

CANNABINOIDS: DEFENDING THE EPILEPTIC BRAIN

The Endogenous Cannabinoid System Regulates Seizure Frequency and Duration in a Model of Temporal Lobe Epilepsy

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Several lines of evidence suggest that cannabinoid compounds are anticonvulsant. However, the anticonvulsant potential of cannabinoids and, moreover, the role of the endogenous cannabinoid system in regulating seizure activity have not been tested in an in vivo model of epilepsy that is characterized by spontaneous, recurrent seizures. Here, by using the rat pilocarpine model of epilepsy, we show that the marijuana extract 9-tetrahydrocannabinol (10 mg/kg) as well as the cannabimimetic, 4,5-dihydro-2-methyl-4(4-morpholinylmethyl)-1-(1-naphthalenyl-carbonyl)-6H-pyrrolo[3,2,1-*i*,*j*]quinolin-6-one [*R*(+)WIN55,212 (5 mg/kg)], completely abolished spontaneous epileptic seizures. Conversely, application of the cannabinoid CB1 receptor (CB1) antagonist, *N*-(piperidin-1-yl-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamidehydrochloride (SR141716A), significantly increased both seizure duration and frequency. In some animals, CB1-receptor antagonism resulted in seizure durations that were protracted to a level consistent with the clinical condition status epilepticus. Furthermore, we determined that during an short-term pilocarpine-induced seizure, levels of the endogenous CB1 ligand 2-arachidonylglycerol increased significantly within the hippocampal brain region. These data not only indicate anticonvulsant activity of exogenously applied cannabinoids but also suggest that endogenous cannabinoid tone modulates seizure termination and duration through activation of the CB1 receptor. Western blot and immunohistochemical analyses revealed that CB1-receptor protein expression was significantly increased throughout the CA regions of epileptic hippocampi. By demonstrating a role for the endogenous cannabinoid system in regulating seizure

activity, these studies define a role for the endogenous cannabinoid system in modulating neuroexcitation and suggest that plasticity of the CB1-receptor occurs with epilepsy.

CB1 Cannabinoid Receptors and On-demand Defense Against Excitotoxicity

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Abnormally high spiking activity can damage neurons. Signaling systems to protect neurons from the consequences of abnormal discharge activity have been postulated. We generated conditional mutant mice that lack expression of the cannabinoid receptor type 1 in principal forebrain neurons but not in adjacent inhibitory interneurons. In mutant mice, the excitotoxin kainic acid (KA) induced excessive seizures in vivo. The threshold to KA-induced neuronal excitation in vitro was severely reduced in hippocampal pyramidal neurons of mutants. KA administration rapidly increased hippocampal levels of anandamide and induced protective mechanisms in wild-type principal hippocampal neurons. These protective mechanisms could not be triggered in mutant mice. The endogenous cannabinoid system thus provides on-demand protection against acute excitotoxicity in central nervous system neurons.

COMMENTARY

It has been known for centuries that exogenous cannabinoids, such as tetrahydrocannabinol (the major constituent of cannabis), have anticonvulsant activity, but little is known about the molecular mechanisms of the cannabinoid system. In mammals, a number of endogenous cannabinoids have been identified, including 2-arachidonoylglycerol (2-AG) and anandamide. Endocannabinoids, like neurotransmitters, are released from neurons after membrane depolarization and Ca²⁺

influx (1). The cannabinoids work through CB1 receptors centrally and CB2 receptors peripherally. The CB1 receptor is the most highly expressed G protein–coupled receptor in the brain and has been implicated in regulation of neuronal excitability (2). Two recent studies have advanced our understanding of the endogenous cannabinoid system and renewed the interest in cannabinoids as a potential treatment for epilepsy. Both studies show that the endogenous cannabinoid system is rapidly activated after seizure activity. Wallace and colleagues demonstrated that exogenous cannabinoids effectively control seizures in a rat epilepsy model. Marsicano and colleagues extended the use of experimental animal models to determine which neuronal circuits are involved in the anticonvulsant effect of cannabinoids. The investigators used conditional knockout mice to show that the glutamatergic neurons of the forebrain are principally responsible for cannabinoid-mediated protection against seizures.

Wallace et al. used a rat model of temporal lobe epilepsy in which animals have seizures for life after treatment with pilocarpine. Unlike standard antiepileptic drugs [AEDs; e.g., phenobarbital (PB) and phenytoin (PHT)], cannabinoids were very effective AEDs in this rat model. This finding implies that cannabinoids may offer unique advantages in treating seizures refractory to currently prescribed AEDs. Blocking the CB1 receptor increased both seizure frequency and duration in epileptic rats but did not cause seizures in control rats, suggesting that CB1 activation is a response to seizure activity rather than a cause. In support of this idea, the authors found that a single pilocarpine-induced seizure increased levels of 2-AG in the hippocampus within 15 minutes.

Marsicano et al. also measured levels of endogenous cannabinoids in the hippocampus of mice after kainic acid–induced seizures. Unlike in the pilocarpine rat model, they saw no increase in 2-AG, but another cannabinoid, anandamide, showed transiently increased levels, which peaked 20 minutes after injection. Different processing of endocannabinoids in different species and/or different experimental conditions (e.g., kainic acid vs. pilocarpine) may be responsible for these differences. However, both studies showed rapid endocannabinoid increase in response to seizures. This finding further supports the involvement of endogenous cannabinoids in protection against seizure activity. The mechanisms causing this increase in endocannabinoid levels in the brain are still to be determined, but it could be due to enhanced production, decreased degradation, or enhanced synthesis of cannabinoid precursors.

In addition to elevated endocannabinoid levels, Wallace et al. reported that seizure activity increased levels of CB1-receptor protein in the CA1 through CA3 regions of the hippocampus. This supports the idea that plasticity of the endogenous cannabinoid system occurs in the hippocampus in

response to seizures. Animals were studied for up to 1 year, suggesting the increase in CB1-receptor expression is prolonged and probably permanent. To determine which neural circuits were involved in CB1-receptor activation in response to seizures, Marsicano et al. produced a conditional knockout of the CB1 receptor, in which CB1 was deleted in principal glutamatergic neurons of the forebrain but still expressed in cortical γ -aminobutyric acid (GABA)ergic interneurons. Complete knockout of the CB1 receptor resulted in increased seizure severity. This finding also was observed in the conditional knockouts, implying that the principal neurons of the forebrain are necessary for neuroprotection.

How does CB1-receptor activation provide neuroprotection? In vitro, exogenously applied cannabinoids decrease neuronal excitability and inhibit glutamatergic transmission (3). Marsicano et al. measured glutamatergic excitation of CA1 pyramidal neurons in an in vitro hippocampal slice preparation. Kainic acid significantly increased the glutamatergic excitation of neurons obtained from conditional CB1 knockout mice, compared with controls. Therefore endogenously released cannabinoids might provide neuroprotection by CB1 receptor–mediated inhibition of glutamatergic transmission. Marsicano and colleagues also found evidence that CB1 receptors activate intracellular signaling cascades, which may contribute to long-term adaptive cellular changes in response to the seizure.

These studies show that seizures rapidly activate the endogenous cannabinoid system, which provides protection against excessive neuronal activity by reducing excitability of hippocampal pyramidal neurons and activating intracellular signaling cascades. Furthermore, CB1 receptors on principal glutamatergic neurons of the forebrain are primarily responsible for this action. These studies have improved our knowledge of the endocannabinoid system, but further investigation is required. What causes endocannabinoid levels to increase in response to seizures? Which endocannabinoid is important in humans: 2-AG, anandamide, or another CB1-receptor ligand? Can we target therapy to the critical circuits in the forebrain? Smoking marijuana is obviously not an appropriate therapy; in addition to the psychoactive effects, the inhalation of smoke poses obvious health risks. To this end, drug companies have already isolated the active ingredients in cannabis and produced them in the form of a pill or a spray. However, most synthetic cannabinoids still have psychoactive effects and are undesirable for therapeutics. It may be more beneficial to target cannabinoid transport or degradation systems to increase the levels of endogenous cannabinoids. Enhancing the cannabinoid system may prove to be an effective treatment for epilepsy, especially in cases in which standard drugs fail to control seizures.

by Robyn Wallace, Ph.D.

References

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