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RAPAMYCIN ATTENUATES AGGRESSIVE BEHAVIOR IN A RAT MODEL OF PILOCARPINE-INDUCED EPILEPSY

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Abstract—Psychiatric disorders are fairly common comorbidities of epilepsy in humans. Following pilocarpine-induced status epilepticus (SE), experimental animals not only developed spontaneous recurrent seizures, but also exhibited significantly elevated levels of aggressive behavior. The cellular and molecular mechanism triggering these behavioral alterations remains unclear. In the present study, we found that aggression is positively correlated with development of spontaneous seizures. Treatment with rapamycin, a potent mTOR (mammalian target of rapamycin pathway)-pathway inhibitor, markedly diminished aggressive behavior. Therefore, the mTOR pathway may have significance in the underlying molecular mechanism leading to aggression associated with epilepsy.

Key words: mTOR, epilepsy, psychiatric comorbidities, rapamycin, seizure.

INTRODUCTION

Psychiatric disorders are fairly common comorbidities of epilepsy (Tellez-Zenteno et al., 2007; Garcia-Morales et al., 2008). The prevalence of psychiatric disorders is around 19–80% depending on the type and severity of epilepsy (Swinkels et al., 2005), being the highest in temporal lobe epilepsy (TLE) (Perini et al., 1996). Psychiatric disorders and epilepsy can reciprocally exacerbate each other. Psychiatric disturbances can occur around the same time as seizures. They are recognized as peri-ictal symptoms, by which the psychiatric behaviors can be initiated or exacerbated immediately before, during or following the seizures (Kanner and Balabanov, 2002; Mula and Monaco, 2011). Psychiatric disorders sometimes also independently precede the onset of spontaneous recurrent seizures, which suggests that the underlying mechanism(s) of psychiatric disorders may trigger seizures (Hesdorffer et al., 2000). Recent studies in animal models suggest that psychiatric symptoms may serve as an indication of development of spontaneous seizures and pharmacological resistance to antiepileptic drugs (Gastens et al., 2008). This reciprocal relationship may reflect that epilepsy and its comorbidities share related cellular neuropathological mechanisms. Indeed, initial neurological insults such as status epilepticus (SE) often induce alterations in several brain regions that are well known to mediate both psychiatric behaviors and seizures (Sheline et al., 2003; Trimble and Van Elst, 2003; Garcia-Morales et al., 2008).

Aggression is one of several psychiatric disorders that have long been observed in epileptic patients including those with TLE (Kligman and Goldberg, 1975; van Elst et al., 2000; Sumer et al., 2007), cortical dysplasia (Granieri and Fazio, 2011) and tuberous sclerosis complex (TSC) (Muzykewicz et al., 2007). This association has been reliably replicated in several animal models including those using pilocarpine (Liu et al., 1994; Rice et al., 1998; Desjardins et al., 2001) and domoic acid (Fuquay et al., 2012), in which aggression develops either in parallel to spontaneous seizures or precedes the development of recurrent seizures. The molecular mechanism underlying this comorbidity remains unclear. In animal models, aggressive behaviors tend to occur in animals with severe brain damage caused by initial SE (Desjardins and Persinger, 1995). Brain damages in the hypothalamus and septum were also reported to be involved in the aggressive behavior (Toth et al., 2010; Comai et al., 2012a).

Recent studies suggest an important role of the mammalian target of rapamycin pathway (mTOR) in epilepsy. Hyperactivation of mTOR is responsible for genetically inherited epilepsies such as TSC and phosphatase and tensin homolog (PTEN) in both patients (Krueger et al., 2010) and animal models (Meikle et al., 2008; Zeng et al., 2008; Zhou et al., 2009). mTOR activity was found to be elevated in animal brains of acquired epilepsy (Buckmaster et al., 2009; Zeng et al., 2009; Huang et al., 2010; Raffo et al., 2011; Sunnen et al., 2011). Inhibition of mTOR by rapamycin has been reported to attenuate epileptogenesis, chronic seizures, and pathological changes in the hippocampus and even the improvement of cognitive functions in experimental animal models (Buckmaster et al., 2009; Zeng et al., 2009; Huang et al., 2010; Raffo et al., 2011; Sunnen et al., 2011), albeit not in a mouse model (Buckmaster and Lew, 2011). During our routine handling of rats for seizure monitoring we found that rats in the pilocarpine-treated group displayed overt struggling and exhibited aggressive behaviors such as biting (see Supplemental Videos 1 & 2). We also noticed that following rapamycin treatment, rats became

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Abbreviations: AEDs, anti-epileptic drugs; mTOR, mammalian target of rapamycin pathway; SE, status epilepticus; TLE, temporal lobe epilepsy; TSC, tuberous sclerosis complex.

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calmer during handling (see Supplemental Video 3). These observations led us to further evaluate the effect of rapamycin on psychiatric behavior. We particularly focused on aggressive behavior as it is a prominent and easily detectable behavioral change in the rat model of pilocarpine-induced epilepsy.

EXPERIMENTAL PROCEDURES

Animals

Sprague–Dawley rats were purchased from Taconic (Taconic, NY). Rats were housed in a room with *ad libitum* access to food and water. All experiments were performed in accordance with National Institutes of Health guidelines for the care and use of laboratory animals and were approved by our Institutional Animal Care and Use Committees.

Induction of SE by pilocarpine

The experiment started with a total of 150 age-matched Sprague–Dawley rats (50–75 g). Of which, 113 rats were first injected i.p. with a solution of LiCl (3 mEq/kg; 1 ml/kg) 16 h prior to i.p. administration of pilocarpine hydrochloride (40 mg/kg; 1 ml/kg) as described (Hirsch et al., 1992). The remaining 37 rats were used as control and were handled and housed in the same manner as the pilocarpine-treated animals and received an equal volume of saline solution (0.9% NaCl). Rats were then put into individual clear plexiglass cages to allow optimal video recording under infrared light. Immediately following injection of pilocarpine seizure activity was continuously recorded by a DVR video surveillance system (The Advanced Security Inc., Chino Hills, CA) for 90 min. Seizure events were graded according to Racine's standard classification (Racine, 1972): stage 1, behavioral arrest with mouth and facial movement; stage 2: head nodding; stage 3: forepaw clonus; stage 4: rearing and stage 5: rearing and falling. The majority (n = 101) of pilocarpine-treated rats developed acute stage 5 seizures. Twelve rats did not initially develop stage 5 seizures, but did after receiving an additional dose of 10 mg/kg of pilocarpine. Seizures were terminated with sodium pentobarbital (25 mg/kg, i.p.) 90 min after administration of pilocarpine. The rats were allowed to spontaneously recover from pentobarbital treatment. Thirty-four rats died within 2 weeks after pilocarpine treatment. All rats were kept in individual cages throughout the experiments.

Behavioral seizure monitoring

Thirty-three pilocarpine-treated rats were monitored for spontaneous recurrent seizures for 12 h/day by DVR video surveillance from weeks 3 to 5 after pilocarpine injection. Videos were then examined by the trained researchers and graded using the Racine scale (Racine, 1972). In the present study only stage 3–5 seizures were counted as they are most easily deciphered from typical rat behavior using video surveillance. After 3 weeks of monitoring, we found 17 (51.5%) of pilocarpine-treated rats developed spontaneous seizures. Pilocarpine-treated rats either with or without spontaneous seizures were then assigned to one of two groups. One group was further treated with rapamycin, the other group was treated with an equal volume of vehicle. EEGs were not performed due to concerns that surgical implantation of EEG electrodes could interfere with behavioral testing. We acknowledge that video recording could miss a small portion of animals that developed mild behavioral seizures or electrographic seizures without a behavioral manifestation. In such a case, those animals would have been assigned to the non-seizure pilocarpine-treated group.

Rapamycin treatment

Immediately before administration, rapamycin (Tecoland) was first dissolved in DMSO (Sigma, St Louis, MO) and diluted in a solution containing 5% tween-20 and 4% ethanol (defined as vehicle). To determine if rapamycin can suppress aggressive behavior, 10 control and 12 pilocarpine-treated rats were given rapamycin 5 mg/kg i.p. for three consecutive days, followed by every other day during a series of behavioral tests. In parallel, 10 additional control rats and 12 pilocarpine-treated rats were given equal volume of vehicle. To determine if the effect of rapamycin is reversible, 12 pilocarpine-treated rats were treated with rapamycin at 5 mg/kg daily for 3 days. Ten pilocarpine-treated and 5 control rats (untreated with pilocarpine) received an equal volume of vehicle daily for 3 days. Rapamycin treatment was administered starting 5–6 weeks after initial treatment with pilocarpine.

Behavioral tests

Excitability and sensory responsiveness of pilocarpine-treated and control rats were evaluated by three behavioral tests which include approach–response, touch–response, and pick up as described (Rice et al., 1998; Gastens et al., 2008). The behavioral test was administrated 5–6 weeks after pilocarpine treatment or 16 h after rapamycin treatment. Each rat was tested every other day between 9:00 and 12:00 pm for a total of five times. With experiments to determine the time course of rapamycin's effect, tests were carried out daily between 1:00 and 5:00 pm. Before each test, individual rats were gently transferred to a designated testing area and habituated for 15 min. All tests were video recorded. The responsiveness to the test was graded using the Racine scale (Racine, 1972). Videos for behavior analysis were encoded so as to prevent the grader from knowing whether individual animals were in the test or control groups.

Approach–response test. A pen held vertically is moved slowly toward the face of the animal. Responses were scored as (1) no reaction; (2) the rat sniffs at the object; (3) the rat moves away from the object; (4) the rat freezes; (5) the rat jumps away from the object; and (6) the rat jumps at or attacks the object.

Touch–response test. The animal is gently prodded in the rump with the blunt end of a pen. Responses were scored as (1) no reaction; (2) the rat turns toward the object; (3) the rat moves away from the object; (4) the rat freezes; (5) the rat jerks around toward the touch; (6) the rat turns away from the touch; and (7) the rat jumps with or without vocalizations.

Pick-up test. The animal is picked up by grasping around the body. Responses were scored as (1) very easy; (2) easy with vocalizations; (3) some difficulty, the rat rears and faces the hand; (4) the rat freezes (with or without vocalization); (5) difficult, the rat avoids the hand by moving away; and (6) very difficult, the rat behaves defensively, and may attack the hand.

Resident–intruder test. The resident–intruder test has been widely used to investigate offensive behavioral such as aggression (Koolhaas et al., 1980; Frye et al., 2002). Intruder rats are temporarily made anosmic (loss of ability to smell) to reduce the likelihood of retaliation toward resident rats. One week before the test, intruder rats (SD, 150 g) received daily intranasal zinc applications for 4 days as described (Frye et al., 2002). Resident rats were habituated in the observation cages for 7 days to facilitate territorial behavior. Before each test, individual rats were gently transferred to a designated testing area and habituated for 15 min. All tests were performed between 9:00 and 12:00 pm. After habituation on the testing day, the intruder was introduced into the resident's cage for 5 min and the test terminated if the intruder receives a maximum of 20 attacks (Frye