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Electrical stimulation of left anterior thalamic nucleus with high-frequency and low-intensity currents reduces the rate of pilocarpine-induced epilepsy in rats

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ABSTRACT

Purpose: Bilateral electrical stimulation of anterior nuclei of thalamus (ANT) has shown promising effects on epileptic seizures. However, bilateral implantation increases the risk of surgical complications and side effects. This study was undertaken to access the effectiveness of a stimulation paradigm involving high frequency and low intensity currents to stimulate the left ANT in rats.
Methods: Male Sprague-Dawley rats were implanted with electroencephalogram (EEG) electrodes, and an additional concentric bipolar stimulation electrode into either the left or right ANT. The stimulus was a train of pulses (90 μs duration each) delivered with a frequency of 200 Hz and a current intensity of 50 μA. Thalamic stimuli were started 1 h before the first intraperitoneal pilocarpine injection (i.p., 300 mg/kg), and were applied for 5 h.
Results: EEG documented seizure activity and status epilepticus (SE) developed in 87.5% of rats treated with no ANT stimulation after a single dose of pilocarpine. Left ANT stimulation significantly increased the tolerance threshold for pilocarpine-induced EEG seizure activity; 20% of rats developed their EEG documented seizure activity after receiving the first dose, whereas 50%, 10% and 20% of rats did not develop seizure activity until they had received the 2nd, 3rd and 4th pilocarpine injection at 1-h intervals. Moreover, left thalamic stimulation reduced the occurrences of both EEG documented seizure activity and SE induced by single-dose pilocarpine to 25%. However, our result demonstrated that little effect on the occurrence rate of seizures and SE was found when rats received right ANT stimulation.
Conclusions: These results suggest that continuously 5-h left ANT stimulation with high frequency and low intensity currents, beginning from 1 h before the pilocarpine administration, may successfully reduce the occurrence rate of EEG documented seizure activity and SE development in rats.

1. Introduction

Epilepsy is one of the most common and devastating neurological disorders. About seventy percent of patients with epilepsy can be well-controlled with currently available anti-epileptic drugs (AEDs), but seizures still persist in 30% of epilepsy patients who do not respond to any of two to three first-line AEDs despite administration of the carefully optimized drug treatment.[1]

Many patients with epilepsy are inadequately controlled by the AEDs and also are not eligible for resective surgery. Alternative therapies, such as vagus nerve stimulation[2,3] and deep brain stimulation (DBS), have been considered for treating refractory epilepsy.

DBS has been used to treat various psychiatric (e.g., depression and obsessive-compulsive disorder)[4–7] and neurological disorders, such as Parkinson's disease (PD)[8] and epilepsy.[9–12] Stimulation of the subthalamic nucleus (STN) improves the cardinal features of PD, and the pedunculopontine (PPN) nucleus has recently emerged as a possible target of DBS for gait disorders in PD.[8] The targeted structures of DBS used for depression include the subthalamic nucleus, internal globus pallidus, ventral internal capsule/ventral striatum, the subgenual cingulated region, and the nucleus accumbens.[6] Amygdala and nucleus accumbens have been

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indicated as the targets of DBS for post-traumatic stress disorder (PTSD)[5] and refractory obsessive-compulsive disorder,[4] respectively. Bilateral electrical stimulation of the anterior nuclei of the thalamus (ANT) is promising in reducing epilepsy in animal experiments[11,12] and human studies.[9,10] The efficiency of the ANT stimulation in treating refractory epilepsy depends on the stimulation paradigm. Low frequency stimulation synchronizes electroencephalic activity in cortex[13,14] and is proconvulsant;[15] whereas high frequency stimulation desynchronizes intrinsic cortical activity[16] and raises seizure threshold.[15] Furthermore, unilateral DBS of the ANT has not been shown to reduce the propensity or latency for developing seizures and status epilepticus (SE).[11] However, bilateral implantation of electrodes into the ANT increases the complexity of surgery, the risk of surgery complication and adverse effects (e.g., intracranial and intracerebral hemorrhage, infection, misplacement of the DBS leads, or suboptimal placement of the leads).[17] We herein reported a stimulation paradigm by employing the relatively high-frequency (200 Hz) and low-intensity currents (50 μA) to unilaterally stimulate the left ANT, which successfully reduced the occurrence rate of pilocarpine-induced seizures and SE in rats.

2. Methods

2.1. Substances

Stock solutions of pilocarpine and methylscopolamine bromide (Sigma–Aldrich, St. Louis, MO, USA) were dissolved in pyrogen-free solution (PFS). These stock solutions were stored at −20 °C until administration. The dose of pilocarpine used in these experiments was 300 mg/kg with intraperitoneal (i.p.) injection. Methylscopolamine bromide (1 mg/kg, ip), an anti-cholinergic that does not cross the blood–brain barrier, was administered to reduce the peripheral cholinergic effects without affecting the central nervous system (CNS). Our personal observation demonstrated that rats would not survive after SE if the injection dose of pilocarpine is over 300 mg/kg. Therefore, we selected the dose of 300 mg/kg pilocarpine to provoke seizures.

2.2. Animals

Male Sprague-Dawley rats (250–300 g; National Laboratory Animal Breeding and Research Center, Taiwan) were used in these experiments. These animals were anesthetized (Zoletil® (Carros, France); 50 mg/kg), and injected with analgesic (morphine) and antibiotic (penicillin G benzathine). All rats were surgically implanted with three electroencephalogram (EEG) screw electrodes (on the right frontal and parietal lobes and the left occipital lobe) as previously described.[18] An additional concentric bipolar electrode (O.D. 0.125 mm, FHC, Bowdoinham, ME, USA) was implanted directly into the left ANT (AP, −2.0 mm from bregma; ML, 1.5 mm; DV, 5.5 mm)[19] in rats of groups 2–5 (see later in the experimental protocol). Insulated leads from EEG electrodes were routed to a Teflon pedestal (Plastics One, Roanoke, VA, USA). The Teflon pedestal was then cemented to the skull with dental acrylic (Tempron, GC Co., Tokyo, Japan). The incision was treated topically with polysporin (polymyxin B sulfate–bacitracin zinc) and the animals were allowed to recover for seven days prior to the initiation of experiments. The rats were housed separately in individual recording cages in an isolated room, in which the temperature was maintained at 23 ± 1 °C and the light:dark rhythm was controlled in a 12:12 h cycle (40 W × 4 tubes illumination). Food and water were available ad libitum. On the second postsurgical day, rats were connected to the recording apparatus (see later) via a flexible tether. Animals were habituated by daily handling timed to coincide with scheduled experimental administrations. We made our best effort to minimize animal suffering and to reduce the number of animals used in current study. All procedures performed in this study were approved by the National Taiwan University Animal Care and Use Committee.

2.3. Recording apparatus and ANT stimulation

Signals from the EEG electrodes were fed into an amplifier (Colbourn Instruments, Lehigh Valley, PA; model V75-01). The EEG was amplified (factor of 5000) and analog bandpass was filtered between 0.1 and 40 Hz (frequency response: ±3 dB; filter frequency roll off: 12 dB/octave). These conditioned EEG signals were subjected to analog-to-digital conversion with 16-bit precision at a sampling rate of 128 Hz (NI PCI-6033E; National Instruments, Austin, TX). The digitized EEG waveforms were stored as binary computer files pending subsequent analyses. Postacquisition determination of the onset of the first EEG seizure occurrence and the latency to SE was done by the visual scoring using AxoScope 10 Software (Molecular Devices, Sunnyvale, CA, USA). We defined EEG documented seizures as the visualization of epileptiform spikes with amplitudes of greater than 1 mV appearing in discharges lasting for at least 30 s. SE was defined as seizure activity associated with continuous epileptiform discharges followed by the periodic epileptiform discharges of at least 5 min duration.[20] EEGs were analyzed with the open-source Chronux algorithms (http://chronux.org/) run by the Matlab Signal Processing Toolkit for the fast Fourier transform (FFT) and multi-taper time-frequency spectrum.

Thalamic stimulation was started 1 h before pilocarpine i.p. injection and lasted for 5 h. A stimulator-isolator unit (A360 Stimulus Isolator, World Precision Instruments, Sarasota, FL, USA) triggered by a main stimulator (Accupulser A310, World Precision Instruments) was used to deliver the ANT stimulation current at a frequency of 200 Hz, pulse width 90 μs, interval 4.1 ms, and intensity 50 μA. The stimulation artifact was not observed during the stimulation time period because of the low sampling rate with which the EEG was acquired. However, we simultaneously determined the stimulation outputs by oscilloscope when the animals received ANT stimuli. The parameters of ATN stimulation with high-frequency and low-intensity currents would deliver the lowest possible total electrical energy delivered (TEED) than that of low-frequency and high-intensity currents.[21] The TEED is calculated as: $TEED_{1\,s}$ = (voltage^2 × frequency × pulse width/impedance) × 1 s.[21] The optimized DBS setting should generate maximal clinical benefit at the lowest possible TEED, which results in fewer stimulation-related complications. Furthermore, the ANT stimulation did not alter the quality of EEG signals as mentioned in the following result section.

2.4. Experimental procedures

A total of 50 Sprague-Dawley rats were used and divided into six groups. Control rats in group-1 ($n = 8$) received a single dose of pilocarpine administration at the second hour of the dark period. EEGs were recorded before and after pilocarpine injection. Rats in group-2 received a similar protocol as those in group-1, except that rats were implanted with a left ANT electrode but electrical stimulation was not delivered. In group-3 ($n = 10$), a 1-h baseline EEG was recorded at the beginning of the dark period. The continuous 5-h left ANT stimulation and EEG recording were simultaneously performed from the 2nd-hour of the dark period. The first i.p. administration of pilocarpine was given 1 h after the initiation of ANT stimulation. The second dose of pilocarpine was given 1 h later if the EEG epileptiform did not occur after the first pilocarpine injection. The third and fourth injections of pilocarpine were given at 1-h interval if the previous dose of pilocarpine did not cause EEG epilepsy. Rats in group-4 ($n = 8$) received the similar protocol as those in group-3, except that pilocarpine was administered once at