

IS COLD THE NEW HOT IN SUDDEN UNEXPECTED DEATH IN EPILEPSY?

Effect of low temperature on heart rate of rats with epilepsy

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Abstract – Sudden unexpected death in epilepsy (SUDEP) is the commonest cause of seizure-related mortality in people with refractory epilepsy. Several risk factors for SUDEP are described; however, the importance of including low temperatures as risk factor for SUDEP was never explored. Based on this, the aim of this study was to evaluate the heart rate of rats with epilepsy during low temperature exposure. Our results showed that low temperature clearly increased the heart rate of rats with epilepsy. Taken together, we concluded that exposure to low temperatures could be considered important risk factors from cardiovascular abnormalities and hence sudden cardiac death in epilepsy.

KEY WORDS: epilepsy, seizure, SUDEP, low temperatures, heart.

É o frio responsável por mortes inesperadas em epilepsia? Efeito de temperaturas baixas na frequência cardíaca de ratos com epilepsia.

Resumo – A morte súbita e inesperada nas epilepsias (SUDEP) é considerada a maior causa de morte em indivíduos com epilepsia refratária. Vários fatores de risco para SUDEP têm sido descritos, no entanto, a inclusão das baixas temperaturas como um possível fator de risco para SUDEP não foi verificada até o momento. Nesse sentido, o objetivo desse estudo foi verificar a frequência cardíaca de animais com epilepsia expostos as temperaturas baixas. Nossos resultados demonstraram que as baixas temperaturas são capazes de aumentar significativamente a frequência cardíaca de animais com epilepsia. Dessa forma, concluímos que as baixas temperaturas podem ser consideradas um importante fator de risco de possíveis alterações cardiovasculares e até mesmo morte súbita cardíaca nas epilepsias.

PALAVRAS-CHAVE: epilepsia, crises epilépticas, SUDEP, baixas temperaturas, coração.

The epilepsies are the most common serious neurological condition. In developed countries, the incidence of epilepsy is around 50 per 100 000 people per year, and is higher in infants and elderly people^{1,2}. The most commonly reported etiological factors are tumors, head injuries, stroke, genetic inheritance and infections of the central nervous system^{1,3}. In resource-poor countries, endemic infections, such as neurocysticercosis and malaria seem to be major risk factors¹. Moreover, people with epilepsy are two- to three-fold increase to die prematurely than those without epilepsy and the most common epilepsy-related category of death is sudden unexpected death in epilepsy (SUDEP).

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 post mortem examination does not reveal a toxicological or anatomical cause of death⁴. Information concerning risk factors for SUDEP is conflicting, but potential risk factors include: age, early onset of epilepsy, duration of epilepsy, uncontrolled seizures, seizure frequency, seizure type and antiepileptic drugs number⁵. The exact pathophysiological causes of SUDEP are unknown, but it is very probable that cardiac abnormalities during and between seizures might play a potential role⁶. Furthermore,

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several studies have related an increase in cardiac events during winter temperatures and it has been known that exposure to low temperatures is considered to be one of the main factors influencing morbidity and mortality from cardiovascular diseases, including sudden death⁷. At the moment, there is no information in the literature that describes with precision a possible relationship between cold weather, cardiovascular abnormalities and SUDEP. In this sense, our research group was the first to hypothesize that low temperatures may lead to cardiovascular abnormalities and hence SUDEP and, consequently, to act as potential risk factor for this epilepsy-related category of death⁸.

In accordance to this hypothesis, the aim of the present study was to evaluate the heart rate (HR) of rats with and without epilepsy during exposition to low temperature.

METHOD

Animals

Adult male Wistar rats (n=15, 220–280g) were housed under standard controlled conditions (7:00 a.m./7:00 p.m. light/dark cycle; 20–22°C; 45–55% humidity) with food and water ad libitum. The SE were induced according to the procedure describes previously⁹. In brief, 30 minutes after methylscopolamine injection (1 mg/Kg, s.c. – used to reduce the peripheral effects of pilocarpine), pilocarpine injection (350 mg/Kg, i.p.) was administered to rats. Only rats that displayed continuous, convulsive seizure activity after pilocarpine treatment were included in these studies. Seizure activity was monitored behaviorally and terminated after 4 h of convulsive SE with an i.p. injection of diazepam (10 mg/Kg; Roche, Brazil). After that, the animals were video-monitored (24 h per day) to detect the first spontaneous recurrent seizures (SRSs) (chronic phase of the pilocarpine model of epilepsy)⁹. Following first SRSs, we performed the HR parameters of rats with epilepsy (n=05) under the following environmental temperature conditions. Firstly, rats with epilepsy were placed in laboratory cages at room temperature (RT) ($22\pm 1^\circ\text{C}$) for

2 hours. They were then quickly transferred to HR device for 25 minutes and we repeat these same procedures for three consecutive days. In the next week, the same rats were placed in laboratory cages at cold temperature (CT) ($14\pm 1^\circ\text{C}$) for 2 hours. They were then quickly transferred to HR device for 25 minutes and we repeat these same procedures for three consecutive days as well. It is important to note that the control animals, control rats that received just saline solution (n=5) and control rats that received just methylscopolamine (1 mg/Kg, s.c.) (n=5) were submitted to the same procedures for RT and CT as described above.

Heart rate device

To measure the HR of rats with epilepsy, we used a HR apparatus developed in our laboratory (Fig 1). This apparatus allows to monitor the electric activity of the heart without fix the electrodes on animal's body¹⁰. 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 cleaning with acid acetic (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 cables, with the objective to obtain a sign that makes possible the recognition of the R-R interval of each animal (Fig 2). This procedure is simple, taking approximately 15 minutes.

Statistical analysis

To determine if differences between groups were significant, we used in this study the two-way ANOVA and the Bonferro-ni post-test. We considered statistical differences among the groups with $p < 0.05$.

RESULTS

Pilocarpine treatment sequentially induced the following behavioral changes: akinesia, facial automatisms,

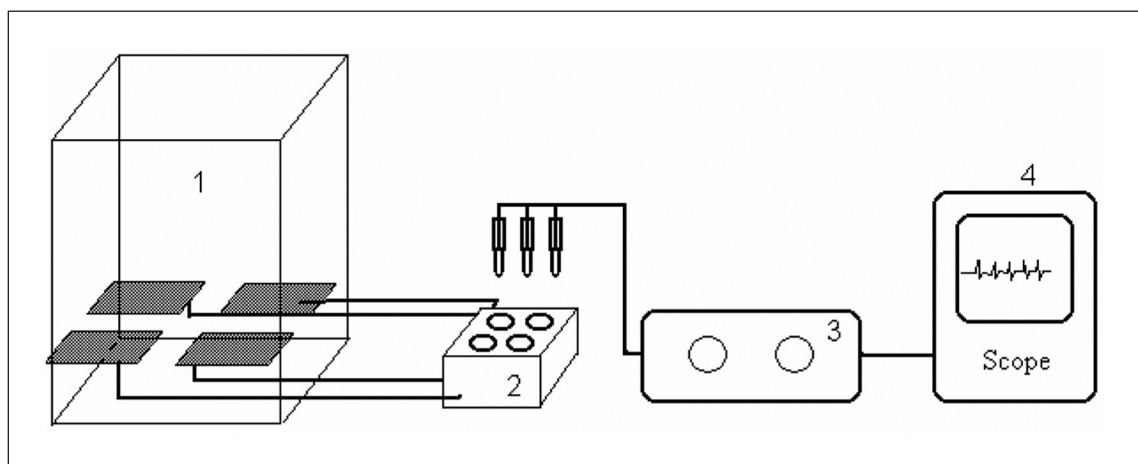


Fig 1. Heart rate measurement apparatus: 1) acrylic box with electrodes; 2) switch of the electrodes; 3) amplifier; 4) scope.

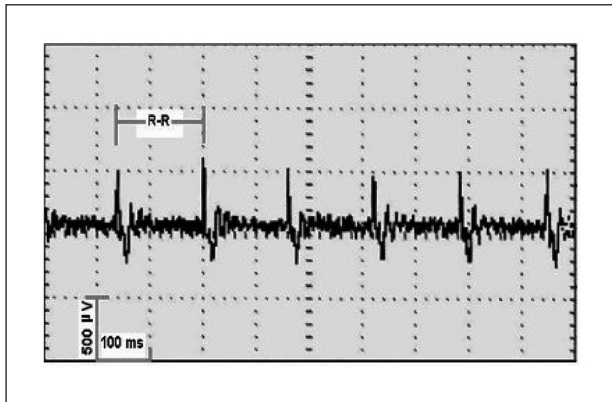


Fig 2. Typical signal obtained in adult male Wistar.

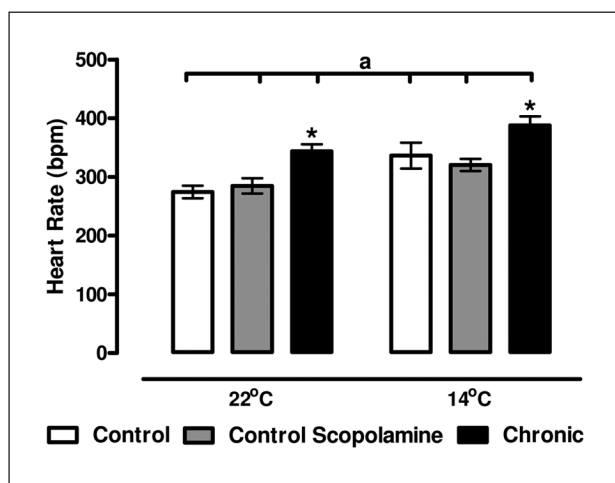


Fig 3. The figure shows the mean and standard deviation of heart rate of all groups under different temperatures. a represents that the heart rate of all groups are significantly higher at 14°C ($p < 0.01$). The * show that the heart rate of rats with epilepsy are higher under both temperatures when compared with other groups ($p < 0.01$).

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^{9,11,12}. SRSs 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 40–60s⁹. In the next step, the behavioral and HR analysis of rats with epilepsy exposed to RT and CT conditions were evaluated. At RT condition, rats with epilepsy presented exploratory activity during the first minutes, followed by a period of habituation, when the animals remained quiet and the HR measurement performed (343 ± 12 bpm) (Fig 3).

In the CT condition, rats exhibited moderate shivering, pilomotor responses and vigorous movements in the cage. Following 10 minutes (habituation of the rats in the box) the animals remained still and then the HR was measured (388 ± 15 bpm) (Fig 3). Control animals (rats that received just saline solution and rats that received just methylscopolamine) presented similar behavior of animals with epilepsy to RT and CT conditions. The HR of control rats that received saline solution was 274 ± 10 bpm to RT and 336 ± 22 bpm to CT (Fig 3). The HR of control rats that received just methylscopolamine was 284 ± 13 bpm to RT and 320 ± 10 bpm to CT (Fig 3).

DISCUSSION

This study evaluated the HR of rats with and without epilepsy during low temperature exposure. Our results showed that low temperature clearly increased the HR of all rats studied. As mentioned before, epilepsy is a chronic disorder that is associated with increased mortality and a common epilepsy-related category of death is sudden unexpected death. The exact pathophysiological causes of SUDEP are unknown, but it is very probable that cardiac abnormalities during and between seizures play a potential role⁶. In accordance to this reasoning, our group started some studies aimed to explore this question. We evaluated the HR (*in vivo* and isolated *ex vivo*) and the ventricular pressure isolated *ex vivo* of rats with epilepsy¹⁰. The results of our previous study showed a significant increase of heart rate *in vivo* in the animals with epilepsy when compared with control group. In contrast, we did not find differences during isolated *ex vivo* experiments, suggesting a central nervous system modulation on the heart, which could explain the sudden unexpected death in epilepsy. In the present study, we also found an increase of heart rate of rats with epilepsy during *in vivo* situation that was exacerbated when the animals were exposed to cold temperatures. In these lines, as cardiac abnormalities play an important cause in SUDEP, we postulated that low temperatures may lead some cardiac abnormalities and hence SUDEP. As research in this field must be guided by the potential for the prevention of SUDEP, a number of arguments might be put forward.

It has been established that exposure to low temperatures is considered one of the main risk factors from cardiovascular abnormalities and hence sudden death⁷. In this way, it is interesting to review some findings that explain an increase in cardiac events to cold temperatures: 1) There is 53% more cases of acute myocardial infarction reported during the winter compared with the summer¹³. In these lines, most of reports, but not all, have demonstrated a significant winter increase in cardiovascular abnormalities and cardiac death, especially in the

northern hemisphere, where there are winter temperature extremes^{14,15}. Quite interesting, sudden cardiac death have been also reported in regions with most comfortable winter.^{16,17} 2) During the winter, increases in haemoconcentration (erythrocyte count, plasma cholesterol and plasma fibrinogen levels) have been reported, which could contribute to arterial thrombosis¹⁸. In this way, rapid coronary deaths could result from rupture of atheromatous plaques during hypertension and cold-induced coronary spasm^{19,20}. 3) Cold weather can induce a higher systemic vascular resistance with an increase of the blood pressure (thus increasing oxygen demand). This study was evaluated by Argiles and colleagues²¹ that determined, during a four-year period, the influence of climate on blood pressure in 53 patients with end-stage renal disease treated with haemodialysis. The authors showed that the mean (\pm SE) systolic and diastolic blood pressure was highest during the winter ($153\pm 3/82\pm 2$ mm Hg) and lowest during the summer ($141\pm 3/75\pm 2$ mm Hg). 4) The winter temperatures may be associated with flu season and an increase of upper respiratory tract infections could place stress on the heart²². Based on this fact, an interesting explanation developed recently²³ should be considered. According to Meyers²³, cardiovascular abnormalities (acute myocardial infarction and atherothrombotic stroke) and hence sudden cardiac death have their peak incidence in winter months. Similarly, the incidence of upper respiratory infections (URIs), 38% of which are due to influenza, also peaks in winter period (November and December). Based on this, as influenza vaccination is an extremely safe procedure and three epidemiologic and one small clinical trial showed that influenza vaccination is associated with a 50% reduction in incidence of cardiovascular abnormalities and sudden cardiac death, influenza vaccination (each year) is able to prevent this cardiac pathogenesis²³. Taken these data together and the results of our present experimental study, we believe that cold weather could be considered a new potential risk factor of sudden cardiac death in patients with epilepsy. Moreover, we are sure that there is an urgent need for several studies (large-scale, prospective, community-based and international) of sudden cardiac death in epilepsy to explore deeply the risk factors to plan preventive strategies.

At the moment, some risk factors for sudden unexpected death in patients with epilepsy have been proposed⁵, but there is very little information in the literature that describes the relationship between winter temperatures and sudden cardiac death in epilepsy⁸. In accordance with this reasoning, we believe that as the modifiable risk factors for cardiovascular abnormalities and sudden death are so prevalent within the epileptic population, it is important for clinicians treating patients with epilepsy to

know what these risks are and understand how they can contribute to increased mortality in these patients.

In the mean time, some actions (medical or non-medical therapies) may help to prevent sudden cardiac death in epilepsy. For that, Kloner²⁴ described some very interesting commonsense and prudent tactics that the physician should consider during the winter time (called "Merry Christmas Coronary" and "Happy New Year Heart Attack"), especially for patients with established cardiac disease or for those with known risk factors for cardiac disease. Despite considerable suggestions and evidences, Keating and Donaldson have also suggested that reduction of outdoor cold stress has been largely ignored in official campaigns to control winter mortality²⁵. In these lines, they purposed that heating of waiting areas for public transports, and at least windproof shelters on bus routes subject to unscheduled delays, are obvious measures that would help²⁵.

Finally, the next logical steps to us, epileptologists, are to understand and associate the mechanisms by which cold weather could influence the cardiovascular system of patients with epilepsy. These mechanisms are likely to be important for developing new strategies in the prevention of sudden cardiac death in epilepsy.

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