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Endocannabinoids block status epilepticus in cultured hippocampal neurons

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Abstract

Status epilepticus is a serious neurological disorder associated with a significant morbidity and mortality. Antiepileptic drugs such as diazepam, phenobarbital and phenytoin are the mainstay of status epilepticus treatment. However, over 20% of status epilepticus cases are refractory to the initial treatment with two or more antiepileptic drugs. Endocannabinoids have been implicated as playing an important role in regulating seizure activity and seizure termination. This study evaluated the effects of the major endocannabinoids methanandamide and 2-arachidonylglycerol (2-AG) on status epilepticus in the low-Mg²⁺ hippocampal neuronal culture model. Status epilepticus in this model was resistant to treatment with phenobarbital and phenytoin. Methanandamide and 2-AG inhibited status epilepticus in a dose-dependent manner with an EC₅₀ of 145±4.15 nM and 1.68±0.19 μM, respectively. In addition, the anti-status epilepticus effects of methanandamide and 2-AG were mediated by activation of the cannabinoid CB₁ receptor since they were blocked by the cannabinoid CB₁ receptor antagonist AM251. These results provide the first evidence that the endocannabinoids, methanandamide and 2-AG, are effective inhibitors of refractory status epilepticus in the hippocampal neuronal culture model and indicate that regulating the endocannabinoid system may provide a novel therapeutic approach for treating refractory status epilepticus.

Keywords

Endocannabinoids; Low Mg²⁺ model of status epilepticus; Patch clamp; Epilepsy

1. Introduction

Status epilepticus is a life-threatening neurological disorder associated with a significant morbidity and mortality (DeLorenzo, 2006; DeLorenzo et al., 1995; Hauser and Hesdorffer, 1990). Seizure events that last greater than 30 min or intermittent seizures without regaining consciousness lasting for 30 min or longer are classified as status epilepticus (DeLorenzo et al., 1995; Hauser and Hesdorffer, 1990). Status epilepticus has been shown to cause significant neuronal damage especially in the limbic system in both animals and man (Drislane, 2000). Benzodiazepines including diazepam and lorazepam are the initial drugs of choice for treatment

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of status epilepticus (Treiman et al., 1998). Despite their effectiveness, at least 1/3 of the patient population with generalized convulsive status epilepticus is refractory to benzodiazepines (Chen and Wasterlain, 2006; Treiman et al., 1998). In addition, benzodiazepines such as diazepam rapidly develop pharmacoresistance and lose their effectiveness in treating status epilepticus as the seizure duration increases (Goodkin and Kapur, 2003). Other antiepileptic drugs that can be administered intravenously, such as phenobarbital, divalproex and phenytoin, have also been effective in treating status epilepticus (Chen and Wasterlain, 2006). However, despite aggressive treatment with these agents, over 20% of status epilepticus cases are refractory to treatment with two or more medications (Mayer et al., 2002; Treiman et al., 1998). These cases of status epilepticus are referred to as refractory status epilepticus since they do not respond to the first two major antiepileptic agents used to treat status epilepticus. Due to the high mortality associated with prolonged seizure activity from status epilepticus (Towne et al., 1994), refractory status epilepticus is treated aggressively with the induction of coma using pentobarbital, midazolam and propofol (Chen and Wasterlain, 2006).

The endocannabinoid system has recently been implicated as an important endogenous mechanism for terminating seizures (Lutz, 2004; Smith, 2005; Wallace et al., 2003). This system is comprised of G protein-coupled cannabinoid receptors (CB₁ and CB₂), endogenous cannabinoids (endocannabinoids) and the associated enzymatic systems involved in their synthesis, transport and degradation (Mackie, 2006). Cannabinoids, such as marijuana and other derivatives, have been used since antiquity for the treatment of seizures (Adams and Martin, 1996) and have also been shown to possess anticonvulsant properties (Karler et al., 1974). Search for the endogenous ligands for the cannabinoid receptors first led to the discovery of anandamide (Devane et al., 1992) and then 2-arachidonylglycerol (2-AG) (Stella et al., 1997). It has been shown that physiological or pathological stimulation of neurons stimulate endocannabinoid synthesis that regulate neuronal excitability by activating presynaptic cannabinoid CB₁ receptors and ultimately inhibiting neurotransmitter release in a retrograde fashion. Endocannabinoids are thought mediate their anticonvulsant effects by activating cannabinoid CB₁ receptors. We also recently demonstrated that synthetic cannabinoid CB₁ receptor agonist WIN 55,212-2 blocked both status epilepticus and acquired epilepsy in hippocampal neuronal cultures (Blair et al., 2006). The hippocampus is rich in cannabinoid CB₁ receptors and is also known to be involved in generation of seizures. Despite the powerful neuromodulatory and anticonvulsant effects of cannabinoid CB₁ receptor activation in regulating seizure termination, the role of endocannabinoids in modulating status epilepticus remains unexplored.

This study employed patch clamp electrophysiology to evaluate the role of the endocannabinoids methanandamide and 2-AG in blocking electrographic status epilepticus using the well-characterized hippocampal neuronal culture model of status epilepticus. Removal of Mg²⁺ from culture medium induces tonic high-frequency epileptiform discharges in hippocampal neurons in culture and represents a well-characterized in vitro model of status epilepticus that has been routinely used to carry out biochemical, electrophysiological and molecular investigations on status epilepticus (DeLorenzo et al., 1998, DeLorenzo et al., 2005; Sombati and DeLorenzo, 1995). The spike frequency and epileptiform discharges manifested in this model are essentially identical to the electrographic features of status epilepticus observed with in vivo animal models and in human status epilepticus and represent an excellent model to study status epilepticus and refractory status epilepticus (DeLorenzo et al., 2005; Mangan and Kapur, 2004; Sombati and DeLorenzo, 1995; Goodkin et al., 2005). The results from this study indicate that endocannabinoids inhibit status epilepticus by activating cannabinoid CB₁ receptors. While conventional antiepileptic drugs were ineffective in completely blocking status epilepticus at the high micromolar range, the endocannabinoids were very potent and prevented status epilepticus at nanomolar concentrations. The results

suggest that development of novel therapeutic agents that modulate the endocannabinoid system could offer unique pharmacological approaches for the treatment of status epilepticus.

2. Materials and methods

2.1. Materials

Methanandamide, 2-AG and *N*-(piperidin-1-yl)-5-(4-iodo-phenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (AM251) were purchased from Tocris Cookson, Inc. (Ellisville, MO). Methanandamide was dissolved in sterile ethanol. Stocks of 2-AG and AM251 were prepared in dimethyl sulfoxide and stored aliquoted at -20°C . Working solutions were prepared freshly everyday. The final concentration of dimethyl sulfoxide in the bath solution was 0.01%. Phenytoin was dissolved in dimethyl sulfoxide and phenobarbital was dissolved in sterile water. All drugs and chemicals were purchased from Sigma-Aldrich Co. (St. Louis, MO) unless otherwise noted.

2.2. Hippocampal neuronal culture

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 (Sombati and DeLorenzo, 1995). 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/cm² 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% CO₂/95% 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.

2.3. Hippocampal neuronal culture model of status epilepticus

After 2 weeks, cultures were utilized for experimentation. Maintenance medium was replaced with physiological bath recording solution (pBRS) with or without 1 mM MgCl₂ containing 145 mM NaCl, 2.5 mM KCl, 10 mM HEPES, 2 mM CaCl₂, 10 mM glucose and 0.002 mM glycine, pH 7.3, and osmolarity adjusted to 325 ± 10 mOsm with sucrose. Exposing neuronal cultures to pBRS without added MgCl₂ (low Mg²⁺) induced high-frequency epileptiform bursts (status epilepticus). The status epilepticus continued until pBRS containing 1 mM MgCl₂ was added back to the cultures. Thus, low Mg²⁺ treatment was carried out with pBRS without added MgCl₂, whereas sham controls were treated with pBRS containing 1 mM MgCl₂. Unless otherwise indicated as low Mg²⁺ treatment, experimental protocols utilized pBRS containing 1 mM MgCl₂. This represents the hippocampal neuronal culture model of status epilepticus used in this study that has been well characterized as a useful in vitro model of refractory status epilepticus (DeLorenzo et al., 1998; DeLorenzo et al., 2005; Sombati and DeLorenzo, 1995).

2.4. Whole cell current clamp recordings

Whole cell current clamp recordings were performed using previously established procedures in our laboratory (Sombati and DeLorenzo, 1995). Briefly, neuronal culture plate was mounted on the stage of an inverted microscope (Nikon Diaphot, Tokyo, Japan) continuously perfused with pBRS. Patch electrodes with a resistance of 2 to 4 M Ω were pulled on a Brown-Flaming P-80C electrode puller (Sutter Instruments, Novato, CA), fire-polished and filled with a solution containing 140 mM K⁺ gluconate, 1 mM MgCl₂ and 10 mM Na-HEPES, pH 7.2, osmolarity 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 then played back on a DC-500 Hz chart recorder (Astro-Med Dash II, Warwick, RI).

2.5. Data analysis

For concentration–response analysis, suppression of status epilepticus was determined as a percentage decrease in frequency over increasing concentrations of methanandamide or 2-AG. Status epilepticus frequency was determined by counting individual epileptiform bursts over a recording duration of 5 min for each neuron analyzed before and after application of the drug. Spike frequency analysis was carried out on multiple neurons at each concentration of endocannabinoids. Spike frequencies at each concentration of endocannabinoids were then represented as a percentage inhibition from control frequency (status epilepticus frequency in the absence of endocannabinoids). Least-square linear regression analysis was used to calculate the EC₅₀ (effective concentration that produced 50% of maximal effect) of endocannabinoids for suppression of status epilepticus burst discharges. Data were plotted using SigmaPlot analysis software 8.02 (SPSS Inc., Chicago, IL).

3. Results

3.1. Methanandamide blocks status epilepticus in the hippocampal neuronal culture model

The hippocampal neuronal culture model of status epilepticus is a well-established model that shares many biochemical and electrophysiological changes observed in animal models and human status epilepticus (DeLorenzo et al., 2005; Mangan and Kapur, 2004; Sombati and DeLorenzo, 1995). Removal of Mg²⁺ from the recording solution resulted in the development of continuous tonic high-frequency epileptiform discharges (Fig. 1B). This hyperexcitable state consisted of repetitive burst discharges with each burst comprising multiple spikes overlaying a depolarization shift (Fig. 1B inset). Running the traces on a faster time scale reveals the paroxysmal depolarization shifts and allows for easy measurements of epileptiform discharges and spikes. This approach is used to calculate spike frequency and analyze effects of various drugs on status epilepticus. Spike frequency in this model of status epilepticus is greater than 3 Hz and meets the criteria of electrographic status epilepticus and has been shown to occur synchronously throughout the neuronal network (Sombati and DeLorenzo, 1995).

Methanandamide is a stable analogue of the major endocannabinoid anandamide and has been shown to regulate cannabinoid CB₁ receptor activity (Mackie, 2006). Thus, we evaluated the effects of methanandamide on status epilepticus discharges in the hippocampal neuronal culture model. Methanandamide (300 nM) produced greater than 50% inhibition of spike frequency (Fig. 2C, top). At 500 nM concentrations, methanandamide inhibited low Mg²⁺-induced high-frequency spike activity by 80% when added during the status epilepticus. At 1 μM concentration, methanandamide completely abolished the status epilepticus elicited by low Mg²⁺ treatment (Fig. 2C, bottom). Thus, methanandamide blocked status epilepticus in a concentration-dependent manner with an EC₅₀=145±4.15 nM (Fig. 3A). Treatment of neurons with ethanol alone did not produce any hyperexcitability (data not shown).

3.2. 2-AG blocks status epilepticus in the hippocampal neuronal culture model

2-AG is a major endocannabinoid that has been shown to activate both cannabinoid CB₁ and CB₂ receptors (Mackie, 2006). We evaluated whether 2-AG had the same effects as methanandamide in inhibiting status epilepticus. As observed for methanandamide, 2-AG also blocked status epilepticus in the hippocampal neuronal culture model (Fig. 2D). At 1 μM dose, 2-AG produced greater than 50% inhibition of spike frequency (Fig. 2D, top). Application of 2-AG (3 μM) inhibited low Mg²⁺-induced spiking by 80%. At 10 μM, 2-AG produced a complete blockade of low Mg²⁺-induced status epilepticus (Fig. 2D, bottom). Thus, 2-AG inhibited status epilepticus spike activity in a concentration-dependent manner with an

$EC_{50}=1.68\pm0.19\text{ }\mu\text{M}$ (Fig. 3B). Treatment of neurons with DMSO alone (0.01%) did not produce any hyperexcitability (data not shown).

3.3. Endocannabinoids block status epilepticus in the hippocampal neuronal culture model via cannabinoid CB₁ receptor activation

AM251 is a well-characterized and highly specific cannabinoid CB₁ receptor antagonist (Lan et al., 1999) and was utilized to determine if methanandamide and 2-AG inhibited status epilepticus via a cannabinoid CB₁ receptor-dependent mechanism. As shown in Fig. 4B, application of AM251 (1 μM) blocked methanandamide-mediated inhibition of status epilepticus (Fig. 4B). Similar results were also obtained when AM251 (1 μM) was applied in the presence of 2-AG (Fig. 4C).

The blockade mediated by AM251 was competitive in nature and it took higher concentrations of endocannabinoids to overcome AM251 antagonism. As shown in Fig. 5, in the presence of AM251 (1 μM) the dose–response curves for both the endocannabinoids were shifted to the right. Thus, in the presence of 1 μM AM251 methanandamide blocked status epilepticus spike activity with an EC_{50} of $1.03\pm0.01\text{ }\mu\text{M}$ in comparison to an $EC_{50}=145\pm4.15\text{ nM}$ in the absence of AM251. 2-AG blocked status epilepticus spike activity with an EC_{50} of $17.81\pm1.44\text{ }\mu\text{M}$ in the presence 1 μM AM251 compared to an $EC_{50}=1.68\pm0.19\text{ }\mu\text{M}$ in the absence of AM251. AM251 (1 μM) alone had no effect on low Mg^{2+} -induced status epilepticus spike activity. These results provide strong evidence that methanandamide and 2-AG are blocking status epilepticus through activation of a cannabinoid CB₁ receptor-dependent mechanism.

3.4. Comparison of endocannabinoids with other antiepileptic drugs against status epilepticus in the hippocampal neuronal culture model

The hippocampal neuronal culture model of status epilepticus has been used as a model of refractory status epilepticus (Goodkin and Kapur, 2003) since it has been difficult to control continuous epileptiform discharges in this model with conventional antiepileptic drugs used to treat status epilepticus in humans. We compared the effectiveness of the endocannabinoids and other antiepileptic drugs in controlling status epilepticus. Phenobarbital and phenytoin are widely used agents in the treatment of status epilepticus (Chen and Wasterlain, 2006). However, up to 20% of status epilepticus cases are refractory to treatment with these antiepileptic drugs (Treiman et al., 1998). Effective therapeutic concentrations of phenytoin in controlling seizures are achieved at concentrations of 10–20 $\mu\text{g/mL}$ (Chen and Wasterlain, 2006) and phenytoin has been shown to block epileptic discharges at 10 μM concentration in hippocampal neuronal culture model of acquired epilepsy (Sombati and DeLorenzo, 1995). We recently demonstrated that phenobarbital and phenytoin does not block low Mg^{2+} -induced status epilepticus (Blair et al., 2006). However, it is important to compare other anticonvulsants to the effectiveness of the endocannabinoids in the same system under the conditions used in this study. Phenytoin had no effect in reducing status epilepticus even at concentrations up to 50 μM (Fig. 6B). Phenobarbital was also not able to inhibit continuous epileptiform discharges at concentrations up to 50 μM (Fig. 6C). The data for drug effects with both phenytoin and phenobarbital were analyzed by counting the spike frequency as outlined in Materials and methods section. No significant changes in spike frequency were observed upon application of phenytoin or phenobarbital. Although a little slowing of status epilepticus is sometimes observed at highest concentrations, the spike frequency is still greater than 3 Hz that meets the criteria of electrographic status epilepticus. Effective therapeutic concentrations of phenobarbital are achieved at level of 20–40 $\mu\text{g/mL}$ (Chen and Wasterlain, 2006) and phenobarbital has been shown to block epileptic discharges at 100- μM concentrations in hippocampal neuronal culture model of acquired epilepsy (Sombati and DeLorenzo, 1995).

Both methanandamide and 2-AG, in contrast, were extremely potent in blocking the low Mg^{2+} -induced continuous epileptiform discharges. As shown in Fig. 2C and D, total suppression of status epilepticus by methanandamide and 2-AG was observed at 1 μM and 10 μM , respectively. All the drugs were added in the first 10 min after removal of extracellular Mg^{2+} and were controlled for time after seizure onset. These findings further underscore the powerful neuromodulatory effects of cannabinoid CB_1 receptor activation by endocannabinoids against status epilepticus and suggest that endocannabinoids may serve as important agents in treating refractory status epilepticus and in providing an endogenous mechanism in the brain to prevent the transition from seizure activity to status epilepticus.

4. Discussion

This study demonstrates that the endocannabinoids methanandamide and 2-AG can effectively block status epilepticus in the hippocampal neuronal culture model in a dose-dependent and cannabinoid CB_1 receptor-mediated manner. Methanandamide and 2-AG inhibited high-frequency epileptiform discharges with an EC_{50} of 145 ± 4.15 nM and 1.68 ± 0.19 μM , respectively, and completely abolished status epilepticus at 1 and 10 μM . The ability of the highly specific cannabinoid CB_1 receptor antagonist, AM251 (Lan et al., 1999), to completely reverse the effects of the endocannabinoids in inhibiting status epilepticus demonstrate that these endocannabinoids are blocking status epilepticus by acting as agonists at cannabinoid CB_1 receptors. To our knowledge, these results are the first direct evidence that endocannabinoids can cause the cessation of refractory status epilepticus in a cannabinoid CB_1 receptor-dependent manner.

Loss of drug efficacy and development of pharmacoresistant seizure activity represents a major obstacle in the treatment of status epilepticus (Chen and Wasterlain, 2006; Goodkin and Kapur, 2003; Treiman et al., 1998). A retrospective cohort study of patients in convulsive and subtle status epilepticus in a large academic teaching hospital found that 31% of seizures are refractory to a combination of a benzodiazepine and a second line agent, phenytoin, fosphenytoin or phenobarbital (Mayer et al., 2002). The hippocampal neuronal culture model used in this study is a good model for refractory status epilepticus (Goodkin and Kapur, 2003). Clinically used drugs such as phenobarbital or phenytoin even at concentrations up to 50 μM were ineffective in blocking status epilepticus. On the other hand, the endocannabinoids used in this study were very potent and blocked status epilepticus at nanomolar concentrations. This study provides direct evidence that endocannabinoids, by activating cannabinoid CB_1 receptors, produce a potent suppression of excitation resulting in inhibition of status epilepticus. The results also indicate that the endocannabinoids may be more effective in controlling continuous epileptiform discharges than some currently available agents used to treat status epilepticus, such as phenytoin and phenobarbital.

Recurrent spike discharge after the removal of extracellular Mg^{2+} is a commonly used model to study the mechanisms underlying seizures and seizure-induced plasticity (DeLorenzo et al., 2005; Engel et al., 2000; Goodkin et al., 2005; Sombati and DeLorenzo, 1995). Studies in this model have shown that within 10 min of low Mg^{2+} -induced status epilepticus, there is marked internalization of $GABA_A$ receptors (Goodkin et al., 2005). This progressive time-dependent loss of $GABA_A$ receptors is thought to underlie development of pharmacoresistance to conventional antiepileptic drugs such as phenobarbital and diazepam that are initial drugs of choice for the treatment of status epilepticus. In fact, phenobarbital and phenytoin are ineffective in blocking status epilepticus in this model at any time points. Thus, taken together, this model is considered as model of refractory status epilepticus. While this study shows that endocannabinoids are powerful blockers of low Mg^{2+} -induced status epilepticus in neuronal cultures, in order to accurately determine their effectiveness against refractory status

epilepticus it is necessary to study their effects at increasing durations of status epilepticus. It will be insightful to investigate this in future studies.

The cannabinoid CB₁ receptor is one of the most abundantly expressed G-protein coupled receptors in brain. They are preferentially coupled to G_{i/o} proteins (Howlett et al., 2002) and are primarily located on presynaptic nerve terminals (Katona et al., 1999). Their activation leads to the inhibition of adenylyl cyclase activity resulting in decreased c-AMP production, inhibition of N, P/Q-type calcium channels, activation of inwardly rectifying potassium channels and activation of mitogen-activated protein kinase (Howlett et al., 2002). Thus, their location, distribution and activation of signaling cascades indicate that cannabinoid CB₁ receptors are ideally situated to regulate neuronal excitability. The effective concentrations of methanandamide and 2-AG in blocking status epilepticus in the present study are in close agreement with other previously observed effects of these endocannabinoids. Anandamide and 2-AG, in similar concentration ranges, have been shown to attenuate epileptiform burst discharges elicited by elevation of potassium to 8 mM and omission of Mg²⁺ in hippocampal slices (Ameri and Simmet, 2000; Ameri et al., 1999). In addition, in this concentration range 2-AG also blocks Ca²⁺ spikes and synaptic transmission in cultured hippocampal neurons (Kelley and Thayer, 2004) and inhibits adenylyl cyclase activity in mouse neuroblastoma cells (Pinto et al., 1994). Indeed, cannabinoid CB₁ receptor agonists have been shown to regulate synaptic transmission (Hoffman and Lupica, 2000; Shen et al., 1996) and inhibit persistent epileptic neuroexcitation (Wallace et al., 2003).

Endocannabinoids regulate neuronal excitability via depolarization-induced suppression of excitation (Kreitzer and Regehr, 2001) or inhibition (Ohno-Shosaku et al., 2001; Wilson et al., 2001). In response to Ca²⁺ mediated depolarization of the post-synaptic membrane, endocannabinoids are synthesized “on demand” and then diffuse in a retrograde fashion and activate presynaptic cannabinoid CB₁ receptors to inhibit neurotransmitter release and thus modulate neuronal excitability (Vaughan and Christie, 2005). Intense synaptic activity caused by seizure discharges stimulates 2-AG synthesis (Wallace et al., 2003). Such an “on-demand” synthesis of endocannabinoids is thought to produce anticonvulsant effects and ultimately terminate seizures and regulate seizure duration and frequency in the pilocarpine model of epilepsy. There is also evidence that the endocannabinoids, such as anandamide and 2-AG, dampen epileptiform activity in hippocampal brain slice preparations (Ameri and Simmet, 2000; Ameri et al., 1999). Using the maximal electroshock (MES) model of short-term seizure and the rat pilocarpine model of temporal lobe epilepsy, we demonstrated that cannabinoids and endocannabinoids blocked seizure spread via a cannabinoid CB₁ receptor-dependent mechanism (Wallace et al., 2002, 2001). In addition, application of a cannabinoid CB₁ receptor antagonist lowered the electroshock seizure threshold in MES model (Wallace et al., 2002, 2001).

This abundance of evidence indicates that an active endogenous cannabinoid tone maintains chronic activation of presynaptic cannabinoid CB₁ receptors that play an important role in dampening persistent excitation in epilepsy and may prevent the transition of a single seizure into status epilepticus. In the pilocarpine model of acquired epilepsy, inhibiting endogenous cannabinoid tone using a cannabinoid CB₁ receptor antagonist caused a marked increase in seizure frequency and duration and produced status epilepticus like activity (Wallace et al., 2003) suggesting that the endocannabinoid system was playing an important role in preventing the development of status epilepticus. Although considerable research has provided major insights into the mechanisms leading to the initiation of seizure discharge and the development of epileptogenesis (Lothman et al., 1991), there is little information concerning the endogenous mechanisms that terminate seizure activity (Delorenzo et al., 2005). Thus, the role of the endocannabinoid system in regulating seizure termination takes on added significance. Alteration of endocannabinoid activity during brain injury could lead to the development of

status epilepticus. Indeed, genetic ablation of cannabinoid CB₁ receptor rendered knockout mice more susceptible to kainic acid-induced seizures than wild-type littermates (Marsicano et al., 2003). Understanding the role of the endocannabinoid system in the regulation of status epilepticus is an important area for further research.

The components of the endocannabinoid system have been implicated as potential therapeutic targets for the treatment of various disorders including pain, obesity, glaucoma, migraine and epilepsy (Fowler et al., 2005). This study provides direct evidence that endocannabinoids block status epilepticus in hippocampal neuronal cultures in a dose-dependent manner by activating cannabinoid CB₁ receptors. Since status epilepticus is one of the most severe neurological emergencies, studying the use of agents that regulate the endocannabinoid system offer new hope for the effective treatment of status epilepticus.

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Fig. 1. Induction of tonic high-frequency epileptiform bursts (status epilepticus) in cultured hippocampal neurons during exposure to low Mg²⁺. (A) Representative current clamp recording from a control neuron showing occasional spontaneous action potentials. (B) Representative whole cell recordings during low Mg²⁺ treatment showing induction of continuous epileptiform activity (status epilepticus) with burst frequencies greater than 3 Hz. (a) expansion of a segment from B showing the presence of continuous epileptiform burst discharges. (b and c) Further expansion of two segments from a, revealing the individual epileptiform bursts, each consisting of depolarization shifts overlaid with multiple spike activity. Response data in Fig. 3 and Fig. 5 were determined by frequency analysis of epileptiform bursts.

Fig. 2. Endocannabinoids block low Mg²⁺-induced status epilepticus. (A) Representative control neuron displaying intrinsic baseline activity consisting of spontaneous action potentials. (B) Induction of continuous epileptiform status epilepticus activity in a neuron upon low Mg²⁺ treatment. Dose-dependent blocker effects of methanandamide (C) and 2-AG (D) on low Mg²⁺ induced high-frequency epileptiform bursts (status epilepticus). These data were representative of 5–7 experiments.

Fig. 3.

Endocannabinoids inhibit low Mg^{2+} -induced status epilepticus in a concentration-dependent manner. Frequency of epileptiform bursts (see Fig. 1B, b and c) in the presence of varying concentrations of methanandamide (A) or 2-AG (B) were measured over a 5-min period and then plotted as a percentage change (inhibition) from control (absence of methanandamide or 2-AG) status epilepticus frequencies. Methanandamide blocked low Mg^{2+} -induced status epilepticus with an $EC_{50}=145\pm4.15$ nM whereas 2-AG blocked low Mg^{2+} -induced status epilepticus with an $EC_{50}=1.68\pm0.19$ μ M. Each data point represents percentage change from control $\pm$ SEM ($n=6$ neurons/ concentration).

Fig. 4. Endocannabinoids block low Mg^{2+} -induced status epilepticus in a cannabinoid CB_1 receptor-dependent manner. (A) Methanandamide (1 μ M) blocks low Mg^{2+} -induced status epilepticus. (B) Pretreatment with cannabinoid CB_1 receptor antagonist AM251 (1 μ M) completely blocked the effects of methanandamide (1 μ M) against low Mg^{2+} -induced status epilepticus. (C) 2-AG (10 μ M) blocks low Mg^{2+} -induced status epilepticus. (D) Pretreatment with cannabinoid CB_1 receptor antagonist AM251 (1 μ M) blocked the effects of application of 2-AG (10 μ M). These data were representative of 5 experiments.

Fig. 5.

AM-251 acts as a competitive receptor antagonist of endocannabinoid-mediated inhibition of status epilepticus. Low Mg^{2+} -induced status epilepticus activity was recorded as described under Materials and methods. (A) Methanandamide (●) blocked status epilepticus activity with an EC_{50} of 145 ± 4.15 nM. In the presence of 1 μM AM251 (○), methanandamide inhibited status epilepticus activity with an EC_{50} of 1.03 ± 0.01 μM . (B) 2-AG (●) inhibited low Mg^{2+} -induced status epilepticus activity with an EC_{50} of 1.68 ± 0.19 μM . 2-AG inhibited high-frequency epileptiform discharges in the presence of 1 μM AM251 (○) with an EC_{50} of 17.81 ± 1.44 μM . Curves were fit as described under Materials and methods. Each data point represents mean $\pm$ SEM of 5 experiments.

Fig. 6. Evaluation of anticonvulsant drugs on low Mg²⁺-induced status epilepticus. (A) Induction of status epilepticus following removal of Mg²⁺ from culture media. Addition of the clinically used antiepileptic drugs phenytoin (50 μM) (B) or phenobarbital (50 μM) (C) during low Mg²⁺ treatment at high therapeutic concentrations had no inhibitory effect on status epilepticus activity. These data were representative of 5–7 experiments.