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

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ABSTRACT

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 has not been tested in an in vivo model of epilepsy that is characterized by spontaneous, recurrent seizures. Here, 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-methyl-4(4-morpholinylmethyl)-1-(1-naphthalenyl-carbonyl)-6H-pyrrolol[3,2,1-i-j]quinolin-6-one \([R(+)]WIN55,212\) (5 mg/kg)], completely abolished spontaneous epileptic seizures. Conversely, application of the cannabinoid CB\(_1\) receptor (CB\(_1\)) antagonist, \(N\)-(piperidin-1-yl-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamidehydrochloride (SR141716A), significantly increased both seizure duration and frequency. In some animals, CB\(_1\) 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 CB\(_1\) ligand 2-arachidonylglycerol increased significantly within the hippocampal brain region. These data indicate not only anticonvulsant activity of exogenously applied cannabinoids but also suggest that endogenous cannabinoid tone modulates seizure termination and duration through activation of the CB\(_1\) receptor. Furthermore, Western blot and immunohistochemical analyses revealed that CB\(_1\) 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 CB\(_1\) receptor occurs with epilepsy.

Characterized by spontaneously recurrent seizures, epilepsy is one of the most common neurological conditions (Hauser and Hesdorffer, 1990). Understanding the factors that contribute to seizure initiation and termination has important implications for our ability to treat epilepsy and for the potential development of novel anticonvulsant agents. Previous evidence has suggested that the endogenous cannabinoid system may be a novel locus of anticonvulsant activity in the brain (Karler et al., 1974; Wallace et al., 2001). Using the maximal electroshock model of short-term seizure, our laboratory determined that cannabinoid compounds block seizure spread via a cannabinoid CB\(_1\) receptor-dependent mechanism (Wallace et al., 2001, 2002). Further study revealed that application of a CB\(_1\) receptor antagonist lowered the electroshock seizure threshold (Wallace et al., 2002), indicating that elimination of endogenous cannabinoid tone at the CB\(_1\) receptor may increase seizure susceptibility.

The CB\(_1\) receptor is the most highly expressed G-protein-coupled receptor in brain (Herkenham et al., 1990) and has been implicated in regulation of neuronal excitability (Wilson and Nicoll, 2001; Ohno-Shosaku et al., 2002). The endogenous cannabinoids, arachidonylethanolamine and 2-arachidonylglycerol (2-AG) (Devane et al., 1992; Mechoulam et al., 1995), are synthesized “on demand” in response to sustained neuronal depolarization and elevated intracellular calcium levels (Stella et al., 1997); both of these events occur with seizure activity (Hauser and Hesdorffer, 1990; Raza et al., 2001). The neuronal hyperexcitability that accompanies seizure discharge may stimulate endogenous cannabinoid synthesis and subsequently result in CB\(_1\) receptor activation. In light of cannabinoid effects on neurotransmission, increased
CB1 receptor activation could influence seizure activity. However, no studies have evaluated the role of the endogenous cannabinoid system in an intact model of epilepsy. This study was initiated to evaluate the role of the CB1 receptor and the endogenous cannabinoid system in regulating seizure activity in a long-term model of epilepsy. We used the pilocarpine model of temporal lobe, partial-complex epilepsy; a rat model of acquired, refractory epilepsy that produces spontaneous recurrent seizures for the lifetime of the animal (Mello et al., 1993; Rice and DeLorenzo, 1998). The pilocarpine model has been shown to closely resemble human refractory partial-complex epilepsy (Mello et al., 1993; Raza et al., 2001). In this study, seizure frequency and duration were determined by continuous electrographic and video recording of each epileptic animal (Rice and DeLorenzo, 1998). The CB1 receptor agonists R(+)-WIN55,212 and Δ9-tetrahydrocannabinol (THC) were evaluated for anticonvulsant efficacy. In addition to agonist effects on seizure activity, the effect of CB1 receptor antagonism on seizure frequency and duration was evaluated using the specific antagonist SR141716A. Hippocampal levels of 2-AG during short-term, pilocarpine-induced seizures were measured to determine whether a correlation exists between endogenous cannabinoid synthesis and seizure activity. In addition, Western blot and immunohistochemical analyses were used to evaluate hippocampal CB1 receptor protein expression in the brains of chronically epileptic and sham control rats. The findings presented suggest an anticonvulsant role for the endogenous cannabinoid system and demonstrate that long-term plasticity of the CB1 receptor occurs with epilepsy.

Epileptic Seizure Monitoring. Seizures were monitored in freely moving animals via simultaneous electroencephalographic (EEG) and video monitoring at least 3 months after pilocarpine treatment (Rice and DeLorenzo, 1998). Electrographic seizures were detected via skull surface electrodes implanted 2 to 3 weeks after the initial episode of SE or after sham treatment in a manner described previously (Perlin et al., 1993). Briefly, animals were put under general ketamine/xylazine anesthesia (75 mg/kg ketamine i.p., 7.5 mg/kg xylazine i.p.), and a midline scalp incision was made to expose the skull. Four surface screw electrodes were implanted bilaterally 2.5 mm from midline, at 2.5 mm posterior to bregma and 2.5 mm anterior to lambda. Surface screw electrodes were connected via Teflon-coated stainless steel wire (Medwire, Mount Vernon, NY) to a male amphenol pin headset assembly, which was secured to the skull with dental acrylic (Hygenic, Akron, OH). Animals were allowed to recover for a minimum of 1 month before experimental analysis. Both electrographic and behavioral seizures were monitored with EKG and video recording, respectively, using a Biomedical Monitoring System Mobile EEG Unit (Campbell, CA).

Materials and Methods

Pilocarpine-Induced Status Epilepticus. Male Sprague-Dawley rats weighing 200 to 250 g were used in accordance with university animal care and use protocols. Animals were housed in single cages on a 12-h/12-h light/dark cycle (lights on at 7:00 AM) and were provided food and water ad libitum. Animals were made epileptic using a modified protocol of Mello et al. (1993) that is well established in our laboratory (Rice and DeLorenzo, 1998). Before pilocarpine injections, animals were administered methylscopolamine nitrate (1 mg/kg i.p.) to minimize peripheral, parasympathetic effects of pilocarpine treatment. Pilocarpine nitrate (375 mg/kg i.p.) was then administered 30 min later. Onset of status epilepticus (SE) typically occurred within 20 to 40 min after pilocarpine injection and was determined when the animal displayed continuous moderate to severe behavioral seizures characterized by forelimb clonus, rearing, and falling.

SE was defined as continuous seizure activity that lasted 30 min or longer or intermittent seizures without regaining consciousness between seizures that lasted 30 min or longer. The severity of convulsions was evaluated, and only those animals that displayed behaviors consistent with ongoing SE were used in the study (Rice and DeLorenzo, 1998). Seizure activity was terminated by consecutive diazepam injections (5 mg/kg i.p., solubilized in 10% ethanol, 45% propylene glycol, and 45% H2O) at 1, 3, and 5 h post onset of SE. Animals continuing to display seizure activity beyond 6 h post onset of SE were euthanized. Control groups were composed of both naive and sham control animals that received methylscopolamine nitrate and diazepam injections only. Approximately 75% of the SE animals developed epilepsy under these conditions, and the mortality rate from SE was approximately 10%. SE animals that did not stop seizing with diazepam treatment were uncommon and represented less than 2% of the animals injected with pilocarpine.
Electrophoresis was carried out on rat hippocampal neuronal membrane preparations from 1 year after SE, epileptic, and age-matched, sham-treated animals. After monitoring of epileptic animals to verify seizure activity, the rats were sacrificed, and hippocampal tissue was harvested on ice. Hippocampi were homogenized in 50 mM Tris, pH 7.5, 6 mM EGTA, 320 mM sucrose, 1 mM dithiothreitol, and 0.3 mM phenylmethylsulfonyl fluoride, and neuronal membranes were isolated by centrifugation (Morris et al., 2001). Before electrophoresis, membrane samples were thawed on ice, and protein concentration per sample was calculated using the MicroBradford reagent system (Bio-Rad, Hercules, CA) quantified using a UV-2101PC ultraviolet spectrophotometer (Shimadzu, Kyoto, Japan). Samples were balanced to 5 μg protein/gel lane and denatured in β-mercaptoethanol and loading dye buffer. Samples were then heated to 90°C for 5 min in a programmable thermal controller PTC100 (MJ Research, Watertown, MA) and allowed to cool to room temperature before loading onto a 10% Tris-HCl Ready Gel (Bio-Rad). A colorimetric molecular mass marker including standards ranging from 10 to 182 kDa (ProSieve; Cambrex Bio Science Rockland, Inc., Rockland, ME) was loaded onto the last lane of the gel to aid in determining protein size. Gels were assembled into a MiniProtein II Electrophoresis System (Bio-Rad) and resolved for 90 min at 220 V constant in Tris buffer (Bio-Rad). After electrophoresis, gels were Western blot transferred to Immobilon P membrane (Millipore Corp., Bedford, MA) for 2 h at 4°C using a Genie transfer apparatus (IDEA Scientific, Minneapolis, MN) at a constant 200 V. Transfer buffer consisted of Tris-glycine buffer containing 10% methanol. After transfer, the Western blot was stored in phosphate-buffered saline at 4°C overnight. Gels were stained for protein and quantitated for microtubule-associated protein 2 and tubulin protein levels as described previously (Morris et al., 2001).

Immunostaining of the Western blot was performed by first blocking the membrane in buffer composed of 3% blotting grade block (Bio-Rad) and 0.05% Tween 20 in phosphate-buffered saline for 45 min at room temperature. Rabbit (polyclonal) anti-cannabinoid CB1 receptor unconjugated primary antibody (Biosource International, Camarillo, CA) was added to the blocking solution at a concentration of 1 μg/ml, and the membrane was incubated for 90 min at room temperature. After primary antibody incubation, the membrane was washed for a total of 15 min (three times for 5 min each) in phosphate-buffered saline. The membrane was then reblocked in fresh blocking buffer for 30 min. Anti-rabbit IgG-horseradish peroxidase conjugated antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA) was then added to the blocking solution in a 1:2000 dilution, and the membrane was incubated for a final 45 min. Western blots were washed (three times for 5 min each) in PBS and incubated for 5 min in SuperSignal (Pierce Chemical, Rockford, IL) for enhanced chemiluminescent analysis. Chemiluminescent images were visualized using Kodak X-Omat Blue XB-1 X-ray film (Eastman Kodak, Rochester, NY). Using a gel scanner and analyzed by computer-assisted densitometry (Amersham Biosciences Inc., Piscataway, NJ). Using the molecular mass marker as reference, the protein band was determined to correspond to a mass of 64 kDa.

Immunohistochemistry. Four epileptic and four control rats were transcardially perfused with isotonic saline, and brains were quick frozen and stored at −80°C in embedding compound (Sakura Inc., Japan). Cryostat sections (10 μm) were fixed in acetone and prepared for immunostaining using established techniques (Pettit et al., 1998). CB1 receptor protein immunoreactivity for each animal was evaluated using more than 15 tissue sections. Briefly, tissue sections were blocked in bovine serum for 1 h and then incubated with CB1 antiserum at 5.0 μg/ml for 1 h at room temperature. Tissue slices were then washed in PBS (three washes, each for 5 min), followed by biotinylated anti-rabbit IgG at 1:200 dilution for 30 min at room temperature. After again washing in PBS for 15 min, CB1 receptor immunoreactivity was visualized by exposure to avidin-biotin complex and 3,3′-diaminobenzidine (Vector Laboratories, Burlingame, CA). Adjacent tissue sections were evaluated morphologically using Nissl stain. Stained tissue sections were evaluated using a binocular microscope (Olympus America Inc., Melville, NY) and were photographed using a digital camera (Olympus America Inc.). Images were analyzed using Analysis software (Soft Imaging System Corp., Lakewood, CO).

Statistical Analyses. Results are given as means ± S.E.M. Statistical comparisons were made using SigmaStat software (SPSS Science, Chicago, IL). The Student’s t test, one-way analysis of variance (ANOVA), and the repeated measures (RM) one-way ANOVA in conjunction with the post hoc Tukey test were used where appropriate. Graphs were generated using Origin 6.1 software (OriginLab Corp, Northampton, MA).

Results

Modulation of the CB1 Receptor Alters Seizure Frequency in the Rat Pilocarpine Model of Epilepsy. Epileptic rats manifested an average of 3.0 (± 0.9) seizures per 10-h period. Representative control and epileptic seizure EEG patterns are shown in Fig. 1. Control animals never manifested EEG or behavioral seizures. Administration of the CB1 receptor agonists R(+)-WIN55,212 (5 mg/kg i.p.) (Fig. 1A and 2A) and THC (30 mg/kg i.p.) (Fig. 2A), the primary psychoactive active compound in marijuana, completely terminated both behavioral and electrographic seizures in this refractory seizure model (p ≤ 0.05). R(+)-WIN55,212 and THC began having anticonvulsant effects at 0.5 and 5 mg/kg i.p., respectively. The dose response evaluation of these compounds revealed approximate ED50 values of 1 mg/kg i.p. for R(+)-WIN55,212 and 15 mg/kg i.p. for THC. The concentrations of THC and R(+)-WIN55,212 required to inhibit seizures in this model were similar in effect and dose to their ability to inhibit maximal electric shock-induced seizures (Wallace et al., 2001), and this concentration of THC has been shown to have anticonvulsant effects in other seizure models (Wada et al., 1975; Karler and Turkanis, 1980; Colasanti et al., 1982). At the maximal anticonvulsant doses of THC and R(+)-WIN55,212, the animals were not significantly sedated and were alert enough to be able to move freely in their cages. These ED50 values are below the ED50 values for R(+)-WIN55,212 and THC in decreasing spontaneous activity and similar to the concentrations used to cause hyperthermia and analgesia (Wallace et al., 2001). Thus, the anticonvulsant effects of THC and R(+)-WIN55,212 are in the same...
These single-injection experiments directly evaluated the effects of each animals. Only THC and (nbinoids THC (30 mg/kg i.p.) and phenobarbital (PB; 40 mg/kg i.p.) and phenytoin (PHT; 100 mg/kg i.p.) and the cannabinoids but below sedative concentrations. The enantioselectivity of the anticonvulsant effect of SR141716A (SR) (10 mg/kg i.p.), and THC (30 mg/kg i.p.). Data represent mean ± S.E. (n = 6 per drug treatment; *, p ≤ 0.05; **, p ≤ 0.001). B, inhibition of seizure activity at high therapeutic concentrations (Morris et al., 2001) by the anticonvulsants phenobarbital (PB, 40 mg/kg i.p.) and phenytoin (PHT; 100 mg/kg i.p.) and the cannabinoids THC (30 mg/kg i.p.) and R(+)-WIN55,212, (+)-WIN (5 mg/kg i.p.) (n = 6 per drug treatment; *, p ≤ 0.01 in comparison with epileptic animals). Only THC and (+)-WIN completely abolished seizure activity. These single-injection experiments directly evaluated the effects of each agent on seizure frequency in multiple animals.

concentration range of some of the other physiological effects of the cannabinoids but below sedative concentrations. The inactive isomer, S(-)-WIN55,212 (5 mg/kg i.p.), and drug vehicle alone had no effect on seizure frequency (Fig. 1 and 2A). The enantioselectivity of the anticonvulsant effect of R(+)-WIN55,212 strongly indicates that this compound is acting via a CB1 receptor-specific mechanism. Maximally effective doses that produce high therapeutic blood levels of phenobarbital (40 mg/kg i.p.) and phenytoin (100 mg/kg i.p.), well established anticonvulsants, do not completely inhibit seizure activity in this model of refractory epilepsy (Leite and Cavalheiro, 1995; Morris et al., 2001). Because of the inability of these standard anticonvulsants to completely block seizures when used in high therapeutic levels in this model, the pilocarpine model of epilepsy is considered a model of refractory or difficult-to-control seizures with conventional anticonvulsant agents. The cannabinoids were very effective anticonvulsants in this model at a concentration that did not produce sedation but completely abolished seizures. Pheno-
and animals were unresponsive to external stimuli with loss of righting reflex for 30 min or more. SR141716A (10 mg/kg i.p.) has been shown to inhibit the anticonvulsant effects of cannabinoids and endocannabinoids (Wallace et al., 2001, 2002). SR141716A was also effective in blocking the anticonvulsant effects of THC and R(+)-WIN55,212 at 5 mg/kg i.p. We choose to use the higher concentration of SR141716A to obtain a clear antagonist effect. In addition, SR141716A (10 mg/kg i.p.) did not induce seizures in control animals (Fig. 4). The effect of SR141716A was only observed in the epileptic animals, and this compound did not cause hyperexcitability in control or naive animals.

To further evaluate the role of endogenous CB1 receptor activation on seizure termination, we quantified the duration of individual seizure events within each drug treatment period (Fig. 5, A and B). In all animals monitored, EEG seizures directly coincided with behavioral seizures observed on video recording. During vehicle and S(-)WIN55,212 treatments, seizure duration was not significantly altered from baseline. Treatment with the CB1 receptor antagonist, SR141716A, caused a significant increase in seizure duration (p < 0.01; Fig. 5, A and B). Prolongation of seizure discharge by SR141716A is apparent in the EEG patterns of representative seizure events (Fig. 5A).

**Hippocampal Levels of 2-AG Increase during Seizure Activity.** 2-AG synthesis occurs during neuronal depolarization in a Ca2+-dependent manner (Stella et al., 1997). Sustained neuronal depolarization and elevated intracellular Ca2+ are known to accompany seizure activity (Raza et al., 2001). We have previously shown that endogenous cannabinoids are anticonvulsant and this anticonvulsant activity of the endocannabinoids could be blocked by SR141716A (Wallace et al., 2002). If the endogenous cannabinoid system contributes to epileptic seizure termination, seizure activity in an intact animal would be expected to increase synthesis of endogenous cannabinoids. The hippocampal brain region is a locus of epileptic seizure activity (Lothman et al., 1991); therefore, we sought to determine the effect of status epilepticus on hippocampal levels of 2-AG. Pilocarpine-injected animals were sacrificed after 15 min of pilocarpine-induced seizure activity along with sham controls. Levels of 2-AG in hippocampal extracts were determined according to previously published methods (Di Marzo et al., 2000). In acutely seizing animals, endogenous 2-AG levels were significantly increased compared with controls (Fig. 5C; p ≤ 0.05). The data demonstrate that a single pilocarpine-induced seizure can increase the level of the endogenous cannabinoid 2-AG in hippocampal tissue. We have also demonstrated that...
SR141716A (10 mg/kg i.p.) inhibited the anticonvulsant effects of endocannabinoids (Wallace et al., 2002).

Increased Hippocampal CB1 Receptor Expression in Epileptic Rats. Because CB₁ receptor activation was shown to alter seizure frequency and duration, we sought to evaluate possible changes in CB₁ receptor expression in the hippocampi of epileptic animals. Using Western blot analyses, we compared sham control with epileptic hippocampal neuronal membranes and found a significant increase in epileptic brains of the expression of the 64-kDa molecular mass CB₁ receptor protein (Cichewicz et al., 2001) (Fig. 6A). Quantification of the bands shown in Fig. 6A revealed that expression of this protein was increased 183% in the hippocampi of epileptic rats compared with sham-treated animals (Fig. 6B; *p ≤ 0.01; Student’s t test).

To evaluate the anatomical distribution of this increase in CB₁ receptor expression in epileptic brains, we conducted immunohistochemical staining of CB₁ receptor protein on coronal hippocampal sections using established techniques (Pettit et al., 1998). Figure 7, A and B, shows representative patterns of cellular Nissl staining in sham control and epileptic hippocampi. No apparent changes in hippocampal morphology were observed in epileptic versus sham control animals, with the exception of minimal cell loss in the CA1 region (less than 10%), as described previously (Rice and DeLorenzo, 1998). Representative pseudocolor-enhanced images of CB₁ receptor protein staining of epileptic and sham control hippocampal sections illustrate a dramatic increase in CB₁ receptor expression in epileptic hippocampi (Fig. 7, C and D). To correct cannabinoid protein levels to internal protein standards, we still observed a significant increase in the CB₁ receptor expression in epileptic animals (data not shown).

Fig. 5. CB₁ receptor-dependent regulation of seizure duration in epileptic rats. A, a representative EEG recording of a seizure in an epileptic (Base) and an epileptic animal treated with SR141716A (SR) (10 mg/kg i.p.) demonstrating increased seizure duration produced by SR. B, mean seizure duration for the treatments shown in Fig. 3B. Data represent the mean ± S.E. (n = 8 animals; RM ANOVA; *p ≤ 0.01). C, hippocampal endogenous 2-AG levels in control and seizure animals (15 min after seizure onset). The data represent the mean ± S.E. (n = 7; *p ≤ 0.01; Student’s t test).

Fig. 6. CB₁ receptor expression is increased in hippocampal neuronal membranes of epileptic rats. A, representative Western blot of sham control (C) and epileptic (E) hippocampal neuronal membranes. The band visualized corresponded to the 64-kDa CB₁ receptor protein (Cichewicz et al., 2001). B, mean optical densities (arbitrary units) of CB₁ receptor protein expression in hippocampal membranes of epileptic and sham control rats (n = 6; *p ≤ 0.01; Student’s t test). Epileptic CB₁ receptor protein was increased by 183%.

Discussion

In the present study, we report that the endogenous cannabinoid system plays a critical role in modulating seizure activity in epilepsy. Both cannabinoids, THC and
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The findings presented in this study demonstrate that a significant change in CB1 receptor expression occurs with the epileptic phenotype. The animals used in this study had been epileptic for nearly 1 year, indicating that this change in CB1 receptor expression is prolonged and probably permanent. CB1 receptor expression has also been shown to increase in an animal model of stroke (Jin et al., 2000), a condition, like epilepsy, that is associated with excessive glutamate release and the development of seizures. The observation that the cannabinoid receptor is up-regulated in ischemia and epilepsy implies a compensatory role for the receptor in mitigating excitotoxicity. In light of the anticonvulsant effect of both $R^+\text{WIN}55,212$ and THC, as well as the proconvulsant action of the CB1 receptor antagonist in this epilepsy model, we propose that the increase in CB1 receptor expression displayed in epileptic brains was a compensatory rather than a causal factor of seizure manifestation and served to dampen seizure activity. The increased CB1 receptor expression displayed in the hippocampi of epileptic animals was regionally specific, occurring in the CA dendritic field and not in the dentate gyrus. This increase in CB1 receptor expression was demonstrated up to 1 year after the induction of epilepsy and thus demonstrates a long-lasting or permanent plasticity change in the brain that may play a role in the pathophysiology of epilepsy. The functional relevance of this differential increase in CB1 receptor expression may be revealed by further study.

Anticonvulsant Action of Cannabinoids. Recent discoveries in the cannabinoid field have demonstrated that cannabinoids ameliorate symptoms associated with neuronal hyperexcitability. In models of multiple sclerosis (Baker et al., 2000) and Huntington’s disease (Lastres-Becker et al., 2002), CB1 receptor activation significantly reduced spasticity and tremor, and exogenous application of 2-AG has been shown to be neuroprotective after traumatic brain injury (Panikashvili et al., 2001). Furthermore, in in vitro and in vivo studies of ischemia, cannabinoids significantly decreased excitotoxic neuronal cell death that resulted from excessive glutamatergic transmission (Abood et al., 2001). These cannabinoid actions are believed to involve attenuation of glutamate release. At the molecular level, the anticonvulsant mechanism of cannabinoids is unknown. However, because modulation of presynaptic neurotransmitter release is believed to be a primary result of CB1 receptor activation, we believe that this mechanism may underlie cannabinoid anticonvulsant properties. CB1 receptor activation is known to decrease calcium influx through N- and P/Q-type $\text{Ca}^{2+}$ channels (Mackie and Hille, 1992), the result of which is decreased Ca$^{2+}$-dependent glutamate release. Glutamate is the primary excitatory neurotransmitter of the central nervous system. Although critical for normal neurotransmission, elevated levels of glutamate are associated with excitotoxicity and excessive glutamatergic transmission is a hallmark of epilepsy (Lothman et al., 1991). With elevated levels of glutamate detected in epileptic tissue (Lothman et al., 1991), decreased release of this neurotransmitter would be a logical cannabinoid anticonvulsant mechanism. CB1 receptor activation also increases the conductance of presynaptic A-type (Hampson et al., 1995) and G-protein coupled inward rectifying K$^+$ channels (Mackie et al., 1995). Increased K$^+$ channel permeability attenuates neuronal bursting and stabilizes membrane potential, additional factors that would contribute to decreased epileptiform discharge. Preliminary data from our group indicates that CB1
knockout animals have spontaneous seizures, further suggesting an endogenous role for the CB₁ receptor in controlling neuronal excitability.

CB₁ receptor activation has also been shown to decrease GABAergic function in the hippocampus. In particular, endogenous cannabinoids are believed to be retrograde mediators of depolarization-induced suppression of inhibition (DSI) (Wilson and Nicoll, 2001). The overall effect of DSI at the synapse is disinhibition of the postsynaptic neuron and, therefore, facilitation of excitatory transmission. In light of the increased neuronal excitability that may result from this action, decreased GABAergic tone most probably does not mediate the anticonvulsant mechanism of cannabinoids. However, Cohen et al. (2002) recently demonstrated that the GABAergic system, normally an inhibitory neurotransmitter, can become a depolarizing force capable of synchronizing abnormal bursting in human epileptic, temporal lobe, brain slice preparations. If this phenomenon were to occur within the brains of animals with pilocarpine-induced epilepsy, then a cannabinoid-mediated decrease in GABAergic tone may indeed be anticonvulsant.

A more probable explanation for the anticonvulsant action of cannabinoids lies in the possibility that the pathology of epilepsy causes a compensatory shift to occur in the balance between CB₁ receptor-mediated inhibition of presynaptic glutamate and GABA release. In support of this, recent studies have shown that, in a manner similar to DSI, depolarization-induced suppression of excitation can be induced in hippocampal tissue (Ohno-Shosaku et al., 2002). The induction of this phenomenon was dependent on the sensitivity of the presynaptic neuron to cannabinoids as well as the duration of postsynaptic depolarization. With extended depolarization, the result of CB₁ receptor activation was a shift from DSI to depolarization-induced suppression of excitation. Therefore, the extended neuronal depolarization of an epileptiform discharge may cause a switch from suppression of GABA release to suppression of glutamate release.

Synthesis of the endogenous cannabinoid 2-AG is believed to occur in a calcium-dependent, “on-demand” fashion from arachidonic acid-enriched membrane phospholipids. During a seizure, elevated intracellular Ca²⁺ results from prolonged neuronal depolarization (Raza et al., 2001). Because increased hippocampal levels of 2-AG were detected 15 min into a seizure and CB₁ receptor antagonism resulted in prolonged seizure duration, we believe that seizure-induced increases in intracellular calcium result in the de novo synthesis of endogenous cannabinoids that then bind the CB₁ receptor to terminate seizure discharge, forming a negative feedback loop. This increase in 2-AG occurred in comparison with sham animals and was shown to be dependent on seizure activity and not manipulation of the animals or drug-specific effects. Additional evidence of compensatory endogenous cannabinoid release during seizure activity is provided by studies that show elevated 2-AG after injection of the chemoconvulsant picrotoxin (Sugiura et al., 2000). 2-AG is known to bind the CB₁ receptor with high affinity in a manner that is blocked by coadministration of SR141716A.

Several factors in addition to increased production of 2-AG could explain seizure-induced increase in the levels of this compound. Alternatively, increased 2-AG levels during seizures may be the result of decreased function of the fatty acid amidohydrolase enzyme that is known to be responsible for the catalysis of the compound. Increased receptor sensitivity and reducing cannabinoid catabolism during seizure activity could also account for the net increase in 2-AG observed after seizure activity. Further study may reveal which mechanism generates this increase in hippocampal 2-AG.

**Therapeutic Implications for Cannabinoids in the Treatment of Epilepsy.** Seizures in patients with refractory, partial-complex epilepsy can be difficult to control despite the use of currently available anticonvulsant medications and surgical interventions. Therefore, there is a clear need for the development of more effective anticonvulsant agents. Some epilepsy patients, seeking alternative treatments, have perceived improvement with marijuana (Consroe et al., 1975). This has prompted several countries to consider the legalization of marijuana for epilepsy treatment (National Institutes of Health, 1997; R. v. Parker, 1997; House of Lords Select Committee on Science and Technology, 1998). The pilocarpine model represents a refractory epileptic condition that is not readily treated by conventional anticonvulsants (Leite and Cavalheiro, 1995; Morris et al., 2001). Our results demonstrate that activation of the CB₁ receptor by cannabinoid drugs and possibly endogenous ligands significantly alters seizure activity and is more effective than conventional anticonvulsants in treating the refractory seizures produced in the pilocarpine model. Although the dose dependence and long-term effects of cannabinoid administration on epilepsy must be further investigated, the results presented here provide evidence that warrants a comprehensive assessment of cannabinoid use in the control of refractory epilepsy via the use of animal models and placebo-controlled clinical trials. Although the psychoactive side effects of cannabinoids make their use in the treatment of epilepsy impractical, understanding the mechanisms of endogenous cannabinoid-mediated anticonvulsant action may lead to the development of novel compounds that do not manifest behavioral toxicity. Further investigation of the cannabinoid anticonvulsant phenomenon may illuminate novel therapeutic targets for the treatment of temporal lobe epilepsy as well as more clearly define the physiological function of the endogenous cannabinoid system in brain.

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