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Original Research Paper

Adeno-associated viral vector-mediated preprosomatostatin expression suppresses induced seizures in kindled rats

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ABSTRACT

Somatostatin is expressed widely in the hippocampus and notably in hilar GABAergic neurons that are vulnerable to seizure neuropathology in chronic temporal lobe epilepsy. We previously demonstrated that sustained bilateral preprosomatostatin (preproSST) expression in the hippocampus prevents the development of generalized seizures in the amygdala kindling model of temporal lobe epilepsy. Here we tested whether sustained preproSST expression is anticonvulsant in rats already kindled to high-grade seizures. Rats were kindled until they exhibited 3 consecutive Racine Grade 5 seizures before adeno-associated virus serotype 5 (AAV5) vector driving either eGFP (AAV5-CBa-eGFP) or preproSST and eGFP (AAV5-CBa-preproSST-eGFP) expression was injected bilaterally into the hippocampal dentate gyrus and CA1 region. Retested 3 weeks later, rats that received control vector (AAV5-CBa-eGFP) continued to exhibit high-grade seizures whereas 6/13 rats that received preproSST vector (AAV5-CBa-preproSST-eGFP) were seizure-free. Of these rats, 5/6 remained seizure-free after repeated stimulation sessions and when the stimulation current was increased. These results suggest that vector-mediated expression of preproSST may be a viable therapeutic strategy for temporal lobe epilepsy.

1. Introduction

Over 20 million people continue to have seizures despite pharmacotherapy, leaving seizure-free rates unchanged for the last 15 years (Annegers et al., 1979; Brodie, 2005; Cascino, 2008; Kwan et al., 2010; Kwan and Brodie, 2000; Kwan et al., 2011). Developing novel strategies for these individuals is critical because drug resistant epilepsy is a potentially life-threatening condition accompanied by progressive cognitive impairment that compromises quality of life (Cramer, 1994; Kwan and Brodie, 2001, 2002). New surgical techniques, laser thermal ablation and responsive devices that detect seizures and automatically stimulate the brain to preempt them are exciting new options for these individuals, but they are in their infancy and have not yet substantially changed seizure-free rates (Cascino, 2008; Fisher et al., 2010; Jobst and Cascino, 2015; Morrell and RNS System in Epilepsy Study Group, 2011).

Viral vector-mediated neuropeptide gene delivery may open a promising treatment avenue for temporal lobe epilepsy (TLE; Riban et al., 2009; Vezzani, 2004) for individuals that are resistant to antiepileptic drug treatment or deemed not good candidates for resective epilepsy surgery. The temporal lobe structures involved in seizure genesis and propagation are permissive to neurotropic vector-mediated gene transfer, which has already demonstrated safety in clinical trials for a variety of human disorders (www.clinicaltrials.gov and Freese et al., 1997; McCown, 2004, 2010;

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O'Connor et al., 1997; Riban et al., 2009; Vezzani, 2004, 2007; Weinberg and McCown, 2013; Weinberg et al., 2013). Some neuropeptides exhibit properties that could contribute to the effective treatment of seizures. For example, they may be neuroprotective and reduce excitability when released during high-frequency neuronal activity (Baraban and Tallent, 2004; Hökfelt, 1991). The endogenous expression and synaptic release of some neuropeptides (Csaba et al., 2004; Schwarzer et al., 1996; Simonato et al., 1998; Sperk et al., 1992) and the expression of their receptor subtypes (Csaba et al., 2005, 2004) is altered by seizure activity, suggesting a role for dysregulated neuropeptide signaling in seizure development and maintenance. Neuropeptides have been shown to suppress seizures in experimental epilepsy (Mazarati and Wasterlain, 2002; Zafar et al., 2012). Sustained adeno-associated viral (AAV) vector-mediated expression of galanin, neuropeptide Y or preprosomatostatin (preproSST) has been shown to delay epileptogenesis or suppress seizures in several animal models of TLE (Haberman et al., 2003; Kanter-Schlifke et al., 2007; Lin et al., 2003; McCown, 2006; Noè et al., 2008; Richichi et al., 2004; Sørensen et al., 2009; Woldbye et al., 2010; Zafar et al., 2012).

Somatostatin (SST) is a particularly attractive treatment candidate for TLE (Brazeau et al., 1973; Epelbaum, 1986; Tallent and Qiu, 2008; Vezzani and Hoyer, 1999). The 116 amino acid prepro-hormone preproSST is cleaved by proteases into biologically active SST-14, SST-28 and neuronostatin neuropeptides (Billova et al., 2007; Galanopoulou et al., 1995; Goodman et al., 1983; Samson et al., 2008; Tavianini et al., 1984; Winsky-Sommerer et al., 2000). In the naïve hippocampus, SST is predominantly expressed in CA1 region, CA3 region and hilar GABAergic interneurons (Freund and Buzsáki, 1996) although one report describes SST immunoreactivity in hippocampal granule neurons and pyramidal neurons (Billova et al., 2007). SST levels are responsive to neuronal activity and are altered across neurological diseases that include experimental and human TLE (Riekkinen and Pitkänen, 1990; Robbins et al., 1991). Specifically, SST expression and release is modulated by seizures (Csaba et al., 2004; Simonato et al., 1998; Tallent and Qiu, 2008; Vezzani and Hoyer, 1999). Moreover, a highly selective loss of SST-containing hilar GABAergic neurons occurs in both animal models and humans with TLE (Buckmaster and Dudek, 1997; Robbins et al., 1991; Sloviter, 1987; Sun et al., 2007). This neuronal loss along with changes in the morphology and connectivity of surviving SST-containing neurons has been postulated to mediate the chronic hyper-excitability associated with epileptogenesis (Peng et al., 2013; Zhang et al., 2009). SST knockout mice demonstrate increased severity of induced seizures (Buckmaster et al., 2002) and specific SST receptor (SSTR) agonists have been shown to effectively treat status epilepticus in experimental epilepsy (Aourz et al., 2011; Kozhemyakin et al., 2013). Together these data support the hypothesis that sustained hippocampal SST expression could ameliorate TLE. To this end, we previously demonstrated that sustained AAV5 vector-mediated hippocampal preproSST expression prevented the development of generalized or high-grade seizures in the majority of adult rats (Zafar et al., 2012).

The primary objective of the current study was to test whether sustained AAV5 vector-mediated hippocampal preproSST expression suppressed seizures when initiated *after* a stable seizure state was established in the rat amygdala kindling model (Goddard et al., 1969; McNamara et al., 1980; Sato et al., 1990). We hypothesized that sustained preproSST transgene expression in the hippocampi of *kindled* rats may be anticonvulsant and therefore a promising TLE treatment strategy. To test this hypothesis, an AAV serotype 5 vector was employed to bilaterally express the preproSST gene in the dentate gyrus and CA1 region of amygdala kindled adult rats. Our results showed that sustained hippocampal preproSST expression significantly reduced seizures in this experimental TLE model and that the preproSST anticonvulsant effect persisted over time.

2. Methods

2.1. Subjects

This study was conducted in accordance with Federal and University of Florida Institutional Animal Care and Use Committee policies regarding the ethical use of animals in research. Adult male Sprague Dawley rats (n = 23; 250–275 g upon arrival from Harlan) were housed in pairs in corncob-lined ventilated shoebox cages located in a standard colony room maintained at 24 ± 1 °C on a 12:12 h light:dark cycle (lights on at 0600 h). The rats were given Harlan Teklad Rodent Food Diet #7912 and reverse osmosis-filtered water *ad libitum* for the duration of the experiment.

Two weeks after arrival, rats were implanted with local electrical field potential recording and stimulating electrodes and allowed 10 days to recover from surgery. A baseline kindling session was employed to determine the after-discharge (AD) threshold current used for daily kindling sessions until rats reached the criterion of exhibiting 3 consecutive Racine Grade 5 seizures (Racine, 1972a,b). The following week, rats were randomly assigned to groups injected with either AAV5 vector driving preproSST and eGFP expression or control AAV5 vector driving eGFP expression. Three weeks later rats were retested to determine the stability and endurance of vector effects.

2.2. Bipolar electrode and connector strip preparation

Connector strips were 3D printed at the University of Florida Infinity Fabrication laboratory (http://fablab.arts.ufl.edu/). Bipolar twisted stimulating and recording electrodes were custom made in-house by cutting quadruple Teflon-coated 316 stainless steel wires (Sigmund Cohn Corp.; Mount Vernon, NY) into 6 cm lengths, removing the insulation at both ends and then soldering the male Amphenol gold pins (A-M systems; Sequim, WA) to both ends. Following this, the electrode wires with the Amphenol pins at either end were twisted. The loop generated at the end of the twisted wires was cut to produce an uninsulated tip that would make contact with the target tissue and deliver the administered current. For ground and reference screw electrodes, quadruple Teflon-coated 316 stainless steel wires (2.0 cm and 2.5 cm respectively), uninsulated at both ends were soldered to male Amphenol gold pins at one end. Stainless steel bone screws (FHC Inc.; Bowdoin, ME) were connected with a Unitek spot welder to the other end. Electrode wires were checked for continuity and impedance tested with an LCR/ESR meter (B&K Precision, Yorba Linda, CA). Only electrodes with impedance <1.8 Ω were implanted.

2.3. Surgical implantation of electrodes

Surgical procedures were conducted as described previously (Zafar et al., 2012). Rats were sedated with xylazine (10 mg/kg, subcutaneous) before anesthesia was induced with 4% isoflurane in 1 L/min oxygen and maintained at 1.5% isoflurane in 0.5 L/min oxygen. Anesthetized rats were placed in a Kopf stereotaxic frame and their shaven heads sterilized with alternating scrubs of 1% povidone-iodine solution and 70% ethanol. A midline incision exposed bregma and lambda. Two bipolar electrodes (330 μm d) were implanted bilaterally in the amygdala (−2.2 mm AP, ±4.8 mm ML, −8.3 mm DV; Paxinos and Watson, 2007) to stimulate and record activity in the left and right hemispheres counterbalanced randomly across groups. Two small diameter plastic hex nuts containing removable screws were affixed to the skull over dentate gyrus (−3.8 mm AP, ±1.8 mm ML) and CA1 region (−3.8 mm AP, ±1.8 mm ML) target coordinates (Paxinos and Watson, 2007) to keep the skull free of dental cement for later vector delivery. Ground and reference metal screw electrodes were