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Original Articles

Anti-epileptogenic Effect of High-frequency Stimulation in the Thalamic Reticular Nucleus on PTZ-induced Seizures

C.R. Pantoja-Jiménez [a,b], V.M. Magdaleno-Madrigal [a,b,*], S. Almazán-Alvarado [a], R. Fernández-Mas [a]

[a] Laboratorio de Neurofisiología del Control y la Regulación, Dirección de Investigaciones en Neurociencias, Instituto Nacional de Psiquiatría Ramón de la Fuente Muñiz, Mexico
[b] Carrera de Psicología, Facultad de Estudios Superiores Zaragoza-UNAM, Ciudad de México, Mexico

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ABSTRACT

Background: Deep brain stimulation, specifically high-frequency stimulation (HFS), is an alternative and promising treatment for intractable epilepsies; however, the optimal targets are still unknown. The thalamic reticular nucleus (TRN) occupies a key position in the modulation of the cortico-thalamic and thalamo-cortical pathways.
Objective: We determined the efficacy of HFS in the TRN against tonic-clonic generalized seizures (TCGS) and *status epilepticus* (SE), which were induced by scheduled pentylenetetrazole (PTZ) injections.
Methods: Male Wistar rats were stereotactically implanted and assigned to three experimental groups: Control group, which received only PTZ injections; HFS-TRN group, which received HFS in the left TRN prior to PTZ injections; and HFS-Adj group, which received HFS in the left adjacent nuclei prior to PTZ injections.
Results: The HFS-TRN group reported a significant increase in the latency for development of TCGS and SE compared with the HFS-Adj and Control groups ($P < 0.009$). The number of PTZ-doses required for SE was also significantly increased ($P < 0.001$). Spectral analysis revealed a significant decrease in the frequency band from 0.5 Hz to 4.5 Hz of the left motor cortex in the HFS-TRN and HFS-Adj groups, compared to the Control group. Conversely, HFS-TRN provoked a significant increase in all frequency bands in the TRN. EEG asynchrony was observed during spike-wave discharges by HFS-TRN.
Conclusion: These data indicate that HFS-TRN has an anti-epileptogenic effect and is able to modify seizure synchrony and interrupt abnormal EEG recruitment of thalamo-cortical and, indirectly, cortico-thalamic pathways.

Introduction

Epilepsy is a neurological disorder that affects 1–2% of the population. Epilepsy treatment is normally initiated after two or more unprovoked epileptic seizures. One-third of epilepsy patients do not respond effectively to anti-epileptic drugs and approximately 30–40% of adult patients remain refractory [1,2]. Surgical resection of the epileptogenic zone is only an option for a minority of patients [3]. Thus, an alternative treatment is needed for the remaining refractory patients. Recent studies have found that deep brain stimulation (DBS) is a safe and beneficial technique to treat medically intractable epilepsies [4].

The goal of DBS in epilepsy patients is to modify the excitability of a variety of structures involved in the initiation, propagation and maintenance of epileptic activity. Experimental studies of DBS in the cerebellum [5], thalamus [6], hippocampus [7], basal ganglia [8] and *nucleus tractus solitarii* [9,10], have been used to delay or abolish the secondary generalization of seizures. Human DBS studies have been performed in the cerebellum [11], the anterior and centromedian nuclei of the thalamus [12,13], the hippocampus [14,15], the *locus coeruleus* [16] and the subthalamic nucleus [17]. However, there are no current studies that have favored one target over another [18].

Although the optimal stimulation parameters are undetermined [19]; experimental [6,20] and clinical [21] studies suggest that high-frequency stimulation (HFS) $\geq$ 100 Hz produces a palliative effect

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* Corresponding author. Instituto Nacional de Psiquiatría Ramón de la Fuente Muñiz, Calz México-Xochimilco No. 101, Col. San Lorenzo Huipulco Del. Tlalpan, Ciudad de México, Mexico CP 14370. Tel.: +52 55 41605088; fax: +52 55 56559980.
E-mail addresses: maleno@imp.edu.mx, malenomad@gmail.com (V.M. Magdaleno-Madrigal).

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on seizures. Anatomical structures critical for arousal and EEG desynchronization could be potential targets for antiepileptic DBS [13,22]. However, there are few data available regarding the effect of thalamic reticular nucleus (TRN) electrical stimulation on epileptic seizures.

The TRN is composed of GABAergic cells [23,24] and occupies a key position in the cortico-thalamic (CT) and thalamo-cortical (TC) pathways [25,26]. The TRN receives monosynaptic glutamatergic inputs from the cerebral cortex and the thalamus and sends GABAergic projections to the thalamus [25,27,28]. The reticular neurons operate with two different firing modes [29]. The *relay* mode is associated with the processing of sensory information, corresponding to a desynchronized EEG state. The *oscillatory* mode is involved in cortical synchronization through the generation of rhythmic discharges, which are associated with rhythmic bursts of high frequency action potentials separated by silent periods that filter the incoming sensory information [30]. These oscillatory rhythmic properties may lead to a hypersynchronous activity pattern and generate spike-wave discharges (SWD) [31].

Chemical stimulation [32] and lesion studies [33] have demonstrated that the TRN generates and modulates the occurrence of SWD in different rat strains [34]. In hippocampal kindling, electrical stimulation of the TRN at 60 Hz induces an EEG desynchronization and can act to suppress limbic motor seizures [35].

Pentylenetetrazole (PTZ) is a model of generalized seizures, displaying typical absence seizures, generalized myoclonic seizures and tonic-clonic generalized seizures (TCGS). This model represents a routine test for screening anticonvulsants [36] and may be used to induce *status epilepticus* (SE) in immature, adult and old rats [37]. The current study analyzes the efficacy of HFS in the TRN on the development of SWD, TCGS and SE provoked by gradual PTZ-doses.

Materials and methods

Animals

Sixteen Wistar male rats (280–320 g) were used in this study. They were born and raised in the vivarium of the Neurosciences Research Division at Instituto Nacional de Psiquiatría Ramón de la Fuente Muñiz, México, D.F. The experiments were performed in accordance with the technical guidelines for the production, care and use of animals in the laboratory issued by SAGARPA (NOM-062 ZOO-1999) and approved by the ethics committee of the Instituto Nacional de Psiquiatría Ramón de la Fuente Muñiz.

Surgical procedure

Surgery was performed using ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (5 mg/kg) i.m. All animals were placed in a stereotactic apparatus (Mod. 1430, David Kopf Instruments, Tujunga Ca) to expose the cranium and to determine the electrode positions. Stainless steel tripolar electrodes were implanted into the left TRN at the following dimensions, according to Paxinos and Watson [38]: AP, −1.4; L, 1.7; H, 6.0. Epidural EEG recording screws were implanted in the motor cortices (MCx) for EEG recordings and another was connected as a ground in the parietal bone. The electrodes were welded into a mini-connector and fixed to the skull with dental acrylic. The animals were treated with an analgesic (Butorphanol 0.4 mg/kg) and an antibiotic (Amoxicillin, 0.6 mg/kg) after the surgical procedures. After surgery, the animals were individually housed in cages (50 × 27 × 30 cm) with an ambient temperature of 23–25 °C and a 12:12-h light–dark cycle. All animals were allowed to recover for 7 days and were provided with food and water *ad libitum*.

Electrical stimulation and recording

Electrical stimulation was delivered using a Grass Instruments S88 stimulator (Grass, Massachusetts) controlled by a device designed in our laboratory that allows the automatic firing of the stimulator [10]. Stimulation parameters consisted of a 10 min sequence of biphasic square wave pulses at the following levels: frequency, 100 Hz; pulse width, 0.5 ms; and intensity, 200 μA. Brain electrical activity recordings were collected using polygraphic equipment model 78-E (Grass, Massachusetts).

Experimental procedure

The animals were assigned to three experimental groups: the Control group ($n = 6$), which received only PTZ injections; the HFS-TRN group ($n = 5$), which received HFS in the left TRN prior to PTZ injections; and the HFS-Adj group ($n = 5$), which received HFS in the left adjacent nuclei prior to PTZ injections. A baseline EEG recording was performed for 15 min. To gradually increase the intensity of induced seizures, we chose the systemic PTZ injection schedule employed by Lüttjohann et al. [39]. Rats received an initial dose of 20 mg/kg PTZ i.p. Every 15 min additional dose of 10 mg/kg were administered until a TCGS lasting 5 min (SE) was observed. Sessions were conducted between 14:00 and 18:00.

Behavioral and EEG analysis

All animal behavior was videotaped during the basal EEG and behavioral seizure stages. The videos were analyzed off-line using a double-blind technique, following the classification scheme proposed by Lüttjohann et al. [39]: Stage 1, sudden behavioral arrest and/or motionless staring; Stage 2, facial jerking with muzzle or muzzle and eye; Stage, 3 neck jerks; Stage 4, clonic seizure in a sitting position; Stage 5, convulsions including clonic and/or tonic-clonic seizures while lying on the belly and/or pure tonic seizures; and Stage 6, convulsions, including clonic and/or tonic-clonic seizures, while lying on the side and/or wild jumping. The highest score after each PTZ injection was assigned for each animal. The mean values obtained from each group were quantified to determine seizure intensity stages.

The EEG signals were amplified and the band-pass was filtered between 3 and 60 Hz. Then, the signals were digitized at 300 samples/second and stored on a hard disk. Off-line spectral analyses by the Fast Fourier Transform (FFT) and wavelets methods at 1 min periods after PTZ injections were performed with computer software developed in our laboratory [40,41]. The power spectrum of both the left- and right-motor cortices (L-MCx; R-MCx, respectively) and the left TRN recordings, showing the evolution of the EEG in the frequency domain, was computed. The variables quantified in the experiments were the latency and duration of the SWD, TCGS and SE.

Histology

The animals were sacrificed after *status epilepticus* was observed. A transcardial perfusion with saline solution (0.9%) and paraformaldehyde (10%) was performed. Brains were removed and cut into coronal slices of 60 μm. The histological verification of the placement of the stimulation and recording electrodes was conducted using the rapid procedure method [42].

Statistical analysis

Seizure intensity stage changes induced by PTZ, duration, latency, cumulative doses of PTZ to SE, the SWD frequency and the