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Pro-excitatory alterations in sodium channel activity facilitate subiculum neuron hyperexcitability in temporal lobe epilepsy

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ABSTRACT

Temporal lobe epilepsy (TLE) is a common form of adult epilepsy involving the limbic structures of the temporal lobe. Subiculum neurons act to provide a major output from the hippocampus and consist of a large population of endogenously bursting excitatory neurons. In TLE, subiculum neurons are largely spared, become hyperexcitable and show spontaneous epileptiform activity. The basis for this hyperexcitability is unclear, but is likely to involve alterations in the expression levels and function of various ion channels. In this study, we sought to determine the importance of sodium channel currents in facilitating neuronal hyperexcitability of subiculum neurons in the continuous hippocampal stimulation (CHS) rat model of TLE. Subiculum neurons from TLE rats were hyperexcitable, firing a higher frequency of action potentials after somatic current injection and action potential (AP) bursts after synaptic stimulation. Voltage clamp recordings revealed increases in resurgent (I_{NaR}) and persistent (I_{NaP}) sodium channel currents and pro-excitatory shifts in sodium channel activation and inactivation parameters that would facilitate increases in AP generation. Attenuation of I_{NaR} and I_{NaP} currents with 4,9-anhydro-tetrodotoxin (4,9-ah TTX; 100 nM), a toxin with increased potency against $Na_v1.6$ channels, suppressed neuronal firing frequency and inhibited AP bursting induced by synaptic stimulation in TLE neurons. These findings support an important role of sodium channels, particularly $Na_v1.6$, in facilitating subiculum neuron hyperexcitability in TLE and provide further support for the importance of I_{NaR} and I_{NaP} currents in establishing epileptiform activity of subiculum neurons.

1. Introduction

Temporal lobe epilepsy (TLE) is a common type of adult epilepsy and is characterized by the occurrence of spontaneous, recurrent seizures that originate from limbic structures of the temporal lobe (Spencer and Spencer, 1994). Seizures associated with TLE can be difficult to treat with up to 30% of patients being considered therapy resistant (Kwan and Sander, 2004). While the hippocampus proper and the entorhinal cortex have been extensively examined in TLE (Buckmaster and Dudek, 1997; Hargus et al., 2013; Hargus et al., 2011; Sanabria et al., 2001), the subiculum remains largely understudied in the disease. The subiculum receives input from the CA1 and entorhinal cortex layer II/III. In turn, the subiculum provides excitatory input to CA1, deep layers of the entorhinal cortex and other subcortical and cortical regions (O'Mara et al., 2001; Shao and Dudek, 2005; Swanson and Cowan, 1977; Witter and Groenewegen, 1990). In addition to propagating information out of the hippocampus proper, the subiculum can also prevent neuronal signaling from spreading and acts as a gate, via $GABA_A$ receptor signaling, to control hippocampal and parahippocampal interactions. This characteristic has even been shown to shunt epileptiform activity, preventing it from spreading to the entorhinal cortex in *in vitro* models of TLE (Benini and Avoli, 2005). A large population of subiculum neurons is endogenously bursting (Staff et al., 2000). This bursting characteristic, along with the many reciprocating inputs between the subiculum, the hippocampus proper and the entorhinal cortex, have implicated the subiculum in not only amplifying synaptic information received, but to also provide loop circuits within the hippocampal/entorhinal cortex network, facilitating neuronal synchronization (Harris and Stewart, 2001; Naber et al., 2001).

Several lines of evidence support a role for the subiculum in initiating seizure generation in both human patients and animal models of TLE. Firstly, subiculum neurons are spared in patients with TLE and may even increase in density (Alonso-Nanclares et al., 2011; Dawodu and Thom, 2005; Fisher et al., 1998), unlike hippocampal neurons

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where significant neuronal loss occurs (Bernasconi, 2003; Houser, 1990; Mathern et al., 1995). Recordings from resected brain tissue obtained from patients with refractory TLE revealed synchronous spontaneous inter-ictal like epileptiform bursts within the subiculum, but not the hippocampus (Cohen et al., 2002; Wozny et al., 2005). Studies using animal models of TLE support the human patient observations, demonstrating early preictal increases in action potential (AP) firing of subiculum excitatory and inhibitory neurons (Fujita et al., 2014; Toyoda et al., 2013). An increase in the number of bursting subiculum neurons along with augmented post-burst after-depolarizations has also been reported in TLE, (Wellmer et al., 2002), although this may be region specific (Knopp et al., 2005). Increased sprouting from surviving CA1 neurons onto subiculum neurons could lead to a further enhancement of synchronized epileptiform activity (Cavazos et al., 2004; de Guzman et al., 2006).

In addition to alterations in synaptic connectivity, intrinsic alterations are also likely to occur in TLE, driving neuronal network hyperexcitability. Alterations in voltage-gated sodium channel physiology have been implicated in facilitating and maintaining increases in neuronal excitability in epilepsy (Agrawal et al., 2003; Aronica et al., 2001; Hargus et al., 2013; Hargus et al., 2011; Ketelaars et al., 2001; Vreugdenhil et al., 2004; Whitaker et al., 2001) and subiculum neurons isolated from patients with intractable TLE exhibit increased persistent sodium currents (Vreugdenhil et al., 2004). The sodium channel isoform $Na_v1.6$ has received much attention in the development of neuronal hyperexcitability since it is highly expressed along the axon initial segment (AIS) (Hu et al., 2009) where it plays a significant role in the initiation of APs (Royeck et al., 2008) and also along nodes of Ranvier, facilitating saltatory conduction (Boiko et al., 2001; Kaplan et al., 2001). Increases in $Na_v1.6$ activity have been implicated in facilitating neuronal hyperexcitability in entorhinal cortex neurons (Hargus et al., 2013; Hargus et al., 2011) and is increased in kindled animals (Blumenfeld et al., 2009). Moreover, reducing $Na_v1.6$ levels impairs the initiation and development of kindled seizures (Blumenfeld et al., 2009), inhibits spontaneous firing and chemically induced seizures, and reduces firing frequencies in various neurons (Makinson et al., 2014; Raman and Bean, 1997; Royeck et al., 2008; Van Wart and Matthews, 2006). Furthermore, gain-of-function mutations of $Na_v1.6$ lead to pro-excitatory alterations in channel function (Barker et al., 2016) and spontaneous seizures in human patients and in a knock-in mouse model (Veeramah et al., 2012; Wagnon et al., 2015).

The importance of sodium channels in facilitating increases in subiculum neuron excitability in epilepsy has yet to be determined. In the current study we investigated if sodium channel function was altered in subiculum neurons in TLE using the continuous hippocampal stimulation (CHS) rat model. Additionally, we wanted to see if persistent (I_{NaP}) and resurgent (I_{NaR}) sodium channel currents were increased in TLE. I_{NaP} currents are slow inactivating currents that arise in sub-threshold voltage ranges and are capable of amplifying a neuron's response to synaptic input (Stafstrom, 2007). I_{NaR} currents occur from channel re-opening during the repolarization phase of the AP and both I_{NaR} and I_{NaP} have been shown to facilitate bursting APs and high frequency AP firing (Lewis and Raman, 2014; Raman and Bean, 1997; Stafstrom, 2007; van Drongelen et al., 2006; Yue et al., 2005) Here we show that bursting subiculum TLE neurons are hyperexcitable and display increased persistent (I_{NaP}) and resurgent (I_{NaR}) sodium currents. Pro-excitatory alterations in sodium channel activation and inactivation gating were also detected. Using a tetrodotoxin metabolite, 4,9-anhydro-TTX to inhibit mainly $Na_v1.6$ channels (Rosker et al., 2007), we show that inhibition of I_{NaP} and I_{NaR} currents leads to attenuation of subiculum neuronal hyperexcitability and burst firing associated with TLE. We propose that increases in I_{NaP} and I_{NaR} currents and pro-excitatory changes in sodium channel physiology, together with synaptic network changes, contribute to the hyperexcitability of subiculum neurons in TLE, which aid in seizure initiation and seizure spread throughout the temporal lobe.

2. Materials and methods

2.1. Animals

All animal experiments were conducted in accordance with the guidelines established by the National Institutes of Health guide for the Care and Use of Laboratory Animals and were approved by the University of Virginia's Institute of Animal Care and Use Committee. Adult male Sprague–Dawley rats (250–300 g) received a bipolar twisted pair of stainless steel electrodes to either hemisphere unilaterally in the posterior ventral hippocampus for stimulation and recording (coordinates from bregma AP ~ −5.3 mm, ML ~ 4.9 mm, DV ~ 5.0 mm, bite at ~−3.5 mm) (Paxinos and Watson, 2006). Electrodes were attached to Amphenol connectors and secured to the skull with jeweler's screws and dental acrylic. One week following surgery, rats were stimulated through the hippocampal electrode to induce limbic *status epilepticus* using a protocol previously described (Lothman et al., 1989). In brief, animals were stimulated for 90 min with 10-s trains of 50 Hz, 1 ms biphasic square waves with a maximum intensity of 400 μA peak to peak delivered every 11 s. Following the induction of and recovery from limbic *status epilepticus*, rats were placed in standard laboratory housing. Three months after the induction of *status epilepticus*, animals were evaluated for the presence and frequency of spontaneous temporal lobe seizures (Bertram and Cornett, 1994). During the monitoring phase, rats were placed in specially designed cages, which allowed full mobility of the animals, good visualization for video monitoring, and a stable recording environment. Animals had free access to food and water, as well as a standard 12 h light–dark cycle. Seizures were recorded and documented using a commercial computerized EEG program (Harmonie, Stellate Systems). All data were reviewed at an offline reading station connected to the vivarium computers via a local area network. The time of occurrence, behavioral severity (Racine 5 point scale) and duration for all seizures were noted.

2.2. Seizure determination

Electrographic seizures in the rats were characterized by the paroxysmal onset of high frequency (> 5 Hz) increased amplitude discharges that showed an evolutionary pattern of a gradual slowing of the discharge frequency and subsequent post-ictal suppression. Seizure duration was measured from the onset of the high frequency activity or initial spike to the cessation of the terminal regular electrographic clonic activity.

3. Brain slice preparation and action potential recordings

Horizontal brain slices (300 μm) were prepared from adult male Sprague–Dawley rats (350–450 g). Rats were euthanized with isoflurane, decapitated, and brains rapidly removed and placed in chilled (4 °C) artificial cerebrospinal fluid (ACSF) containing (in mM): 125 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 2 $CaCl_2$, 1 $MgCl_2$, 0.5 L-Ascorbic acid, 10 glucose, 25 $NaHCO_3$, and 2 Pyruvate (oxygenated with 95% O_2 and 5% CO_2). Slices were prepared using a Vibratome (Vibratome 1000 Plus), transferred to a chamber containing oxygenated ACSF, incubated at 37 °C for 45 min, and then stored at room temperature. For recordings, slices were held in a small chamber and superfused with heated (32 °C) oxygenated ACSF. Subiculum bursting neurons were found to be in high density in the distal pyramidal layer just adjacent to the presubiculum. The subiculum pyramidal layer was identified by infra-red video microscopy (Hamamatsu, Shizouka, Japan) using a Zeiss Axioscope microscope. Whole-cell voltage and current clamp recordings were performed using an Axopatch 700B amplifier (pCLAMP 10 software, Molecular Devices) and a Digidata 1322A (Molecular Devices). Electrodes were fabricated from borosilicate glass using a Brown-Flaming puller (model P97, Sutter Instruments Co) and had resistances of 3.5–4.0 MΩ when filled with an intracellular recording solution