Available online at www.sciencedirect.com
ScienceDirect
Neuroscience Letters 411 (2007) 11–16
Neuroscience Letters
www.elsevier.com/locate/neulet

Cannabinoid CB1 receptor antagonists cause status epilepticus-like activity in the hippocampal neuronal culture model of acquired epilepsy

Laxmikant S. Deshpande [a], Sompong Sombati [a], Robert E. Blair [a], Dawn S. Carter [c], Billy R. Martin [b], Robert J. DeLorenzo [a,b,d,*]

[a] *Department of Neurology, Virginia Commonwealth University, Richmond, Virginia 23298, USA*
[b] *Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, Virginia 23298, USA*
[c] *Department of Anatomy and Neurobiology, Virginia Commonwealth University, Richmond, Virginia 23298, USA*
[d] *Department of Molecular Biophysics and Biochemistry, Virginia Commonwealth University, Richmond, Virginia 23298, USA*

Received 28 June 2006; received in revised form 14 September 2006; accepted 20 September 2006

Abstract

Status epilepticus (SE) is a major medical emergency associated with a significant morbidity and mortality. Little is known about the mechanisms that terminate seizure activity and prevent the development of status epilepticus. Cannabinoids possess anticonvulsant properties and the endocannabinoid system has been implicated in regulating seizure duration and frequency. Endocannabinoids regulate synaptic transmission and dampen seizure activity via activation of the presynaptic cannabinoid receptor 1 (CB1). This study was initiated to evaluate the role of CB1 receptor-dependent endocannabinoid synaptic transmission towards preventing the development of status epilepticus-like activity in the well-characterized hippocampal neuronal culture model of acquired epilepsy using patch clamp electrophysiology. Application of the CB1 receptor antagonists SR141716A (1 μM) or AM251 (1 μM) to "epileptic" neurons caused the development of continuous epileptiform activity, resembling electrographic status epilepticus. The induction of status epilepticus-like activity by CB1 receptor antagonists was reversible and could be overcome by maximal concentrations of CB1 agonists. Similar treatment of control neurons with CB1 receptor antagonists did not produce status epilepticus or hyperexcitability. These findings suggest that CB1 receptor-dependent endocannabinoid endogenous tone plays an important role in modulating seizure frequency and duration and preventing the development of status epilepticus-like activity in populations of epileptic neurons. The regulation of seizure activity and prevention of status epilepticus by the endocannabinoid system offers an important insight into understanding the basic mechanisms that control the development of continuous epileptiform discharges.

Keywords: CB1 receptor; Status epilepticus; Cannabinoid; Epilepsy; Endocannabinoid tone

Epilepsy is one of the most common neurological disorders affecting approximately 1–2% of the world population [5]. It is characterized by the occurrence of spontaneous recurrent epileptiform discharges (SREDs) or seizures [9,16]. Status epilepticus (SE) is a major neurological emergency characterized by prolonged seizures [3] and is associated with significant morbidity and mortality [17]. Seizure initiation and termination are governed by complex synaptic regulation of neuronal excitability [4,9]. However, little is known about the molecular mechanisms that mediate seizure termination and prevent development of SE in populations of epileptic neurons.

Cannabinoids, such as marijuana and other derivatives, have been used since ancient times for the treatment of seizures [1] and have also been shown to possess anticonvulsant properties [6]. The endocannabinoid system consists of at least two cannabinoid receptors (CB1 and CB2), its endogenous ligands (endocannabinoids: anandamide and 2-AG) and the protein machinery for their synthesis, transport and degradation [11]. It is well documented that in response to physiological (and pathological) synaptic stimulation, endocannabinoids are synthesized and released "on demand" and travel in a retrograde manner to activate the presynaptic CB1 receptors to inhibit neurotransmitter release [15]. It is thought that endocannabinods tonically activate CB1 receptors to generate an "endocannabinoid tone"

* Corresponding author at: Virginia Commonwealth University, School of Medicine, P.O. Box 980599, Richmond, VA 23298, USA.
Tel.: +1 804 828 8969; fax: +1 804 828 6432.
E-mail address: rdeloren@hsc.vcu.edu (R.J. DeLorenzo).

0304-3940/$ – see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2006.09.046

that modulates neuronal excitability [18]. Recent studies in animal models and neuronal cultures have demonstrated that both cannabinoids and the endocannabinoid system may act to regulate seizure duration and termination [2,12,18,19]. The CB1 receptor has been shown to mediate many of the anticonvulsant effects of cannabinoids [20] and to play an important role in regulating synaptic transmission [15]. Thus, in the epileptic phenotype, the endocannabinoid system may provide an intrinsic mechanism for terminating seizure activity and preventing the development of SE.

This study was initiated to evaluate the role of the CB1 receptor-dependent endocannabinoid tone in preventing the development of SE in populations of epileptic neurons. The endocannabinoid tone was disrupted by utilizing the CB1 receptor antagonists, *N*-(piperidin-1-yl-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamidehydrochloride (SR141716A) and *N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (AM251), in the well characterized hippocampal neuronal culture (HNC) model of acquired epilepsy using patch clamp electrophysiology [16]. The results indicate that application of CB1 receptor antagonists caused "epileptic" neurons to develop SE-like activity, characterized by essentially continuous epileptiform discharges. Our findings provide the first direct evidence that CB1 receptor-dependent endocannabinoid tone is essential for maintaining tonic inhibition of continuous seizure activity and prevention of SE.

R(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolol[1,2,3 de]-1,4-benzoxazinyl]-(1-naphthalenyl)methanone (WIN55,212-2) was purchased from Sigma Chemical (St. Louis, MO). AM251 was purchased from Tocris Cookson Inc. (Ellisville, MO). SR141716A was supplied through the NIDA Chemical Synthesis and Drug Supply Program. Stocks of WIN55,212-2 and SR141716A were made up in a vehicle stock solution of absolute ethanol, Emulphor-620 (Rhone-Poulenc, Inc., Princeton, NJ) and 0.9% saline at a ratio of 1:1:18 that was then diluted at a minimum of 1:500 to a final working concentration in the physiological bath recording solution (pBRS). Stock solutions of AM251 were prepared in DMSO and stored aliquoted at −20 °C. The final working concentration of DMSO was 0.01%. The pBRS consisted of (in mM): 145 NaCl, 2.5 KCl, 10 HEPES, 2 $CaCl_2$, 1 $MgCl_2$, 10 glucose, and 0.002 glycine, pH 7.3, and osmolarity adjusted to 325 ± 5 mOsm with sucrose. All the drugs were bath-applied using a multi valve perfusion assembly (Warner Instrument Corp., CT, USA).

All animal use procedures were in strict accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and approved by Virginia Commonwealth University's Institutional Animal Care and Use Committee. Studies were conducted on primary mixed hippocampal neuronal cultures prepared as described previously with slight modifications [16]. In brief, hippocampal cells were obtained from 2-day postnatal Sprague–Dawley rats (Harlan, Frederick, MD) and plated at a density of 2.5×10^4 cells/cm^2 onto a glial support layer previously plated onto poly-L-lysine coated (0.05 mg/ml) 35-mm grid cell culture dishes (Nunc, Naperville, IL). Cultures were maintained at 37 °C in a 5% CO_2/95% air atmosphere and fed twice weekly with NeuroBasal-A medium supplemented with B-27 (Invitrogen Corp., San Diego, CA) containing 0.5 mM L-glutamine. All other reagents were purchased from Sigma–Aldrich Co. (St. Louis, MO) unless otherwise noted.

After 2 weeks, cultures were utilized for experimentation. Neuronal cultures were rendered "epileptic" by exposing them for 3 h to a solution containing no added $MgCl_2$ (low Mg^{2+}) [2,16]. Briefly, after the removal of maintenance media, neurons were gently washed with 3 × 1.5 ml of pBRS (±1 mM $MgCl_2$) and then allowed to incubate in this solution at 37 °C under 5% CO_2/95% air atmosphere. At the end of the 3-h period, cultures were restored to the physiological concentration (1 mM) of $MgCl_2$ by gently washing with 3 × 1.5 ml of minimum essential medium, returned to the maintenance medium and incubated at 37 °C under 5% CO_2/95% air atmosphere. Thus, low Mg^{2+} treatment was carried out with pBRS without added $MgCl_2$, whereas sham controls were treated with pBRS containing 1 mM $MgCl_2$.

Whole cell current clamp recordings were performed using previously established procedures in our laboratory [2,16]. Briefly, cell culture medium was replaced with pBRS, mounted on the stage of an inverted microscope (Nikon Diaphot, Tokyo, Japan), continuously perfused with pBRS. Patch electrodes with a resistance of 2–4 MΩ were pulled on a Brown-Flaming P-80C electrode puller (Sutter Instruments, Novato, CA), fire-polished, filled with a solution containing (in mM): 140 K^+ gluconate, 1 $MgCl_2$, and 10 Na-HEPES, pH 7.2, and the osmolarity was adjusted to 290 ± 10 mOsm with sucrose. Intracellular recordings were carried out using an Axopatch 200B amplifier (Axon Instruments, Foster City, CA) in current clamp mode. Data were digitized and transferred to videotape using a PCM device (Neurocorder, New York, NY) and played back on a DC-500 Hz chart recorder (Astro-Med Dash II, Warwick, RI).

Employing the HNC model of acquired epilepsy, we evaluated the effects of CB1 receptor antagonists on seizure activity. Fig. 1A is a representative current clamp recording from a control neuron showing occasional spikes. The induction of acquired epilepsy by the low Mg^{2+} treatment is shown in Fig. 1B. Recordings from neurons in cultures 1-day after a 3 h, low-Mg^{2+} treatment demonstrated SREDs, a characteristic of acquired epilepsy (Fig. 1B). These SREDs or "seizure episodes" occurred for the life of the neurons in culture and demonstrated paroxysmal depolarization shifts, a pathophysiological characteristic of epilepsy (Fig. 1B) [9,16]. Two SREDs lasting between 1 and 1.5 min are observed in this epileptic neuron in a continuous 30-min recording (Fig. 1B). Each SRED started and stopped spontaneously. Multiple recordings ($n = 6$) from adjacent neurons demonstrate that these SREDs were synchronous events occurring in populations of neurons. SREDs were never observed in control neurons ($n = 45$). There were no significant differences in membrane potential and input resistance between control and epileptic neurons. Control neurons exhibited a mean membrane potential of −60.2 ± 1.5 mV and a mean input resistance of 119.6 ± 7.4 MΩ, whereas the epileptic neurons demonstrated a mean membrane potential of −59.4 ± 1.1 mV and a mean input resistance of 109.8 ± 8.9 MΩ. The HNC model of