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Retigabine calms seizure-induced behavior following status epilepticus

A.M. Slomko, Z. Naseer, S.S. Ali, J.P. Wongvravit, L.K. Friedman *

Department of Cell Biology & Anatomy, New York Medical College, Valhalla, NY 10595, USA

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ABSTRACT

In adult rats, intraperitoneal injection of kainate (KA) results in sustained status epilepticus and persistent behavioral comorbidities such as hyperexcitability, anxiety, and altered response to environmental cues. Intrahippocampal KA also results in sustained status epilepticus and continuous high frequency oscillations in the electroencephalograph (EEG), although subsequent behavioral side effects are unknown. We hypothesized that retigabine, a recently discovered anticonvulsant and potent positive modulator of Kv7 channels, may attenuate seizure-induced behavioral abnormalities. Status epilepticus was induced by administration of KA either intraperitoneally (15 mg/kg) or by single intrahippocampal injection (1.0 μg/0.5 μL). After 24 h, half of systemically KA-treated animals that reached stage 6 seizures were injected once daily with retigabine (5 mg/kg) for 14 continuous days. All groups underwent three behavioral tests — capture and handling, open field, and elevated plus maze — 24 h following the last retigabine treatment and were sacrificed at 25–28 days. In the capture and handling test, systemic KA treatment resulted in frisky behavior and resistance to capture with wild attempts to escape during the 1st, 2nd, and 3rd weeks of the observation period. In contrast, these behaviors were attenuated in KA + retigabine-treated animals. In the open-field test, KA-treated animals spent more time in the center zone, but KA + retigabine-treated rats had greater overall activity compared with those having vehicle, KA, or retigabine-only treatment. In the elevated plus maze, KA + retigabine-treated animals traveled greater distances in open and closed arms (proximal and distal) compared with controls, also signifying anxiety reduction. Retigabine-only-treated rats traveled more in the open proximal arms compared with controls, indicating increased hyperlocomotion in normotensive rats. Although treatment with KA + retigabine resulted in anxiolytic-like effects in all three behavioral tasks compared with vehicle, this group did not significantly differ from systemically KA-treated rats in most measurements in open-field and elevated plus maze tasks, suggesting that retigabine may also cause hyperlocomotion unrelated to anxiety level. Despite that intrahippocampal KA-treated rats displayed comparable seizure behavior, epileptiform activity, and hippocampal injury, their behavior resembled the controls, suggesting that molecular and subsequent cellular changes are also partially responsible for anxiolytic-like effects and that these results are likely independent of the hippocampus.

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1. Introduction

Patients with epilepsy are at a high risk of experiencing or developing anxiety and other behavioral and/or cognitive abnormalities [1]. Voltage-gated K^+-channels play a critical role in regulating neuronal excitability. Activation of these channels leads to anticonvulsant effects, whereas diminished activity may promote neuronal hyperexcitability. Therefore, K^+-channel regulation may signify a potential target for therapeutic intervention of seizure activity. Retigabine [*N*-(2-amino-4-(4-fluorobenzylamino)-phenyl)-carbamic acid ethyl ester] is the first slow K^+ current neuronal channel opener indicated for adjunctive treatment for partial seizures in adult patients [2]. This agent has successfully reduced the incidence of partial seizures; however, its effects on hyperactive behaviors associated with kainate (KA)-induced status epilepticus remain unknown. Unlike other anticonvulsants, retigabine has been shown to potentiate neuronal M-currents by opening Kv7.2–7.5 potassium channels [3]. These neuronal M-currents occur in various structures of the brain, including the amygdala, which is known to be an important structure in fear conditioning and generalized anxiety [4]. Retigabine can raise the seizure threshold in amygdala-kindled rats by decreasing seizure duration and severity [5]. Additionally, retigabine reduces hyperexcitability in prepared hippocampal slices from KA-treated rats, further indicating its therapeutic potential [6].

Resistance to handling and performance in the elevated plus maze and open field are routinely used to quantify anxiety-like activity, particularly when pharmacological drugs are being evaluated [7,8]. In the open-field test, rats tend to stay close to the walls, a phenomenon termed "thigmotaxis", whereas movement into the center zone indicates a decreased level of anxiety [8]. The elevated plus maze assesses approach–avoidance conflict by measuring distance traveled or time

* Corresponding author at: Department Cell Biology & Anatomy, New York Medical College, 15 Dana Rd, Valhalla, NY 10595, USA. Tel.: +1 914 594 3893.
E-mail addresses: linda_friedman@nymc.edu, lfriedma@aol.com (L.K. Friedman).

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spent in open arms vs. closed arms. Untreated rats typically exhibit anxiety-like behaviors (e.g., urination, defecation, and freezing) and spend most of the time in the closed arms of the maze [9]. In humans and experimental models, neuronal activation of the amygdala vs. the hippocampus can be distinguished by fear, anxiety, and contextual learning stimuli [10,11]. In rats, it is well established that both the amygdala and the hippocampus play pivotal roles in Pavlovian fear conditioning and extinction, allowing the association between innocuous stimuli and normal fear responses [4,12,13]. However, while both the amygdala and the hippocampus are activated during a fear conditioning response, the location of the neuronal lesion may differentially affect the acquired fear response. Hippocampal lesions disrupt the acquisition of the contextual fear response but do not affect the fear response to conditioned stimuli. In contrast, amygdala lesions disrupt both responses [14]. In addition, a recent study showed that hemodynamic signals in the amygdala vs. the hippocampus can distinguish opposite differences in neuronal activity when comparing aversive vs. neutral cues [15].

In adult rats, intraperitoneal injection of KA results in sustained status epilepticus and persistent behavioral comorbidities such as hyperexcitability, anxiety, and altered response to environmental cues; however, these symptoms only occur in rats that achieve status epilepticus after KA injection. In contrast, intrahippocampal injection of KA also induces sustained status epilepticus with rapid bursting as seen in its concordant hippocampal electroencephalograph (EEG) (in all microinjected rats) but without subsequent behavioral impairments observed in intraperitoneal treatment with KA [16]. The negative behavioral side effects reappear when spontaneous seizure activity commences from systemic KA treatment; however, it was also shown that the route of injection was important since incidence of spontaneous recurrent seizures following intracerebral (intrahippocampal or intraamygdala) injection of KA was comparably very low [17].

By analyzing retigabine's effect on the acute seizure threshold of KA-treated rats in our ongoing study, we discovered that onset to stage 5–6 seizures is attenuated or delayed; therefore, we explored retigabine's potential therapeutic uses for treating seizure-induced behavioral abnormalities which may have therapeutic advantage for patients suffering from epilepsy.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (200 g) (Charles River, St Louis, MO) were used. Animals were housed in single cages and were given food and water ad libitum until sacrifice. Animals were kept on a 12-h light/dark cycle at room temperature (55% humidity) in the NYMC accredited animal facility with full IACUC approval. All animal procedures were in accordance with the NIH guidelines. Animals were divided into control and three experimental groups: control vehicle (N = 8), intraperitoneal KA (N = 9), intraperitoneal KA + retigabine (N = 9), intraperitoneal retigabine only (N = 7), and intrahippocampal KA (N = 6).

2.2. Drugs

Retigabine (5 mg/kg) was administered daily (i.p.) to a subset of systemically KA-treated rats for two weeks 24 h following KA-induced status epilepticus and then discontinued until sacrifice at 25 or 28 days. Control animals received vehicle (dimethyl sulfoxide, DMSO, 10% in PBS) or retigabine only. Retigabine stocks of 20 mg were dissolved in DMSO (100%) then diluted by 50% to a 20-mg/mL stock and then further diluted to a 5-mg/mL stock. Final dosage administered was 5 mg/kg, and the final concentration of DMSO was ≤10%. To a separate group, KA was microinfused into the hippocampus manually with a 5-μL syringe and cannula assembly at a flow rate of 1.0 μg/0.5 μL over 5 s (0.5 μL of 2-μL/2-μg stock) as described [18]. Intrahippocampal KA-treated rats did not receive retigabine. These animals served as an internal control for the behavioral and histological tests.

2.3. Electrode implantation for EEG recordings

Acute EEG recordings were obtained from both systemic and intracranial injected animals. Kainate-treated rats were first anesthetized with a mixture of 70-mg/kg ketamine and 6 mg/kg of xylazine and then stereotaxically implanted with either bipolar electrodes or dual bipolar electrode/cannula assembly into the right hippocampus 48 h prior to the KA treatment (coordinates in mm with respect to bregma: AP: −3.4, L: 2.6, D: −3.0; incisor bar at −3.5) [19,20]. The electrodes were perpendicularly oriented angled at 0° from the vertical plane. After surgery, dental acrylic was used to close the wound and stabilize the electrode assembly. Rats recovered from anesthesia and became active 1–2 h following the surgery. Animals were kept warm at 30 °C in a clean cage box under an incandescent lamp and returned to their cages until sacrifice (25 or 28 days after status epilepticus). In order to obtain EEG recordings, KA-treated and KA + retigabine-treated animals were paired, placed in an insulated chamber, and connected to the recording setup through flexible low noise leads (Plastics One), which permitted free movement [21]. Baseline EEG recordings were obtained from KA-treated and KA + retigabine-treated animals for 2 h following drug administration to affirm status epilepticus. For off-line analysis, Data Wave software was used to quantify wave frequency, amplitude, and number of spike and burst events in the EEG traces. The program uses digital filtering with a Butterworth 3-pole filter and a 6-dB-per-octave roll-off. Electroencephalograph traces were filtered prior to being subjected to burst, Fourier, and spectral analyses to quantify changes in oscillation frequency and duration (0-500 Hz, 3 s sweep time). The EEG spectral power was monitored from six frequency bands: delta (0.1–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), beta (12–30 Hz), low gamma (30–70 Hz), and high gamma (70–180 Hz). Epileptic spike discharges were recorded in the low- and high-gamma range. Phosphate buffered saline (PBS)/DMSO-injected control rats were also used for baseline comparison (n = 3). Electrode placement into the CA1 was verified histologically with Nissl staining.

2.4. Induction of status epilepticus and seizure scoring

Status epilepticus was induced with KA intraperitoneally (15 mg/kg) or by a single intrahippocampal injection (1.0 μg/0.5 μL) to male adult rats (200–250 g). As previously described, a modified Racine method was used to assess seizure severity [22,23]. After the onset of behavioral seizures (~60 min), the number, frequency, and duration of different seizure behavior manifestations were recorded every 5 min for 3 h. In order for the rats to remain in the study, periodic presentation of a specific stage 6 behavior, such as rearing and forelimb clonus with frothing, had to be present for at least 1 h of the observation period. Approximately 90% of injected animals at the dose used achieved this level of status epilepticus. After 24 h, half of the animals received retigabine once daily for 14 days. Equal number of injections of retigabine was administered to a separate group to compare in the behavioral tests. Seizure behaviors were scored on a scale of 0–7, with 0 representing normal behavior and 7 representing death. Stage 1 = mild scratching and hyperexcitability; Stage 2 = continuous scratching and circling; Stage 3 = wet-dog shakes; Stage 4 = salivation, wet-dog shakes, and unilateral clonus of forepaw; Stage 5 = continuous wet-dog shakes, rearing and/or standing tonus, and bilateral clonus of forepaws; Stage 6 = tonic–clonic seizures, heavy drooling and frothing, continuous head bobbing, rearing with forelimb clonus with continuous head bobbing, circling, running, and loss of postural control. These typical behaviors were recorded after early signs emerged (e.g., scratching), and seizure behavior scores were calculated for each animal. Stages 1–2 were classified as nonconvulsive; Stages 3–6 were classified as