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Effects of transcranial focal electrical stimulation alone and associated with a sub-effective dose of diazepam on pilocarpine-induced status epilepticus and subsequent neuronal damage in rats

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ABSTRACT

Experiments were conducted to evaluate the effects of transcranial focal electrical stimulation (TFS) applied via tripolar concentric ring electrodes, alone and associated with a sub-effective dose of diazepam (DZP) on the expression of status epilepticus (SE) induced by lithium-pilocarpine (LP) and subsequent neuronal damage in the hippocampus. Immediately before pilocarpine injection, male Wistar rats received TFS (300 Hz, 200-μs biphasic square charge-balanced 50-mA constant current pulses for 2 min) alone or combined with a sub-effective dose of DZP (0.41 mg/kg, i.p.). In contrast with DZP or TFS alone, DZP plus TFS reduced the incidence of, and enhanced the latency to, mild and severe generalized seizures and SE induced by LP. These effects were associated with a significant reduction in the number of degenerated neurons in the hippocampus. The present study supports the notion that TFS combined with sub-effective doses of DZP may represent a therapeutic tool to induce anticonvulsant effects and reduce the SE-induced neuronal damage.

1. Introduction

Epilepsy is one of the most common brain disorders worldwide with no age, racial, social class, national nor geographic boundaries. It affects about 67 million people, 85% of whom live in developing countries [1]. Despite decades of research, new antiepileptic drugs (AEDs) and advances in surgical therapy, many patients suffer from refractory epilepsy or the side effects of AEDs and surgical treatment [2].

Pharmacoresistant epilepsy, as well as other conditions such as brain tumors, ischemic brain injury and alcohol withdrawal, is associated with an increased likelihood of status epilepticus (SE), a neurologic emergency that requires immediate vigorous treatment in order to prevent brain injury [3–6]. Yet, strategies to prevent SE and its consequences in patients at high risk for SE are limited. Notably, the efficacy of diazepam (DZP) and similar first-line abortive SE treatments is incomplete and SE often continues after administration of these drugs [7,8]. Indeed, many of the medications used to stop SE have several well-known and potentially serious adverse effects, such as respiratory depression, sedation, hypotension and cardiac dysrhythmias [9].

The use of brain stimulation in the treatment of pharmacoresistant epilepsy has a long history, but few studies have focused on its acute effects to prevent SE and its consequences. Indeed, it is suggested that the protocols effective in the termination of SE are different from those used in the prevention of seizures [10].

We previously demonstrated that noninvasive transcranial focal electrical stimulation (TFS) was able to reduce the expression of pilocarpine-induced SE in Sprague–Dawley rats when applied during seizure activity via tripolar concentric ring electrodes (TCREs) (Fig. 1) [11]. The effects of TFS may last for hours and are associated with desynchronization at the beta and gamma frequencies, but not with motor contractions or pain [12,13]. These results support the notion that TFS has the potential to be a viable noninvasive therapy for SE. However, at present, it is unclear if TFS is able to prevent the SE and the subsequent neuronal damage.

The identification of therapeutic strategies that prevent SE and its consequences constitutes a major clinical need. Therefore, for the present study, we investigated if TFS associated with DZP may represent a good approach to avoid the expression of this disorder and the subsequent neuronal damage. Experiments in rats were designed to investigate if TFS alone or associated with a sub-effective dose of DZP was able to prevent the lithium-pilocarpine-induced (LP) SE and consequent cell damage in the hippocampus when applied before the pilocarpine injection. We studied the hippocampus because it is an area of the brain prone to generating seizure activity [14] and presents

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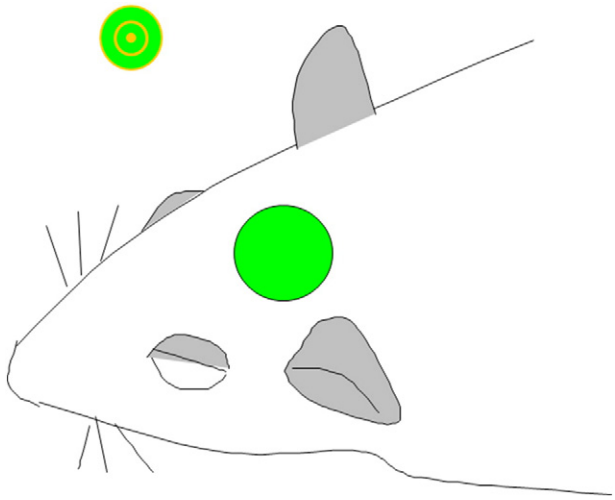

Fig. 1. Schematic representation of the electrode placement. The TFS was applied between the outer ring and the central disc of electrode.

early and late cellular events associated with neuronal damage and cognitive impairment following pilocarpine-induced SE [15,16].

2. Methods

2.1. Animals

Adult male Wistar rats initially weighing 250–300 g were used in the present study. They were individually housed at 22 °C and maintained on a 12-h light/dark cycle. Rats had free access to food and water. Procedures involving animal care were conducted in agreement with the Mexican Official Standard (NOM-062-ZOO-1999) and the Ethical Committee of the Center for Research and Advanced Studies (Protocol #222/04).

2.2. Transcranial focal electrical stimulation

The TFS consisted of 200-μs symmetrical biphasic square charge-balanced constant current pulses at a rate of 300 Hz and at an intensity of 50 mA. We employed a custom stimulator designed and built by our group, with programmable frequency, phase, and time duration of the TFS output signals. The TFS was controlled by a Parallax Basic Stamp 2P24® which had the specific TFS pattern pre-programmed to run automatically for 2 min when triggered [11,12].

The TCRE was placed on the shaved scalp centered on the top of the head, as close to 5 mm behind the bregma as possible. Approximately 2 mm of Ten-20 electrode paste was used for skin-to-electrode impedance matching. Then, TFS was applied through the outer ring (external diameter of 1 cm) and disc of a TCRE (with the middle ring floating).

2.3. Experimental groups

2.3.1. LP-TFS + DZP group ($n = 13$)

Rats received daily administration of saline solution (1 ml/kg, i.p.) for 5 days to habituate them to manipulations. Twenty-four hours after the last saline injection, the animals received lithium chloride (3 mEq/kg, i.p.). Twenty-four hours later, the scalp was shaved and TFS was applied as described above. Immediately after, the animals received the administration of pilocarpine (35 mg/kg, i.p.) and a sub-effective dose of DZP (0.41 mg/kg, i.p.). This sub-effective dose of DZP, defined as the dose reducing 30% or less the number of animals presenting LP-induced severe generalized seizures, was determined from dose–response studies carried out in our laboratory (data not shown). Then, the following parameters were assessed during 3 h of continuous behavioral monitoring by an author blinded to the treatment condition: latency to the first forelimb clonus and generalized seizure, as well as establishment of SE, and percentage of animals presenting mild (rearing and upper extremity clonus) and severe generalized seizures (rearing, upper extremity clonus, and falling), as well as SE. We utilized the definition of SE commonly used in the rat pilocarpine model, i.e., continuous motor seizures (stage 3 to 5 seizures according to Racine [17]) persisting for at least 30 min and associated with unresponsiveness to any environmental stimuli [18].

2.3.2. LP-DZP group ($n = 10$)

Rats were manipulated as indicated previously for LP-TFS + DZP group, except that they did not receive TFS.

2.3.3. LP-TFS group ($n = 14$)

Animals were manipulated as described above for LP-TFS + DZP group, except that they received vehicle administration instead of DZP.

2.3.4. LP group ($n = 24$)

Animals were manipulated as described earlier for LP-DZP group, except that they received vehicle administration instead of DZP.

2.3.5. TFS group ($n = 5$)

Rats received TFS as described above for LP-TFS group followed by saline injection, instead of LP.

2.3.6. Control group ($n = 5$)

Animals were manipulated as described above for LP group, except that they received vehicle administration instead of LP.

Rats from all pilocarpine-treated groups that went into SE received an injection of DZP (10 mg/kg i.p.) 2 h after its onset to stop the seizures, standardize the duration of continuous seizure activity and reduce the mortality rate.

2.4. Histology

Animals that survived 24 h after LP-induced SE or manipulation were injected with an overdose of pentobarbital and were transcardially perfused with 0.1-M phosphate buffered saline (PBS) and 4% paraformaldehyde solution in PBS. Then, the brains were removed and postfixed for one week at 4 °C and processed for embedding in paraffin. Coronal sections were then cut (12-μm thickness) with the aid of a microtome (Leica RM2125 RT, Germany) and mounted onto gelatin-coated slides. The sections were deparaffinized and hydrated in water for their subsequent processing for Nissl and Fluoro-Jade (FJ) staining. Fluoro-Jade is a fluorescent marker that binds to irreversibly damaged neurons and allows identification of degenerating neurons [19].

Fluoro-Jade staining was performed as follows. The slides were first immersed in a solution containing 1% sodium hydroxide in 80% alcohol for 5 min. This was followed by 2 min of incubation in 70% alcohol and 2 min in distilled water. The slides were then transferred to a solution of 0.06% potassium permanganate for 20 min and then rinsed in distilled water for 2 min. Thereafter, the slides were incubated in FJ for 2 h. The 0.0001% working solution of FJ was prepared by adding 1 ml of stock FJ solution (0.01%) to 99 ml of 0.1% acetic acid in distilled water. Then, the slides were rinsed for 1 min in each of three distilled water washes and dried. The slides were immersed in xylene for 1 min and mounted in synthetic resin (Merck Lab.). Sections from the dorsal and ventral hippocampus corresponding to 3.30 mm and 5.60 mm from bregma, respectively, [20] were examined.