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Effects of TRPV1 activation on synaptic excitation in the dentate gyrus of a mouse model of temporal lobe epilepsy

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ABSTRACT

Temporal lobe epilepsy (TLE) is a condition characterized by an imbalance between excitation and inhibition in the temporal lobe. Hallmarks of this change are axon sprouting and accompanying synaptic reorganization in the temporal lobe. Synthetic and endogenous cannabinoids have variable therapeutic potential in treating intractable temporal lobe epilepsy, in part because cannabinoid ligands can bind multiple receptor types. This study utilized in vitro electrophysiological methods to examine the effect of transient receptor potential vanilloid type 1 (TRPV1) activation in dentate gyrus granule cells in a murine model of TLE. Capsaicin, a selective TRPV1 agonist had no measurable effect on overall synaptic input to granule cells in control animals, but significantly enhanced spontaneous and miniature EPSC frequency in mice with TLE. Exogenous application of anandamide, an endogenous cannabinoid that acts at both TRPV1 and cannabinoid type 1 receptors (CB1R), also enhanced glutamate release in the presence of a CB1R antagonist. Anandamide reduced the EPSC frequency when TRPV1 were blocked with capsazepine. Western blot analysis of TRPV1 receptor indicated protein expression was significantly greater in the dentate gyrus of mice with TLE compared with control mice. This study indicates that a prominent cannabinoid agonist can increase excitatory circuit activity in the synaptically reorganized dentate gyrus of mice with TLE by activating TRPV1 receptors, and suggests caution in designing anticonvulsant therapy based on modulating the endocannabinoid system.

Introduction

Temporal lobe epilepsy (TLE) is a common neurological condition affecting approximately 1% of the human population. It is associated with characteristic changes in neuronal circuitry, which render the brain more susceptible to seizure generation (Ben-Ari et al., 1981; Babb et al., 1991; Dudek and Spitz, 1997). Circuit modification includes hippocampal cell loss and axon sprouting, which have been associated with the development of TLE in animal models and humans (Ben-Ari, 1985; Tauck and Nadler, 1985; Sutula et al., 1989; Shibley and Smith, 2002; Smith and Dudek, 2001). Mossy fibers of the granule cells sprout into the inner molecular layer of the dentate gyrus where they form excitatory synapses with the other granule cells, resulting in a recurrent excitatory circuit associated with TLE (Sutula et al., 1989; Franck et al., 1995; Wuarin and Dudek, 1996; Winokur et al., 2004).

Recent evidence suggests that cannabinoids and endocannabinoids play a protective role under excitatory conditions in the brain and in modulating circuits activated during seizures (Wallace et al., 2002, 2003; Marsicano et al., 2003). Release of the endocannabinoids, arachydonoylethanolamide (anandamide; AEA) and 2-arachydonoylglycerol (2-AG) is increased during seizures and the synthetic cannabinoid receptor agonist WIN 55,212–2 can suppress seizure development in animal models of TLE (Marsicano et al., 2003; Wallace et al., 2003). Thus, agonist binding to cannabinoid type 1 receptors (CB1R) tends to suppress seizure activity in experimental epilepsy.

In addition to binding CB1R centrally, several eicosanoid endocannabinoid agonists, including AEA, also functionally activate TRPV1. TRPV1 receptors are non-specific cation channels and are believed to be molecular integrators of various stimuli, including capsaicin, pH, and high temperature, being well characterized in peripheral pain pathways. TRPV1 is also expressed in the brain, including the hippocampus and the dentate gyrus (Mezey et al., 2000; Roberts et al., 2004; Toth et al., 2005; Cristino et al., 2006), but little is known about the physiological functions of central TRPV1 receptors. Increasing evidence suggests activation of both TRPV1 and CB1R by AEA centrally (Al-Hayani et al., 2001; Derbenev et al., 2006; Kauer and Gibson, 2009; Kofalvi et al., 2007; Starowicz et al., 2008), implying a dual role for endocannabinoid agonists in brain areas containing both receptors. It is necessary to understand such dual roles for endocannbinoids to design effective therapies based on the endocannabinoid system.

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In preliminary studies, we observed a transient increase in EPSC frequency in many granule cells treated with AEA in mice with TLE (Bhaskaran and Smith, 2007). AEA activates TRPV1 as well as cannabinoid receptors, a mixed AEA response due to TRPV1 and CB1R activation is observed in other brain systems where both receptors are located (Derbenev et al., 2006), and blockade of endocannabinoid activity actually exacerbates seizures (Wallace et al., 2003; Braakman et al., 2009). We therefore hypothesized that TRPV1 activation and expression would be observed in the new recurrent excitatory circuitry formed between granule cells in association with TLE development in pilocarpine-treated mice.

Methods

Animals

Adult male CD1 mice (Harlan, Indianapolis, IN, USA) weighing 25–30 g were housed individually on a 12-hour day and night cycle. Food and water were available ad libitum. The animals were housed at least 7 days prior to beginning treatment. All procedures were approved by the Tulane University and University of Kentucky Animal Care and Use Committees.

Pilocarpine injection

Mice were administered an intraperitoneal injection (i.p.) of methylscopolamine in sterile saline (1 mg/kg) 15–30 min prior to injection of pilocarpine to reduce the peripheral cholinergic effects of the pilocarpine. Experimental animals were then injected i.p. with a single dose of pilocarpine hydrochloride (280–290 mg/kg) in sterile saline vehicle (0.9% NaCl; 0.1 ml total volume) as described previously (Shibley and Smith, 2002). Control mice were age-matched with treated mice and were administered a comparable volume of vehicle or were not injected after the initial methylscopolamine treatment.

Seizure behavior was observed for at least 2 h starting immediately after the pilocarpine injection. The category and the number of generalized convulsive seizures in each 1/2-hour period were tallied. A modified version of the seizure scale described by Racine (1972) was used to identify seizure severity, with primary attention given to convulsive seizures (i.e., categories 3 to 5) as they were correlated with the eventual development of spontaneous seizures, mossy fiber sprouting, and increased recurrent excitation in the dentate gyrus (Shibley and Smith, 2002; Winokur et al., 2004). Convulsive seizures in mice most often included unilateral limb myoclonus, loss of postural control and repetitive jumping. Seizures were typically of 30–90 s duration and were separated by variable duration periods of relative inactivity. The periods between convulsive seizures were marked by continuous low-level seizure-like activity (i.e., categories 1–2) that typically included continuous head bobbing, chewing, wet dog shakes, and stiff tail. A mouse that experienced a minimum of 3 generalized convulsive seizure events within the 2 h following pilocarpine injection was considered to have undergone status epilepticus (SE). In addition to standard rodent chow and water, mice were supplied with water-moistened chow and a 5% sucrose solution in water in a petri dish inside the cage for 4 days after SE induction to help replenish fluids.

Slice preparation

Mice were anesthetized to effect by halothane inhalation and then decapitated. The brains were rapidly removed and immersed in oxygenated (95% O_2/5% CO_2) ice-cold (0–4 °C) artificial cerebrospinal fluid (ACSF) containing (in mM): 124 NaCl, 3 KCl, 26 $NaHCO_3$, 11 glucose, 2 $CaCl_2$, and 1.3 $MgCl_2$, pH = 7.3–7.4, with an osmolality of 290–305 mOsm/kg. Brains were then blocked and glued to a sectioning stage and sliced in the horizontal plane such that transverse slices (300–400 μm thickness) of the ventral ~2/3 of the hippocampal formation were cut in cold oxygenated ACSF using a vibrating microtome (Vibratome Series 1000; Technical Products Intl, St Louis, MO, USA). The hippocampus was then separated from the surrounding tissue, being sure to completely remove any entorhinal cortex from the slice. Slices were then transferred to a storage chamber, where they were perfused with warm (32–35 °C) and oxygenated ACSF.

Whole-cell patch-clamp recordings

After an equilibration period of 1–2 h, granule cells in the dentate gyrus were targeted for recording under a 40× water-immersion objective (NA = 0.8) with infrared-differential interference contrast (IR-DIC) optics (Olympus, BX51WI) using a CCD video camera. Whole-cell voltage-clamp recordings from granule cells of the dentate gyrus were obtained using pipettes with open tip resistance of 2–5 MΩ using a Multiclamp 700A or Axopatch 200B amplifier (Axon Instruments, Union City, CA, USA). Signals were low-pass filtered at 2–5 kHz, digitized at 88 kHz (Neurocorder, Cygnus Technology, Delaware Water Gap, PA, USA), and recorded onto videotape as well as to a PC-style computer (Digidata 1440A or 1320A, Axon Instruments). Data were captured using pCLAMP programs (Axon Instruments) and analyzed using pCLAMP or Mini-Analysis (Synaptosoft, Decatur, GA, USA). Recording pipettes were pulled from borosilicate glass (1.65 mm outer diameter and 0.45 mm wall thickness, Garner Glass Co., Claremont, CA, USA) with a P-87 puller (Sutter Instruments) and were filled with (in mM): 130 K^+-gluconate , 10 HEPES, 1 NaCl, 1 $MgCl_2$, 1 $CaCl_2$, 3 KOH, 5 EGTA, 2 Mg-ATP. Seal resistance was typically 1–4 GΩ and series resistance, measured from brief voltage steps (10 mV, 5 ms) applied through the recording pipette was typically <30 MΩ, uncompensated. Spontaneous excitatory postsynaptic currents (sEPSCs) were examined at a holding potential of −70 mV. Synaptic events were characterized by a typically fast rising phase and exponential decay phase, and only currents with amplitudes greater than twice peak-to-peak noise level were included for analysis. Recordings in which a >20% change in series resistance was measured during drug application were excluded from the analysis. Input conductance was estimated by measuring the current at the end of brief (20–400 ms) voltage pulses of 5–10 mV. Resting membrane potential was determined by periodically monitoring the voltage at which no current was measured (i.e. briefly removing voltage-clamp control of the neuron by switching to $I = 0$) during the recording.

Drug application

Whole-cell recordings were usually done in the absence of added Mg^{2+} in the ACSF in order to remove the Mg^{2+}-dependent blockade of the NMDA receptors near resting membrane potential and expose network excitability (Mayer et al., 1984; Smith and Dudek, 2001, 2002; Winokur et al., 2004). The $GABA_A$ receptor antagonist, bicuculline methiodide (30 μM; Sigma) was added to ACSF to reduce synaptic inhibition. Tetrodotoxin (TTX; 1 μM) (Sigma or Alomone labs, Jerusalem, Israel) was applied to study action-potential-independent synaptic events (i.e., 'miniature' EPSCs). The selective TRPV1 agonist (*E*)-*N*-[(4-Hydroxy-3-methoxyphenyl) methyl]-8-methyl-6-nonenamide (capsaicin; 1–10 μM; Tocris) and the endogenous cannabinoid/vanilloid ligand, AEA (1–10 μM; Tocris) were used to activate TRPV1 and/or CB1R. The CB1R antagonist/inverse agonist, *N*-(Piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (AM 251; 10 μM; Tocris) and selective TRPV1 antagonist, *N*-[2-(4-Chlorophenyl)ethyl]-1,3,4,5-tetrahydro-7,8-dihydroxy-2*H*-2-benzazepine-2-carbothioamide (capsazepine; CPZ; 10 μM; Tocris) were used as antagonists to isolate receptors involved in agonist responses. Capsaicin, capsazepine, and AM251 were dissolved in 0.01% DMSO; AEA was dissolved in Tocrisolve®. Vehicle applied alone at identical final concentrations in preliminary analyses ($n = 5$ cells each) was without effect.