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Effects of uridine on kindling

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Abstract

The anticonvulsant effect of the nucleoside uridine has been studied for several decades with controversial results. One of its attractive properties is that as a natural endogenous molecule, it lacks the serious side effects of common antiepileptic drugs used today. In the current study, we examined the potential antiepileptogenic effect of uridine in the hippocampal kindling model, using once-daily stimulations. Uridine was administered once or three times daily; levetiracetam was administered as a positive control; and normal saline was used as a negative control. Rats receiving uridine or levetiracetam had slower kindling rates and shorter afterdischarge durations than the normal saline controls. These results are consistent with previous work using a rapid kindling model and suggest that uridine has antiepileptogenic properties. Because of its combination of low toxicity and efficacy, uridine is a possible candidate for the treatment of epilepsy.
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1. Introduction

Uridine, a pyrimidine nucleoside, is essential for the synthesis of DNA, RNA, and numerous other molecules critical for cell metabolism [1,2]. During the past several decades, uridine has been used to treat a rich variety of disorders including anti-retroviral therapy-associated lipoatrophy [3], vascular dementia [4], and diabetic neuropathy [5] in humans, depression in rats [6], intestinal toxicity caused by 5-fluorouracil in mice [7,8], anti-leukemic and immunosuppressive activities of 5-azacytidine in mice [9], and hypoglycemic brain injury in mice [10]. Uridine modulates the steady state and turnover rate of striatal D_2 dopamine receptors in rats treated with *N*-ethoxycarbonyl-2-ethoxy-1, 2-dihydroquinoline [11]. Uridine also exhibits a dose-dependent, antidepressant-like effect in the forced swim test in rats, a model used in depression research [6]. In an unpublished clinical study on bipolar disorder, uridine appeared to have an antidepressant effect (Rusche, unpublished, 2007).

Uridine has also been shown to have an anticonvulsant effect in the penicillin- and penicillin plus pentylenetetrazole-induced seizure models [12,13], the pentylenetetrazole-induced seizure model [14], and electroconvulsive models [15]. Uridine was found to inhibit the firing rate of hippocampal neurons exposed to kainate [16]. However, there are several other reports indicating that uridine does not have an anticonvulsant effect. Dwivedi et al. [14] reported uridine did not protect against maximal electroshock-induced convulsions. Uridine was also found not to be effective in blocking seizures induced by 4-aminopyridine, a potassium channel blocker [17]. In a previous study from our laboratory [18], uridine did not show significant neuroprotective effects in the lithium–pilocarpine-induced status epilepticus model, although there was a trend toward

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reduced EEG spike frequency, improved visual spatial memory, and better histology scores in rats treated with uridine.

Currently there are still a large number of patients with epilepsy who remain refractory to the currently available AEDs and susceptible to seizure-induced brain injury. Some patients who have achieved seizure control with AED therapy develop intolerable side effects. Identifying effective and safe antiepileptic compounds therefore remains a high priority in epilepsy. An endogenous antiepileptic modulator would be an attractive candidate as a therapeutic agent.

Because of the discrepancies in the literature regarding its efficacy in preventing or aborting seizures, we studied uridine in a rapid hippocampal kindling model and showed that uridine slowed the rate of kindling [18]. In addition, uridine was demonstrated to have an anticonvulsant effect, reducing seizure severity in rats previously kindled. To further study the possible antiepileptogenic properties of uridine, we compared the rate of kindling in a once-daily kindling model. We administered uridine either once or three times daily, and used normal saline (NS) and levetiracetam (LEV) as negative and positive controls. LEV was chosen to be a positive control as it has been well accepted to have anti-kindling effects [19].

2. Methods

The experimental procedures were approved by the Animal Care Committee of Dartmouth Medical School and performed in accordance with National Institutes of Health guidelines for the humane treatment of animals.

2.1. Electrode implantation

Male adult Sprague–Dawley rats (300–350 g) (Charles River Laboratories, Wilmington, MA, USA) were used. After rats were anesthetized with intraperitoneal (IP) pentobarbital (45 mg/kg), electrodes were implanted under sterile conditions. The rat was placed in a stereotaxic frame. The skull was exposed, and anchor screws were placed into the skull over the right olfactory bulb and left frontal cortex. A 2-mm hole was made in both parietal bones. A bipolar electrode (Plastics One Inc. Roanoke, VA, USA) with a tip difference of 400 μm was fixed in the CA1 area of both hippocampi (3.8 mm posterior to bregma, 2.5 mm lateral to midline, and 2.4 mm below dura). Sterile petroleum jelly was used to cover the exposed brain surface. Grip cement was then applied over the jelly to fix the electrode onto the skull and the anchor screws. The right CA1 electrode was used to administer the kindling stimulation and the left CA1 electrode was used to record afterdischarges. The ground electrode for the EEG recordings was a skull screw placed over the cerebellum.

Rats were given 1 week to recover from surgery. They were maintained on a 12/12 light/dark cycle and had free access to food and water.

2.2. Uridine administration during hippocampal kindling

2.2.1. Afterdischarge threshold

EEG (bandwidth 0.3–300 Hz) recordings were performed using a differential AC amplifier (A-M Systems Inc.). The data were digitized at 1 kHz using a Digidata 1322A interface (Axon Instruments Inc.) and analyzed offline with Clamfit 8.1 (Axon Instruments Inc.). The afterdischarge thresholds (ADTs) were recorded in awake, freely moving rats. Increasing intensities of bipolar current pulses were delivered from a Grass stimulation unit (1 second, 50 Hz, 1.0-ms pulsewidth) beginning at 10 μA and increasing in 10-μA increments until an afterdischarge was elicited (operationally defined as bursts of rhythmic spikes with an amplitude at least twice the baseline). At that point the animal was given a 60-minute rest period and the stimulus that caused an afterdischarge was repeated. If this did not result in an afterdischarge, the current was increased in 10-μA increments until two consecutive afterdischarges were elicited. Once two consecutive afterdischarges were elicited, the current intensity was reduced by 5 μA. If an afterdischarge was obtained, that current intensity was defined as the ADT; if the lower intensity did not elicit an afterdischarge, the previously used intensity was designated as the ADT.

2.2.2. Hippocampal kindling and uridine administration

After determination of the ADT, rats ($n = 48$) were divided into four groups, with each group having a similar mean average ADT: (1) uridine administered once daily (U1) ($n = 12$, average ADT = 67.1 ± 1.66 μA); (2) uridine administered three times daily (U3) ($n = 12$, average ADT = 66.4 ± 1.89 μA); (3) LEV administered once daily ($n = 12$, average ADT = 67.1 ± 1.66 μA); (4) NS administered once daily ($n = 12$, average ADT = 67.1 ± 1.86 μA).

During the kindling process, a 2-second, 20-Hz bipolar stimulus train with superthreshold intensity (50 μA higher than ADT) was administered once daily, 5 days a week. The EEGs were recorded prior to and throughout the afterdischarge. Kindling was done at the same time each day.

Behavioral seizure stages were scored as follows: (1) facial clonus; (2) head nodding and wet dog shaking; (3) contralateral (to stimulating electrode) forelimb clonus; (4) rearing; and (5) rearing and falling [20].

Full kindling was operationally defined as two consecutive stage 5 seizures. During the entire kindling process, rats received intraperitoneal injections of uridine (200 mg/kg) either once or three times a day, LEV (50 mg/kg) once per day, or NS (equal in volume to the uridine dose) once daily. Injections were administered 5 minutes prior to the daily stimulation. In the U3 group, three injections were given at 4-hour intervals, and the stimulations were given 5 minutes after the third injection. Rate of kindling and afterdischarge durations were compared among groups.

2.3. Histology

After completion of the kindling experiments, rats were sacrificed with a lethal dose of sodium pentobarbital (100 mg/kg) and perfused transcardially. Their brains were removed and sectioned. Thionin staining was used to verify electrode location and to assess cell loss.

2.4. Statistical analysis

All data are presented as means ± SE. Number of stimulations to reach each kindling stage and afterdischarge durations for each trial were compared with the two-way ANOVA with repeated measures.

3. Results

3.1. Uridine administration during once-daily kindling

Hippocampal kindling was done daily, Monday to Friday, until full kindling was completed. During the early stages of kindling, rats responded to the stimulus with immobility, facial clonus, and chewing (stage 1). With continued stimulations rats progressed to head nodding and frequent wet dog shaking (stage 2), followed by contralateral forelimb clonus (stage 3). Eventually, rearing with salivation (stage 4), and rearing followed by loss of balance (stage 5) occurred. Full kindling was defined as two consec-

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