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The anti-ictogenic effects of levetiracetam are mirrored by interictal spiking and high-frequency oscillation changes in a model of temporal lobe epilepsy

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ABSTRACT

Purpose: Mesial temporal lobe epilepsy (MTLE) is the most prevalent type of partial epileptic disorders. In this study, we have analyzed the impact of levetiracetam (LEV) in the pilocarpine model of MTLE.
Methods: Sprague-Dawley rats ($n = 19$) were injected with pilocarpine (380 mg/kg, i.p.) to induce a *status epilepticus*. Twelve animals were used as controls and seven were treated with LEV. They were implanted with bipolar electrodes in the CA3 subfield of the hippocampus, entorhinal cortex (EC), dentate gyrus (DG) and subiculum and EEG-video monitored continuously from day 4 to day 14 after SE.
Results: Only 29% of LEV-treated animals had seizures compared to all controls following a latent period that was similar in duration. Seizure rates were lower in LEV-treated animals. In LEV-treated animals without seizures, lower interictal spike rates were found in all regions compared to controls. Analysis of interictal high-frequency oscillations (HFOs) revealed that LEV-treated animals without seizures had lower rates of interictal spikes with ripples (80–200 Hz) in CA3, EC and subiculum ($p < 0.01$), whereas rates of interictal spikes with fast ripples (250–500 Hz) were significantly lower in CA3 and subiculum, compared to controls.
Conclusion: Our findings indicate that the anti-ictogenic properties of LEV are mirrored by decreases of interictal spike rate in temporal lobe regions, and are accompanied by subregion-specific decreases of HFO occurrence in CA3 and subiculum. Overall, this evidence suggest that LEV may inhibit neural network activity in regions that are known to play important roles in MTLE.

1. Introduction

Levetiracetam (LEV; [(S)-α-ethyl-2-oxo-1-pyrrolidine acetamide]) is a second generation anti-epileptic drug (AED) that is widely used in patients with either generalized or partial epileptic disorders.[1] LEV also has anti-ictogenic effects in animal models of epilepsy such as in the amygdala kindling model,[2] audiogenic kindling,[3] spontaneously epileptic rats[4] and the kainic acid model.[5] Unlike several other AEDs,[6] LEV does not block voltage-dependent Na^+ but may decrease glutamatergic excitatory transmission,[7] thus preventing excessive neuronal synchronization.[8] Although the exact mechanisms through which LEV controls seizures remain unclear, its anticonvulsive properties are thought to rely on its ability to modulate neurotransmission by binding to SV2A, a synaptic vesicle glycoprotein that interacts with synaptotagmin, a pre-synaptic Ca^{2+} sensor involved in regulating synaptic vesicle exocytosis.[9]

MTLE is the most prevalent type of partial epileptic disorders, with symptoms consisting of partial seizures that originate from the hippocampus, entorhinal cortex or amygdala many years after an initial brain insult such as *status epilepticus* (SE), encephalitis or febrile convulsions.[10] MTLE is also one of the most refractory forms of epilepsy with approximately one third of patients being unresponsive to medication.[11] In these patients, the surgical removal of the seizure onset zone often represents the only therapeutic alternative, which may sometimes necessitate the use of invasive intracranial EEG recordings to properly delineate the seizure onset zone.[12]

Recently, high-frequency oscillations (HFOs, ripples: 80–200 Hz, fast ripples: 250–500 Hz) have been recorded in the EEG of epileptic patients and in animal models of epilepsy.[13] They were shown to occur in association with spikes or independently, and they are thought to reflect the activity of dysfunctional neural

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networks, since the surgical removal of regions with HFOs is associated to good post-surgical outcomes.[14–18] Animal studies have also shown that HFOs may be linked to epileptogenesis and ictogenesis.[8,19,20] The relation between seizures, interictal spikes, HFOs and the effect of anti-epileptic drugs is however still unclear. A study performed in epileptic patients has shown that HFOs may increase after medication reduction.[21] However, these studies were carried out in patients with "well-established" epileptic conditions, and were limited by the variability of AEDs. We analyzed here the effects of LEV on seizures, interictal spikes and HFOs in the pilocarpine model of MTLE, and focused on the 4–14 day period that follows the initial pilocarpine-induced SE.

2. Methods

2.1. Ethical approval

All procedures were approved by the Canadian Council of Animal Care and all efforts were made to minimize the number of animals and their suffering.

2.2. Animal preparation

Male Sprague-Dawley rats (250–300 g) were obtained from Charles River (St-Constant, Qc, Canada) and let habituate for 72 h after delivery before pilocarpine treatment. They were housed in controlled conditions, at 22 (±2) °C and under a 12 h light/12 h dark cycle (lights on from 7:00 AM to 7:00 PM) with food and water *ad libitum*.

2.3. Pilocarpine treatment

Animals were injected with scopolamine methylnitrate (1 mg/kg i.p.; Sigma–Aldrich, Canada) and 30 min later with a single dose of pilocarpine hydrochloride (380 mg/kg, i.p.; Sigma–Aldrich, Canada).[8] Their behaviour was scored according to the Racine scale[22] and *SE* was defined as continuous stage 5 seizures. *Status epilepticus* (SE) was terminated after 1 h by an injection of diazepam (5 mg/kg, s.c.; CDMV, Canada) and ketamine (50 mg/kg, s.c.; CDMV, Canada).[8]

2.4. Treatment with levetiracetam

Approximately 4 h after SE termination, seven animals were anesthetized with isoflurane (2%) in 100% O_2 and an osmotic pump (2 ml, flow rate: 5 μl/h) was installed subcutaneously (2ML2 ALZET osmotic mini-pumps, DURECT Corporation, Cupertino, CA, USA) in order to deliver LEV dissolved in saline at 300 mg/kg/day.[23] These pumps deliver a continuous dosing over 2 weeks, circumventing the need for repetitive invasive blood sampling. Osmotic pumps filled with saline were installed subcutaneously in controls.

2.5. Surgery for the implantation of electrodes

All animals underwent surgery for the implantation of electrodes three days after pilocarpine treatment. Before surgery, animals received topical Lidocain (5%; Odan, Canada). An incision was then made in the skin to expose the skull plate, from bregma to lambda. Four stainless steel screws (2.4 mm length) were fixed to the skull and four small holes were drilled to allow the implantation of bipolar electrodes (20–30 kΩ; 5–10 mm length; distance between exposed tips: 500 μm) (MS303/2-B/spc, Plastics One, VA, USA). Electrodes were implanted in the CA3 subfield of the ventral hippocampus (AP: −4.4, ML: 4, DV: 7.8), medial entorhinal cortex (AP: −8.6, ML: 5.2, DV: 6.8), ventral subiculum (AP: −6.8, ML: 4, DV: 6) and dentate gyrus (AP: −4.4, ML: 2.4, DV: 3.4). Recordings were performed in the right CA3 and EC and in the left DG and subiculum. Screws and electrode pins were connected with a pin connector and fastened to the skull with dental cement. A fifth bipolar electrode was placed under the frontal bone, after the removal of insulating material, and used as reference. During surgery, animals received a preventive antibio-therapy (Enrofloxacine, 10 mg/kg, s.c.). After surgery, animals were injected with Ketoprofen (5 mg/kg, s.c. Merail, Canada), Buprenorphine (0.01–0.05 mg/kg, s.c., CDMV, Canada) and 2 ml of 0.9% sterile saline (s.c.).

2.6. EEG recordings

The pin connector was connected to a multichannel cable and electrical swivel (Slip ring T13EEG, Air Precision, France; or Commutator SL 18C, HRS Scientific, Canada) and EEG-video monitoring (24 h/day) was performed. EEGs were amplified *via* an interface kit (Mobile 36ch LTM ProAmp, Stellate, Montreal, QC, Canada), low-pass filtered at 500 Hz and sampled at 2 kHz per channel. Infrared cameras were used to record day/night video files that were time-stamped for integration with the electrophysiological data using monitoring software (Harmonie, Stellate, Montreal, QC, Canada). Continuous 24/7 EEG-video recordings were performed from day 4 to day 14 after SE.

2.7. Spontaneous seizures and onset patterns

All EEG and video recordings were reviewed manually in order to detect seizures. The time corresponding to the onset and end of each seizure was recorded and the behaviour of the animal was graded on the Racine scale.[22] Seizures were also classified in two groups according to their onset pattern. Low-voltage fast-onset seizures (LVF) were characterized by the occurrence of a positive- or negative-going spike that was followed by the appearance of low amplitude high frequency activity. Hypersynchronous-onset seizures (HYP) were characterized at onset by a pattern of focal periodic spiking at a frequency of approximately 2 Hz. Seizures for which a seizure onset pattern could not be identified were named "unclassified". The duration of seizures was based on the first occurrence of activity in the 5–20 Hz range until the return to baseline activity and normal behaviour. The duration of seizures was calculated from the onset of activity in the 5–20 Hz range (see arrows in Figs. 2 and 3) until the return to baseline EEG activity.

The occurrence of a spontaneous seizure (non-convulsive or convulsive) after SE marked the end of the latent period. Based on the presence or absence of spontaneous seizures between the 4th and the 14th day after SE, LEV-treated animals were divided in two groups, those with seizures (LEV w Sz) and those without seizures (LEV w/o Sz).

2.8. Analysis of interictal spikes and high-frequency oscillations

To reduce the potential variability in interictal spike occurrence due to seizures in the LEV-treated group, we excluded LEV w Sz animals from the analysis of interictal spikes and HFOs. For each control and LEV w/o Sz animal, two epochs of 10 min were selected for each day in order to analyze interictal spikes and HFOs. In order to reduce a possible bias on seizures and interictal activities caused by the light/dark cycle, we selected from each recording day one epoch during the light period (from 7 AM to 7 PM) and a second epoch during the dark period (from 7 PM to 7 AM). Only epochs of non-REM sleep were used for analysis, because of the low rate of movement artefacts and because HFOs are more prominent during this sleep stage.[24] Non-REM sleep epochs were selected based on