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Intense olfactory stimulation blocks seizures in an experimental model of epilepsy

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ABSTRACT

There are reports of patients whose epileptic seizures are prevented by means of olfactory stimulation. Similar findings were described in animal models of epilepsy, such as the electrical kindling of amygdala, where olfactory stimulation with toluene (TOL) suppressed seizures in most rats, even when the stimuli were 20% above the threshold to evoke seizures in already kindled animals. The *Wistar Audiogenic Rat* (*WAR*) strain is a model of tonic–clonic seizures induced by acute acoustic stimulation, although it also expresses limbic seizures when repeated acoustic stimulation occurs – a process known as audiogenic kindling (AK). The aim of this study was to evaluate whether or not the olfactory stimulation with TOL would interfere on the behavioral expression of brainstem (acute) and limbic (chronic) seizures in the *WAR* strain. For this, animals were exposed to TOL or saline (SAL) and subsequently exposed to acoustic stimulation in two conditions that generated: I) acute audiogenic seizures (only one acoustic stimulus, without previous seizure experience before of the odor test) and II) after AK (20 acoustic stimuli [2 daily] before of the protocol test). We observed a decrease in the seizure severity index of animals exposed only to TOL in both conditions, with TOL presented 20 s before the acoustic stimulation in both protocols. These findings were confirmed by behavioral sequential analysis (neuroethology), which clearly indicated an exacerbation of clusters of specific behaviors such as exploration and grooming (self-cleaning), as well as significant decrease in the expression of brainstem and limbic seizures in response to TOL. Thus, these data demonstrate that TOL, a strong olfactory stimulus, has anticonvulsant properties, detected by the decrease of acute and AK seizures in *WAR*s.

1. Introduction

Epilepsy is a chronic neurological disease characterized by the presence of any of the following conditions: (1) at least two unprovoked seizures (or reflex) occurring in >24 h apart; (2) one unprovoked seizure (or reflex) with probability (at least 60%) of occurrence of further similar seizures in the next 10 years; or (3) in case of diagnosis of an epilepsy syndrome [1]. Seizures are characterized by abnormal excessive activity and/or synchrony of brain neurons [2–5], normally self-limited [5].

According to the World Health Organization, the epilepsies affect approximately 50 million people worldwide [6], representing 1–2% of the world population [3,7,8]; being the Temporal Lobe Epilepsy (TLE) as the most common epilepsy syndrome in adults [8–10]. Its occurrence is preceded by an early life insult followed by a latent period, when seizures are not usual, but changes occur in the structure and physiology of the brain that predispose to development of subsequent seizures [3,11].

Abbreviations: AK, Audiogenic Kindling; cm, Centimeters; cSI, Categorized Brainstem Severity Index; EEG, Electroencephalogram; LI, Limbic Index; NMDA, *N*-Methyl-D-Aspartate; PRE, Periods before acoustic stimuli, with duration of 01 min; POST, Periods after acoustic stimuli or TCV, also with duration of 01 min; PTZ, Pentylenetetrazol; s, Seconds; SAL, Saline; SAL-AK 60 group, Animals in which the acoustic stimulation was performed 60 s after SAL exposure; SAL-NS group, Saline group; SAL-SOUND group, Animals exposed to SAL with subsequent acoustic stimulation; SEM, Standard Error of the Mean; SOUND, Periods of acoustic stimuli, with maximum duration of 1 min or until the TCV; TCV, Tonic–Clonic Convulsion; TOL, Toluene; TOL-NS group, Toluene group; TOL-AK 20, Animals in which the acoustic stimulation was performed 20 s after TOL exposure; TOL-AK 60, Animals in which the acoustic stimulation was performed 60 s after TOL exposure; TOL-SOUND group, Animals exposed to TOL with subsequent acoustic stimulation; TLE, Temporal Lobe Epilepsy; TMT, 2,5-dihydro-2,4,5-trimethylthiazoline; *WAR*, *Wistar Audiogenic Rat*; WR, Wild running; $\chi 2$, Chi-Square Test.

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Pharmacological and surgical treatments represent an important therapeutic option for seizure control by preventing seizure recurrence [8,12], reversing motor and cognitive consequences, and enhancing quality of life [8]. Among 20–40% of individuals with TLE are resistant to current pharmacological treatments [6,13–16], and an important part of these is noneligible for surgery [17–19]. Although much has been made toward the improvement of epilepsy diagnosis and treatment, the proportion of patients with refractory seizures (those unresponsive to medical therapy), in addition to the magnitude of the negative socioeconomic impact of the epilepsies, evidences the need of a strongly integrated basic and clinical research to develop new therapies [12].

The involvement of olfactory structures with TLE is known for quite some time [20,21]. Some studies have shown that olfactory sensations could indicate the paroxystic seizure onset in patients with TLE, a symptom described as aura [20–24], and that the peripheral sensory stimulation, such as an olfactory stimulus is able to modulate those seizures: suppressing or inhibiting [9,10,20,25–32] or even inducing them, such as in the reflex epilepsies [29,33–42].

In a landmark report, Efron [28] described that the olfactory stimulation was able to prevent the occurrence of seizures in a patient with epilepsy, producing satisfactory results even when the same patient with an aura of strong olfactory odor (jasmin) conditioned herself to that odor, in such a way that the simple evocation of the olfactory memory was able to prevent her seizure expression [28]. In that scenario, other alternative treatment for seizure control is the use of volatile plant oils, including essential oils [26,30] known as aromatherapy, which has been used to help people who have symptoms suggestive of early seizures (the auras) [26]. The use of certain essential oils can reduce the seizure occurrence or its severity, while others may trigger seizures [9,10]. Moreover, in India and eastern countries, it is described since ancient times—the exotic seizure control using the "shoe-smell" [9,10] as first aid technique, for example, in an emergency situation in the street, to block seizures.

In the electrical kindling of the amygdala, an animal model of TLE, Ebert & Löscher [37] showed that the exposure to olfactory stimulation for 15 s with toluene (TOL) suppressed seizures in most rats, even with electrical stimulation 20% above of the threshold. In other experimental models induced by pentylenetetrazol (PTZ) (200 mg/kg, in mice), the pretreatment with TOL by intraperitoneal injection prevented the occurrence of tonic extension phase and increased the latency to seizure onset depending on the dose. Moreover, TOL inhalation (2 h before injection of PTZ and 0 to 4 h after injection) in subconvulsant concentrations prevented the death of the animals after PTZ injection (110 mg/kg). In fact, in mice, PTZ induced convulsions characterized by the following behaviors: straub tail, clonus, tonic hindlimb extension, and death [32]. Thus, the effects of acute exposure to TOL can change the susceptibility to seizures depending particularly on the dose, as assessed in other models such as electroshock [43], seizures produced by *N*-Methyl-D-Aspartate (NMDA) [25] or those induced by manipulations of different ligand-gated ion channels [44]. In general, lower doses of TOL may protect against seizure events, while higher doses are known to induce convulsive effects [43].

In this context, it is essential to (A) validate the effectiveness of TOL in the therapeutic response (anticonvulsant substance) at least in other animal models of epilepsy, (B) establish parameters for administration, (C) provide insight "tools" for preclinical research to facilitate the development of new therapies, especially for those patients whose seizures are refractory or resistant, and (D) stimulate discussion between the scientific and pharmaceutical communities in order to promote the research on the anticonvulsant action mechanisms of this compound. In fact, more than the TOL molecule itself, the use of the olfactory stimulation (odor therapy) to modulate or even block seizures is an important issue to be understood, as a potential anticonvulsant and antiepileptogenic mechanism.

The induction of seizures with genetic animal models of reflex epilepsy to evaluate new antiepileptic drugs is a strategy widely used [45]. The *Wistar Audiogenic Rat* (*WAR*) [46,47] is an audiogenic reflex epilepsy animal model, derived from inbreeding of genetically selected rats susceptible to audiogenic seizures. In this strain, the acute high intensity acoustic stimulation induces brainstem-dependent seizures which are behaviorally expressed by running in circles, jumping, atonic falling (wild running (WR)), and Tonic–Clonic Convulsion (TCV) with both activation of the inferior and superior colliculus [46,48–52]. Beyond that, with repeated acoustic stimuli (audiogenic kindling (AK)), these animals present brainstem seizures, but there is also recruitment of new circuits over time with the presence of limbic seizures [47,51,53–60] behaviorally expressed by facial automatisms and myoclonic spasms of head, fore-, and hind legs, in addition to rearing and falling. So, the *WAR* strain is an important experimental model with predisposition to two modalities of seizures, representing an important advantage in the study of the neurobiology of epilepsies, particularly to test potential therapies, such as the response to an olfactory stimulus, in this case of TOL.

Considering the evidence from the literature about the control or blockade of seizures by olfactory stimulation, in patients and rodents, we, thus, aimed to evaluate whether or not the olfactory stimulation with TOL would interfere on the behavioral expression of brainstem (acute) and limbic (chronic) seizures in the *WAR* strain.

2. Methods

2.1. Ethics statement

All the experimental protocols of this study were carried out according to the recommendations for animal experimentation of the Brazilian Society for Neuroscience and Behavior that are based on international guidelines on the ethical use of animals, from the Society for Neuroscience. The experimental protocols were approved by the Ethics Committee in Animal Research of Ribeirão Preto School of Medicine, University of São Paulo (Protocol: 172/2010). All efforts were made to minimize the suffering of the animals.

2.2. Animals

Male *WAR*s (n = 70), weighing 250–350 g, were bred and maintained at the Vivarium of the Physiology Department of the Ribeirão Preto School of Medicine. The animals were kept in controlled temperature (23 ± 2 °C) on light/dark cycle of 12/12 h (light on at 7 a.m. and light off at 7 p.m.), with access to food and water ad libitum and stored in polyethylene cages with chrome iron cover (40.5 × 33.5 × 21 cm) in groups of up to 6 rats per cage. After the experimental procedures (described below), the rats were euthanized.

2.3. Protocol 1: TOL effect on acute audiogenic seizures

Male *WAR*s (n = 35) were exposed for 15 s to 0.9% saline (SAL) or TOL. Another group (n = 12) of *WAR*s was only exposed to the odorants [groups: SAL (n = 6) and TOL (n = 6)], to identify and evaluate their behavior. The other 23 animals were exposed to SAL or TOL and, 20 s later, were submitted to acoustic stimulation (see item: 0 [groups: SAL-SOUND (n = 10) and TOL-SOUND (n = 13)]).

2.4. Protocol 2: TOL effect in AK

Thirty-five male *WAR*s underwent the AK (see item: 2.7). Before the 21st acoustic stimulation, each rat inhaled 0.9% SAL, for 15 s (group: SAL-AK 60, n = 6) or TOL, and the acoustic stimulation was performed 20 s or 60 s after TOL exposure (groups: TOL-AK 20, n = 9 and TOL-AK 60, n = 9). Therefore, only the 24 animals that displayed limbic seizures during the AK were tested on the 21st stimulation.