Kg three times day, i.p. of CBZ for two weeks; [C] rats with epilepsy that received just saline solution (n=8); [D] rats with epilepsy that received a dose of 40 mg/Kg three times day, i.p. of CBZ for two weeks (n=8). It is important to note that the efficacy of CBZ dose (120 mg/kg) used in the present study was determined in a previous study evaluated by our research group. All animals used in our study were housed under standard controlled conditions (7:00 AM/7:00 P.M. light/dark cycle; 20-22°C; 45-55% humidity) with food and water ad libitum. All animals were treated according to protocols for animal care established by ethical committee from Federal University of São Paulo, and all efforts were made to minimize animal suffering. The project was approved by the local ethical committee.

**Induction of status epilepticus (SE)**

Adult male Wistar rats (250-300g) were given a subcutaneous (s.c.) injection of methylscopolamine (1 mg/kg; Sigma, St. Louis, MO) followed 30 minutes later by an i.p. injection of picrotoxin hydrochloride (350 mg/kg; Sigma, St. Louis, MO) to induce SE. Seizure activity was monitored considering animal behaviour and terminated with an i.p. injection of diazepam (10 mg/kg; Roche, Brazil) after 4 hr of convulsive SE. Only rats that displayed continuous, convulsive seizure activity for 4 hr after picrotoxin treatment and without significant hypoxia were included in this study. Sixty days after SE induction, all animals were fully recovered from status epilepticus and were selected for treatment with CBZ randomly. The rats treated with CBZ (Sigma, St. Louis, MO) started to receive a daily dose (120 mg/Kg, i.p.) during two weeks.

**Heart rate device**

To measure the HR we used a HR apparatus developed in our laboratory, which allowed us to monitor heart electrical activity without inserting fixed skin electrodes (Fig 1). This characteristic is very important because any contact to the body of the animal can stress it and consequently alter the HR. In brief, the animals were placed inside the box, which was previously cleaned with a solution of 1 mM of acetic acid. The procedure with acid acetate (followed by drying with paper towel) is fundamental.
to eliminate coming scents of the previous animal. Following animal's habituation (approximately 10 minutes) we started the commutation of the plus's cables, with the objective to obtain a sign that makes possible the recognition of the R-R interval of each animal. This procedure is very simple, taking approximately 15 minutes.

**Isolated ex vivo procedures**

**Solutions** – To wash the hearts, we used the Krebs-Henseleit solution (KH) composed by (in mM): sodium chloride 126.4; potassium chloride 4.6; KH$_2$PO$_4$ 1.2; magnesium sulfate 1.2; sodium bicarbonate 13.6; calcium chloride 1.5; GLUCOSE 11.11, pH 7.4 to 36°C, saturated with 95% of O$_2$, 5% of CO$_2$.

**Experimental camera** – The experimental camera used in our study was developed in our lab (Fig 2) and allows the recording of the experiments in a well controlled way. The camera’s principles are:

[A] The isolated heart is maintained under perfusion with physiologic solution in a retrograde way for the aorta (Preparation of Langendorf);
[B] The temperature of the solution is maintained (36°C);
[C] The solution is bubbled continuously with a carbogenic solution (95% O$_2$ + 5% CO$_2$);
[D] The pressure developed by the left ventricle during the systole is monitored.

**Ventricular pressure and heart rate** – As shown in Fig 3, a pressure transducer (TP) is coupled at a derivation of the bath that takes the perfusion solution to the heart in a close point to the stem. The pressure measured by the transducer during the contractions is amplified and registered in a digital oscilloscope (Tektronix, Inc, USA) with interface for printer. With this, we could characterize the mechanical activity of the hearts: the spontaneous heart frequency (Hz) and converted later for bpm,
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and the maximum pressure developed by the left ventricle (mmHg).

Preparation of the sample and experimental protocol – All animals were killed with a guillotine 74 days after the pilocarpine injection. The heart was removed and submerged in KH solution to clean its cameras, arteries and veins. After this, the aorta was canulated and then mounted in the experimental camera. The heart was retrograde perfused with KH (until to stabilize), that typically happened between 15 and 30 minutes. The registration obtained in the oscilloscope was printed and analyzed later.

Statistical analysis
To determine statistical differences between groups, we used a bifactorial variance analysis and the Turkey’s post-test. For each parameter (heart frequency and pressure) we considered statistical differences among the groups with p<0.05.

RESULTS
Pilocarpine treatment sequentially induced the following behavioral changes: akinesia, facial automatisms, and limbic seizures consisting of forelimb clonus with rearing, salivation, and masticatory jaw movements and falling. This type of behavior built-up progressively into motor limbic seizures that recurred repeatedly and rapidly developed into SE. After SE, animals were comatose or unresponsive to their environment and akinetic. Behavior returned to normal over a 3- to 5-day period. Spontaneous recurrent seizures in rats with epilepsy observed during the chronic period of the pilocarpine model of epilepsy were characterized by facial automatisms, forelimb clonus, rearing, loss of postural control and generalized clonic seizures lasting 40-60s. CBZ was introduced six day after pilocarpine injection and had a marked suppressant action on spontaneous seizures. Seizure frequency during baseline period was 1.96 seizure episodes per day per animal, 0.097 during CBZ treatment period and 2.09 during post-treatment period (p>0.05).

In the next step, the heart parameters (in vivo and isolated ex vivo procedures) were evaluated. Our results showed that HR in vivo (under influence of the autonomic nervous system) of animals with epilepsy treated with CBZ (346±28 bpm) and without CBZ treatment (348±16 bpm) were higher when compared with rats of the control groups (with CBZ: 307±20 bpm; and without CBZ: 309±17 bpm; p>0.05) (Fig 3). On the other hand, in the isolated ex vivo experiments, no significant differences in mean HR of rats with epilepsy treated with CBZ (151±50 bpm), without CBZ treatment (180±14 bpm) and control groups (with CBZ: 182±15 bpm; and without CBZ: 177±16 bpm) were observed (Fig 4A). In the same way, a similar result was observed for the parameter of ventricular pressure, i.e., rats with epilepsy and treated with CBZ (58±20 mmHg), without CBZ treatment (58±12 mmHg).
and control groups (with CBZ: 54±5 mmHg; and without CBZ: 56±10 mmHg) did not show statistical differences (Fig 4B).

**DISCUSSION**

The present study evaluated the heart rate (*in vivo* and isolated *ex vivo*) and ventricular pressure (isolated *ex vivo*) of rats with and without epilepsy treated with CBZ. Our results showed that rats with epilepsy had a significant increase in HR when compared to controls, probable secondary to functional autonomic changes that occur in cerebral function. Although, CBZ did not increase in a maintained way significantly the HR of rats studied during *in vivo* situation and did not alter the ventricular pressure during isolated *ex vivo* situation as well.

As we know, SUDEP is responsible for 7.5% to 17% of all deaths in epilepsy and has an incidence among adults between 1:500 and 1:1,000, and AEDs therapy is one of the many risk factors suggested for SUDEP. Although several studies suggested increase risk of SUDEP with increasing number of AEDs used, a very few studies have made attempts to verify if there is an association between specific AEDs, cardiovascular abnormalities and hence SUDEP. Furthermore, it has been shown that CBZ is able to affect the conduction system of the heart and the autonomic nervous system as well. Although our study did not find HR and ventricular pressure differences with CBZ administration during *in vivo* and isolated *ex vivo* situation, a number of arguments might be put forward. To do so, interesting studies developed by Timmings showed that CBZ was disproportionately represented in patients suffering SUDEP. Thus, the authors demonstrated that 11 of the 14 SUDEP (79%) patients were taking CBZ at the time of death. Moreover, the results also indicated that one patient was not taking any drug therapy, and died during his first seizure, reducing the number of evaluable ‘drug usage’ patients to 13, and increasing the proportion taking CBZ at the time of death to 85%. As possible mechanisms, Timmings suggests that CBZ induced lengthening of the ECG Q-T interval combined with a mild pro-arrhythmic effect of epileptic seizure discharges, and consequent transient cardiac instability leading to arrhythmic death. It is important to note that in these studies, no attempts were made to control and associate SUDEP cases for other recognized potential SUDEP risk factors, such as duration of epilepsy, seizure frequency, age, polytherapy and winter temperatures. In parallel, Nilsson and colleagues demonstrated that high CBZ plasma levels (above 40 μmol/l) was a risk factor for SUDEP, point to the risks associated with unstable severe epilepsy. Although this result was not confirmed in the U.S. study, we have to be in mind that CBZ is metabolized by hepatic P450 isoenzyme CYP 3A and when drugs that inhibit CYP 3A are administered together, plasma levels of parent drug may increase, thus leading to further cardiovascular abnormalities (lengthening of the QT interval) and increasing the risk of sudden death.

In contrast to CBZ as potential risk factor to SUDEP, there is a growing body of clinical studies in agreement with our experimental results suggesting that CBZ, at therapeutic levels, has no or minimal effects on the heart conduction system, supporting its safe use in the absence of cardiac disease. In fact, three larger controlled studies described that the use of CBZ was not increased in SUDEP cases when compared with control subjects. Following this, it is reasonable to think that CBZ may not precipitate prolongation of the QT interval into SUDEP, but patients are still at risk for cardiovascular alterations for other reasons such as co-medication with other drugs, electrolyte imbalance and hepatic or renal impairment.

SUDEP is usually unwitnessed and the majority of people dying with SUDEP are found in or near the bed, presumably during sleep. Quite interesting, Hennessy and colleagues described that abrupt withdrawal of CBZ leads to enhanced sympathetic activity in sleep as evidenced by changes in heart rate variability, suggesting that increased sympathetic activity in the setting of seizure-induced hypoxia could predispose to SUDEP. Although our study has only considered the heart rate parameters during CBZ treatment and we did not find any cardiovascular dysfunctions in our set of experiments, this question should not be neglected. As we know, hyponatremia is a usual electrolyte disorder and is defined as serum sodium <135 mmol per liter. Because of the fact that sodium is the dominant electrolyte of the extracellular fluid and the primary determinant of serum osmolality, the disturbances of sodium homeostasis can lead to pathologic alterations in cell functions. It has been determined that diuretics are the most common drugs which induce hyponatremia, but there are a few case reports in literature in which hyponatremia is induced by another therapy, including CBZ treatment. Taken together, this electrolytic anomaly can derive in secondary cardiovascular effects, in so outstanding occasions, as to produce the phenomenon denominated SUDEP.

According to the current knowledge, improved seizure control seems to be one of the most important measures to prevent SUDEP; however, AEDs (mainly polytherapy management) are equally dangerous regarding SUDEP. Finally, further studies are needed to gain a better understanding of the role of CBZ as a potential risk factor to SUDEP, but in the mean time caution with CBZ continuous to be prudent and necessary.

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