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Lithium affects rat hippocampal electrophysiology and epileptic seizures in a dose dependent manner

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ABSTRACT

Lithium, a classic mood stabilizer, prevents apoptosis-dependent cellular death and has garnered considerable interest as a neuroprotective agent that is efficacious in the treatment of many neurological diseases. However, the effects of lithium in epilepsy remain controversial. We found that different doses of lithium affect epileptic seizure activity and bidirectionally modulate the susceptibility to and severity of seizures induced by pilocarpine in rats. Recently, it has been demonstrated that systematically administered lithium affects the powers of hippocampal *gamma* and *theta* oscillations in baseline electroencephalograms. Low-dose lithium (10 mg/kg) administered to pilocarpine-treated rats markedly increased the powers of basal *gamma* (30–80 Hz) and *theta* (4–12 Hz) oscillations, decreased the proportion of Racine stage 4–5 seizures, extended latency until seizure onset, and significantly reduced the frequency of lower-class seizures ($p < 0.05$). Conversely, when the dose was increased to 40 mg/kg, lithium reduced the frequency of lower-class seizures compared to control treatment ($p < 0.05$). Further, at this high dose, lithium reduced the power of basal *gamma* oscillations and markedly increased the susceptibility to and severity of pilocarpine-induced seizures and enhanced ripple rhythms (80–200 Hz) postictally. Our results provide a framework for further investigations of the underlying electrophysiological mechanisms of lithium-induced imbalances in excitatory and inhibitory neural circuits that regulate seizure activity in rats. In conclusion, the observed in vivo changes in the powers of basal *gamma* and *theta* oscillations in response to different doses of lithium may reflect hippocampal neural network responsiveness.

1. Introduction

Epilepsy is one of the most common neurological disorders and is characterized by seizures of abnormal electrical activity and spontaneous recurrent attacks (Staley, 2015). Epileptic seizures are considered to result from hyper-synchronous activity arising from an imbalance between excitation and inhibition in large populations of neurons (Loscher and Brandt, 2010; Staley, 2015). A wide range of brain injuries that damage neuronal membrane stability, including traumatic brain injury, stroke, infections, and the use of chemical anticonvulsants, form the foundation of excitatory and inhibitory neurotransmitter imbalance (Aizawa et al., 2013; Pastore et al., 2014; Moshe et al., 2015).

Lithium prevents apoptosis-dependent cellular death and is widely believed to have clinically relevant neuroprotective effects in an array of neurological disorders. Considering that the procedures for studying lithium vary widely, there is strong evidence for the conjecture that it is neuroprotective (Lazzara and Kim, 2015). Schmidt (1986) reported that in kindled rats, the acute application of lithium dose-dependently decreased seizure susceptibility. On the other hand, in a pilocarpine-induced epilepsy model, lithium reduced the pilocarpine dose required for seizure induction by 10-fold. Therefore, the role of lithium as a treatment for epilepsy remains under debate.

Many in vitro, in vivo, and clinical studies have shown that lithium protects against many types of nervous tissue damage, mainly by preventing apoptosis via the modulation of autophagy, oxidative stress, and inflammatory activity, in addition to the upregulation of

Abbreviations: ANOVA, analysis of variance; EEG, electroencephalogram; FFT, fast fourier transform; LFP, local field potential; GABA, gamma-aminobutyric acid; LSD, least significant difference; NMDA, *N*-methyl-D-aspartic acid; AP, anterior posterior; ML, medial lateral; DV, dorsal ventral

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mitochondrial function and increasing the secretion of neurotrophins (Li et al., 2016; Xu et al., 2016; Zeng et al., 2016; Song et al., 2017). In the current study, we investigated the protective effects of lithium and found that lithium not only modulated multiple pathways, but possibly also had direct effects on different parts of the same pathway (Dell'Osso et al., 2016; Morlet et al., 2018). For example, lithium had been shown to induce the survival signaling pathway and inhibit N-methyl-D-aspartate (NMDA)-receptor-mediated calcium influx and downstream signaling (Chuang et al., 2011; Greenwood et al., 2018). In the current study, we suggest that lithium reduces the neuronal excitation induced by dopamine and glutamate, but increases the inhibition of gamma-aminobutyric acid (GABA)-mediated neurotransmission (Rowe and Chuang, 2004; Li et al., 2011; Motaghinejad et al., 2016).

Glutamate is the main excitatory neurotransmitter in the central nervous system and is a potent regulator of neuronal excitability. It is therefore of interest in studies of neuronal electrical activity (Naylor et al., 2013; Sanger et al., 2013). By inhibiting the influx of NMDA receptor-mediated calcium, therapeutic levels of lithium can protect neurons from the calcium influx following glutamate over-excitation (Nonaka et al., 1998; Chaudhary and Gupta, 2001; Rowe and Chuang, 2004). Lithium has been used to treat bipolar disorder since the mid-1900s. It is thought to have become a classic mood stabilizer because it mediates a reduction in excitability (via dopamine and glutamate) and a concurrent increase in inhibitory (GABAergic) neurotransmission (Malhi et al., 2013). These rapid adjustments in neurotransmission balance the excitation and inhibition within hippocampal networks and affect synchronized, rhythmic hippocampal activity. In rodent electroencephalogram (EEG) studies, researchers have investigated the significance of interictal epileptiform spikes and anomalous transient oscillatory activity in the development of epilepsy, ictogenesis, and related impairments in function (Atallah and Scanziani, 2009; Mann and Mody, 2010; Hiyoshi et al., 2014a, b).

Based on this body of knowledge, we speculated that lithium regulates hippocampal electrophysiology by affecting the energy spectra of local field potentials (LFPs) and that it dose-dependently affects seizure susceptibility. Therefore, we analyzed EEG spectra using a fast Fourier transform (FFT) algorithm and partitioned them using the following common physiological frequency bands: *theta* (4–12 Hz), *gamma* (30–80 Hz), and ripples (80–200 Hz). In addition, we investigated the pharmacological significance of the effects of different doses of lithium on pilocarpine-induced epileptic activity in rats.

2. Experimental procedures

2.1. Animals

All animal tests were performed in accordance with the Chinese Animal Welfare act and were approved by the Animal Research Committee of the North Sichuan Medical College (NSMC201801). All experiments were performed according to national guidelines and Animal Research: Reporting of in vivo Experiments guidelines (http://www.nc3rs.org). We obtained male Sprague-Dawley rats (n = 96, weight = 200–220 g) from the Laboratory Animal Center of North Sichuan Medical College. The rats were individually housed under controlled conditions (23–24 °C, 50–60% humidity, lights on from 8:00 to 20:00) and had ad libitum access to food and water. The experimental scheme and animal grouping are shown in Fig. 1.

2.2. Surgical procedures and electrophysiological recordings

We anesthetized the rats using chloral hydrate (350 mg/kg, intraperitoneal) and placed them in a stereotaxic apparatus (RWD Life science Co, LTD, China). We then fixed ground screws to the anterior and posterior cranium. A microwire array (2 × 8 array of platinum-iridium alloy electrodes, each 25 μm in diameter) was surgically inserted in the right dorsal hippocampus (AP − 3.6, ML 2.8, DV − 3.5),

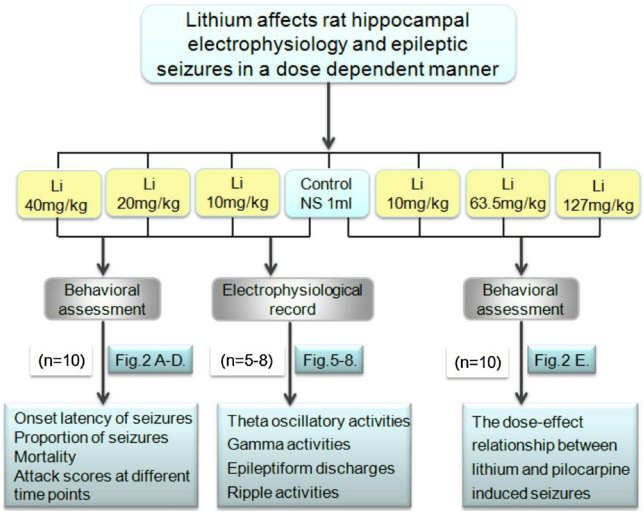

Fig. 1. Experimental scheme. The purpose of this experiment was mainly to (1) assess the dose-effect relationship of lithium on pilocarpine induced seizures, (2) assess the effects of different doses of lithium on epileptic seizures and hippocampal electrical activity, and (3) group the animals based on the different treatments.

as described by Paxinos and Watson (2007). The apparatus was grounded to the ear bar of the stereotaxic structure. The control group and the lithium treatment groups (10, 20, 40 mg/kg) each contained five rats, which were used to obtain electrophysiological recording. All rats were allowed to stabilize for 30 min following electrode implantation before initiating the electrophysiological recording. LFPs were preamplified (×1000), filtered (0.1–1000 Hz), and digitized at 4 kHz using an OmniPlex® D Neural Data Acquisition System (Plexon; Dallas, TX, USA). We used direct current-coupled head stages for electrophysiological recordings, and all recordings were referenced to the two ground screws.

2.3. Analysis of electrophysiological data

NeuroExplorer® software (v4.0, Plexon) and MATLAB software (v7.1, R2009a; MathWorks, Inc.; Natick, MA, USA) were used for the analysis of EEG data. EEG data (LFPs and power spectrograms) were analyzed offline. After digital filtering (0.1–1000 Hz bandpass), an FFT algorithm was applied to each 4-s epoch for power spectrum analysis. The frequency contents of continuous variables or neuronal rate histograms were obtained using power spectral density analysis. NeuroExplorer® is used to evaluate the power spectra of EEG spike trains based on rate histograms. We computed the parameters of the rate histograms using the following formulas: Bin = 1/(2*maximum_frequency) and number_of_bins = 2* number_of_frequency_values. The time axis was separated into periods of length bin*number_of_bins. Subsequently, we calculated the power spectrum for each interval. The final raw power spectrum comprised the average of all spectra for the separate intervals. We calculated the EEG power in the range of 0.5–200 Hz in 1-min and 10-min bins for heatmaps and power spectra, respectively. To determine the time-course changes in EEG power, the total power in each of the three frequency bands, including *theta* (4–12 Hz) and *gamma* (30–80 Hz), were averaged for each 1-min bin and normalized to the average value of the 10-min baseline recording. We calculated the LFP and the area under the curve for every EEG power change from 0 to 40 min after pilocarpine administration to evaluate the effects of different doses of lithium. Thereafter, we measured the power of high-frequency oscillations (ripples, 80–200 Hz) in 10-min intervals from onset to continuous discharge. We computed power and magnitude-squared coherence using native MATLAB functions from the Signal Processing Toolbox. Statistical analysis and