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Assessment of the role of CB_1 receptors in cannabinoid anticonvulsant effects

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Abstract

The cannabinoid CB_1 receptor has been shown to be the primary site of action for cannabinoid-induced effects on the central nervous system. Activation of this receptor has proven to dampen neurotransmission and produce an overall reduction in neuronal excitability. Cannabinoid compounds like Δ^9-tetrahydrocannabinol and cannabidiol have been shown to be anticonvulsant in maximal electroshock, a model of partial seizure with secondary generalization. However, until now, it was unknown if these anticonvulsant effects are mediated by the cannabinoid CB_1 receptor. Likewise, (*R*)-(+)-[2,3-Dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone (WIN 55,212-2), a cannabimimetic compound that has been shown to decrease hyperexcitability in cell culture models via the cannabinoid CB_1 receptor, has never been evaluated for anticonvulsant activity in an animal seizure model. We first show that the cannabinoid compounds Δ^9-tetrahydrocannabinol (ED_{50} = 42 mg/kg), cannabidiol (ED_{50} = 80 mg/kg), and WIN 55,212-2 (ED_{50} = 47 mg/kg) are anticonvulsant in maximal electroshock. We further establish, using the cannabinoid CB_1 receptor specific antagonist *N*-(piperidin-1-yl-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamidehydrochloride (SR141716A) (AD_{50} = 2.5 mg/kg), that the anticonvulsant effects of Δ^9-tetrahydrocannabinol and WIN 55,212-2 are cannabinoid CB_1 receptor-mediated while the anticonvulsant activity of cannabidiol is not. This study establishes a role for the cannabinoid CB_1 receptor in modulating seizure activity in a whole animal model.

Keywords: Cannabinoid; Epilepsy; Maximal electroshock; Seizure; Cannabinoid CB_1 receptor

1. Introduction

Despite marijuana's illegal status in the United States, individuals both here and abroad report its use to be therapeutic in the treatment of a variety of ailments, including epilepsy (Hollister, 1983; Adams and Martin, 1996). Approximately 1% of Americans have epilepsy and 30% of these patients are refractory to conventional antiepileptic drug treatments (Zarrelli et al., 1999). Cannabinoid compounds have been used as a natural remedy for seizures for nearly 2000 years (Adams and Martin, 1996). In 1974, Karler et al. found that Δ^9-tetrahydrocannabinol, the primary psychoactive compound in marijuana, displayed anticonvulsant properties in maximal electroshock-induced tonic–clonic convulsions (Karler et al., 1974). The non-psychoactive marijuana constituent, cannabidiol, was also shown to be protective in this seizure model (Karler et al., 1973). Since this initial research, several cannabimimetic compounds have been synthesized and evaluated in vitro for their effects on neuronal hyperexcitability. (*R*)-(+)-[2,3-Dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone (WIN 55,212-2) attenuated low-Mg^{2+} induced burst-firing in hippocampal culture (Shen and Thayer, 1999). In addition, the endogenous ligands anandamide and 2-Arachidonylglycerol were found to decrease the amplitude of stimulation-induced population spikes, as well as attenuate low-Mg^{2+}-induced epileptiform discharges in rat hippocampal slice preparation (Ameri and Simmet, 2000). The mechanism underlying this dampening of excitability is believed to involve the inhibition of presynaptic excitatory neurotransmitter release (Shen and Thayer, 1999; Takahashi and Linden, 2000), of which glutamate is the most ubiquitous.

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Cannabinoids are known to bind two G protein-coupled 7-transmembrane spanning receptors, CB_1 and CB_2 (Matsuda et al., 1990; Munro et al., 1993). Cannabinoid receptor CB_1 is the type preferentially expressed in brain and is known to mediate the psychoactive effects of cannabinoids. The classic tetrad Δ^9-tetrahydrocannabinol-induced behaviors; ataxia, catalepsy, analgesia and hypothermia show susceptibility to block by the selective cannabinoid CB_1 receptor antagonist, pyrazole compound, *N*-(piperidin-1-yl-5-(4-chlorophenyl)-1-(2,4-dichloro phenyl)-4-methyl-1H-pyrazole-3-carboxamidehydrochloride (SR141716A) (Reche et al., 1996; Fields and Meng, 1998; Smith et al., 1998). Similarly, cannabinoid effects on excessive neuronal excitability, in vitro, are inhibited by pretreatment with SR141716A (Rinaldi-Carmona et al., 1994), indicating a cannabinoid CB_1 receptor-mediated mechanism. Conversely, cannabidiol does not bind the cannabinoid CB_1 receptor with reasonable affinity (Thomas et al., 1998) and does not produce Δ^9-tetrahydrocannabinol like behaviors that are blockable by the antagonist. Although the anticonvulsant activities of Δ^9-tetrahydrocannabinol and cannabidiol in the maximal electroshock model have been recognized for many years, it has not been investigated as to whether the anticonvulsant activity of these compounds is conferred by cannabinoid CB_1 receptor activation. Furthermore, cannabimimetic compounds such as WIN 55,212-2 dampen neuronal hyperexcitability in cultured neurons (Shen et al., 1996), but have never been evaluated for their anticonvulsant activity in whole animals. Therefore, the purpose of this study was to evaluate the cannabinoid compounds Δ^9-tetrahydrocannabinol, cannabidiol, and WIN 55,212-2 for anticonvulsant activity in the maximal electroshock model and determine if their protective activity is cannabinoid CB_1 receptor-mediated.

2. Methods

CF-1 male mice, 20–28 days old, weighing 20–30g (Harlan, Dublin, VA), were housed in the university animal facilities in groups of 4–5 for a minimum of 3 days and a maximum of 2 weeks prior to all experiments. All animals were kept in a temperature-controlled (20–22 °C) environment on a 12 h light–dark cycle (lights on at 7 am) with access to food and water ad libitum. Eight to fourteen animals were assigned to each treatment group. For anticonvulsant testing, animals received an intraperitoneal injection (i.p.) of Δ^9-tetrahydocannabinol, cannabidiol, or WIN 55,212-2 suspended in a vehicle of absolute ethanol, Emulphor-620 (Rhone-Poulenc, Princeton, NJ) and 0.9% saline at a ratio of 1:1:18. All cannabinoid compounds, as well as SR141716A, were obtained from the National Institutes of Health. All cannabinoids were administered 2 h prior to maximal electroshock. For behavioral testing, animals received an i.p. injection of Δ^9-tetrahydrocannabinol 2 h prior to testing. Phenytoin (Sigma Aldrich, St. Louis, MI), a positive control in maximal electroshock experiments, was suspended in a vehicle of polyethylene glycol and 0.9% saline at a ratio of 3:7 and was injected i.p. 30 min prior to shock. To test for antagonism of Δ^9-tetrahydrocannabinol's anticonvulsant effect, animals received an i.p. injection of SR141716A 20 min before receiving an i.p. injection of Δ^9-tetrahydrocannabinol. To test for cannabinoid CB_1 receptor-mediated effects of WIN 55,212-2 and cannabidiol, 10 mg/kg SR141716A was used, a dose found to produce maximal inhibition of Δ^9-tetrahydrocannabinol's anticonvulsant effect. Two hours following cannabinoid injection, electroshock was administered. All injections were administered at a volume of 0.1 ml/10 g (Krall et al., 1978). Appropriate vehicle controls were performed and each animal was used only once.

2.1. Maximal electroshock procedure

Maximal electroshock was produced by a 50 mA current for 0.2 s with a pulse train of 60 Hz (ECT unit model 7801, Ugo Basile, Comerio, Italy) through corneal electrodes. A drop of electrolyte solution containing lidocaine (2% lidocaine in 0.9% saline) was placed in each animal's eyes immediately prior to shock to improve electrode contact and decrease any pain localized to the eye area following shock (Swinyard et al., 1986). The shock administered was sufficient to produce hind limb extension in greater than 97% of control animals. Complete suppression of hind limb extension was considered a positive measure of anticonvulsant activity. All anticonvulsant experiments were carried out between 11:00 a.m. and 3:00 p.m. Data were expressed in terms of percent protection, that is the percentage of animals protected from hind limb extension within a treatment group. Probit analysis was used to calculate the effective dose 50% (ED_{50}) of each compound with 95% confidence limits, as well as the antagonism dose 50% (AD_{50}) of blockade Δ^9-tetrahydrocannabinol's anticonvulsant effect by SR141716A. ED_{50} was defined as the dose of drug at which 50% of the animals showed protection from hind limb extension. The effective dose 84% (ED_{84}) was defined as the dose of drug at which 84% of the animals showed protection from hind limb extension. This cannabinoid dose was used in all antagonism studies in an effort to decrease non-specific drug effects. The AD_{50} was defined as the pretreatment dose of SR141716A that abolished protection from hind limb extension in 50% of the animals treated with Δ^9-tetrahydrocannabinol. Data analysis was performed using the method of Litchfield and Wilcoxon (Litchfield and Wilcoxon, 1949). Statistical significance ($P \leq 0.05$) was determined using the Fisher Exact Test where appropriate. Dose–re-

sponse curves were generated using Microsoft Excel 97 in conjunction with Origin 6.0 software.

2.2. Behavioral testing procedures

Measurement of spontaneous activity in mice occurred in standard activity chambers interfaced with a Digiscan Animal Activity Monitor (Omnitech Electronics, Columbus, OH). A standard tail-flick apparatus (Dewey et al., 1970) and a telethermometer (Yellow Springs Instrument, Yellow Springs, OH) were used to measure antinociception and rectal temperature, respectively. Prior to testing in the behavioral procedures, mice were acclimated overnight to the experimental setting (ambient temperature 22–24 °C). Pre-injection control values were determined for rectal temperature and tail-flick latency (in seconds). Mice were injected i.p. with drug or vehicle and 1 h and 50 min later, were placed in individual activity chambers where spontaneous activity was measured for 10 min, following a 5-min acclimation period. Activity was measured as the total number of interruptions of 16 photocell beams per chamber during the 10-min test and was expressed as the percentage inhibition of activity of the vehicle group. Tail-flick latency was measured at 2 h post-injection. Maximum latency of 10 s was used. Antinociception was calculated as percent of maximum possible effect {%MPE = [(test-control latency)/(10-control)] × 100}. Control latencies typically ranged from 1.5 to 4.0 s. At 2 h and 5 min post-injection, rectal temperature was measured. This value was expressed as the difference between control temperature (before injection) and temperatures following drug administration. Each mouse was tested in each of the three procedures. Based on data obtained from numerous previous studies with cannabinoids, maximal effects of Δ^9-tetrahydrocannabinol in each procedure were estimated as follows: 90% inhibition of spontaneous activity, 100% MPE in the tail-flick procedure, and −6 °C change in rectal temperature. ED_{50}'s were defined as the dose at which half-maximal effect occurred. Graphs were generated using Sigma Plot software and were analyzed using analysis of variance (ANOVA) and Tukey Test where appropriate.

3. Results

3.1. Cannabinoid anticonvulsant activity in maximal electroshock

Fig. 1 illustrates the dose response relationships of Δ^9-tetrahydrocannabinol, WIN 55,212-2, cannabidiol and phenytoin in maximal electroshock. Each point represents data obtained from groups of 8 to 11 animals. Each drug was tested at the time of peak effect that, for each compound, was 2 h post-injection. The resulting ED_{50} values for Δ^9-tetrahydrocannabinol, WIN 55,212-2, and cannabidiol were 42, 47 and 80 mg/kg i.p., respectively. The

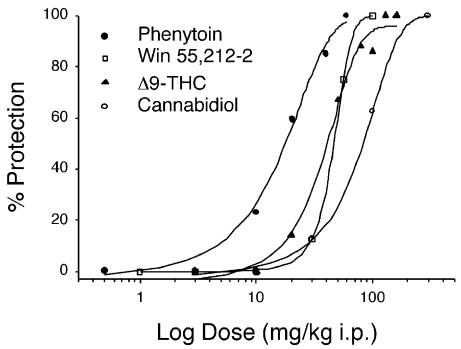

Fig. 1. Log dose response curve of Δ^9-tetrahydrocannabinol (Δ^9-THC), WIN 55,212-2, cannabidiol and phenytoin in the maximal electroshock model of seizure. Percentage of protection defined as number of animals that do not exhibit hind limb extension with electroshock ($n = 8$ per dose, per drug).

dose–response curves for Δ^9-tetrahydrocannabinol, WIN 55,212-2, and cannabidiol indicated that these compounds were equally efficacious to phenytoin in this model (ED_{50} = 18 mg/kg i.p.). Vehicle (1 Emulphor-620:1 ethanol:18 0.9% saline) showed no anticonvulsant activity in maximal electroshock as did animals treated with saline only (0.9% i.p.).

3.2. Effect of SR141716A pretreatment on cannabinoid anticonvulsant activity

The effects of SR141716A pretreatment on Δ^9-tetrahydrocannabinol's anticonvulsant activity are presented in Fig. 2. Δ^9-tetrahydrocannabinol was administered at its effective dose 84% (ED_{84}) to avoid non-specific, receptor-independent effects. With increasing pretreatment doses of SR141716A, up to 10 mg/kg, the anticonvulsant activity of Δ^9-tetrahydrocannabinol significantly decreased from 84% to 0% protection ($P \leq 0.001$ Fisher Exact Test). The AD_{50} of SR141716A was determined to be 2.5 mg/kg. Each data point represents 9–14 animals. Fig. 3 illustrates the effect of SR141716A pretreatment on the anticonvulsant activity of WIN 55,212-2 and cannabidiol when administered at their ED_{84} doses. SR141716A pretreatment completely blocked the anticonvulsant activity of the cannabimimetic WIN 55,212-2 with protection decreasing significantly from 84% to 0% ($P \leq 0.001$ Fisher Exact Test) (Fig. 3(A)). However, SR141716A failed to antagonize the anticonvulsant effect of cannabidiol significantly. The protection produced by cannabidiol in the absence and presence of SR141716A was 84% and 63%, respectively ($P = 0.067$ Fisher Exact Test) (Fig. 3(B)). SR141716A at doses up to 10 mg/kg alone had no anticonvulsant effect. A proconvulsant effect for SR141716A was not observed because the electroconvulsive threshold was not evaluated for this drug. However, the duration of tonic hind limb extension was evaluated and showed that SR141716A-treated animals were not statistically significant from vehicle-treated or control animals (data not shown). Further

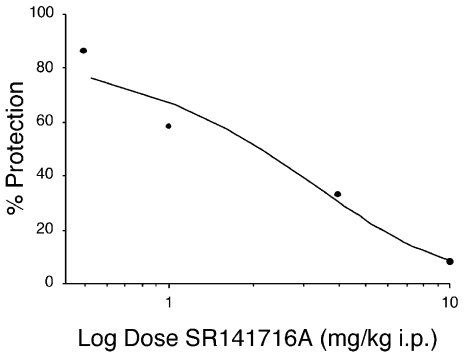

Fig. 2. Log dose inhibition curve of SR141716A in blocking the anticonvulsant effect of Δ^9-tetrahydrocannabinol. SR141716A was administered i.p. 20 min prior to the i.p. injection of Δ^9-tetrahydrocannabinol at its ED_{84} dose (70 mg/kg i.p.). Maximal electroshock was administered 2 h post Δ^9-tetrahydrocannabinol injection ($n = 9–14$ mice/group).

studies evaluating the effect of SR141716A on seizure threshold may elucidate a possible proconvulsant effect.

3.3. Effects of Δ^9-tetrahydrocannabinol on behavior

Cannabinoids produce stereotypic behaviors that include analgesia, hypothermia, and ataxia. Analgesia was measured using the tail-flick test. Spontaneous locomotor activity was quantified by the number of photocell interruptions within a 10-min time period and was expressed as the percentage inhibition of vehicle level activity. Hypothermia was measured by rectal temperature. An n of six animals per group per test was used. The results of our analysis showed that doses of Δ^9-tetrahydrocannabinol that produced anticonvulsant activity also produced behavioral effects. Fig. 4 shows the dose response relationship of

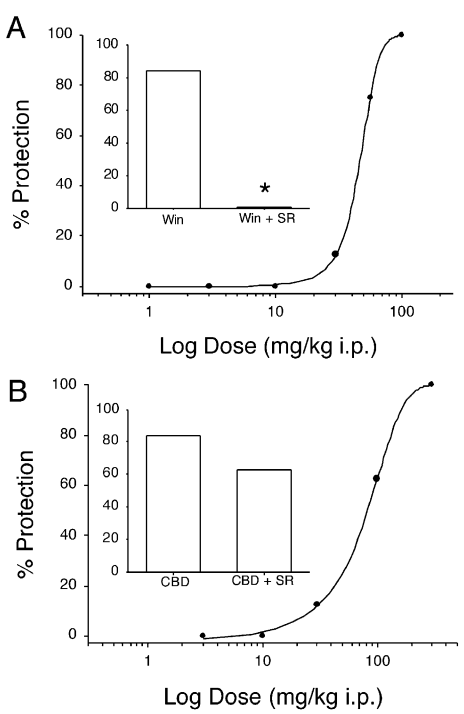

Fig. 3. Effects of SR141716A (SR) (10 mg/kg) pretreatment on anticonvulsant effects of WIN 55,212-2 (Win), or cannabidiol (CBD). The anticonvulsant log dose response curve of WIN 55,212-2 alone is presented in (A). The insert shows the effects of SR141716A (10 mg/kg i.p.) pretreatment on the ED_{84} dose (60 mg/kg i.p.) of WIN 55,212-2 ($P \leq 0.001$ Fisher Exact Test). The anticonvulsant dose response curve of cannabidiol is presented in (B). The insert shows the effects of SR141716A (10 mg/kg i.p.) pretreatment before an ED_{84} dose (160 mg/kg i.p.) of cannabidiol ($P = 0.067$ Fisher Exact Test). At least eight animals per group were tested.

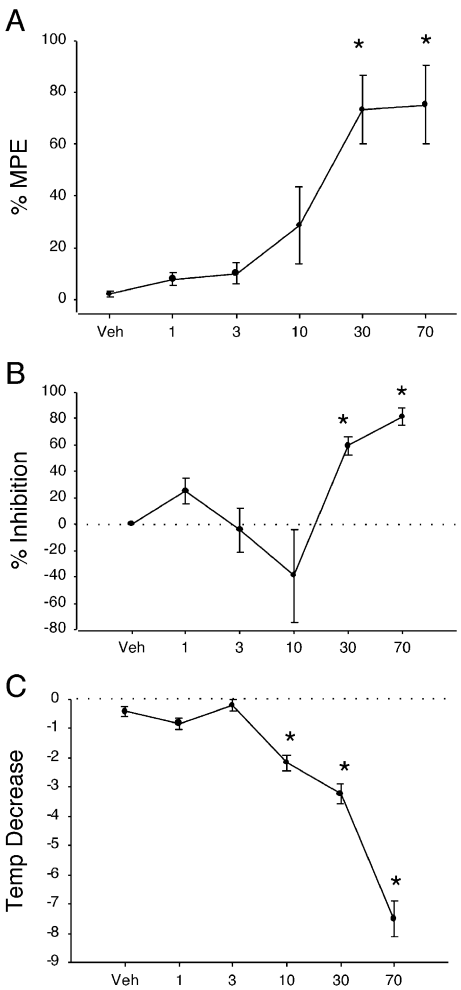

Fig. 4. Dose response of the behavioral effects of vehicle and Δ^9-tetrahydrocannabinol at 1, 3, 10, 30 and 70 mg/kg i.p. Δ^9-Tetrahydrocannabinol at 70 mg/kg i.p., the ED_{84} anticonvulsant dose, produced significant behavioral effects in the three parameters measured. (A) Dose response of Δ^9-tetrahydrocannabinol-induced analgesia as measured by the tail-flick test. Δ^9-Tetrahydrocannabinol-treated animals showed significant analgesia at 30 and 70 mg/kg compared to vehicle animals ($P \leq 0.05$ ANOVA, Tukey Test). (B) Dose response of Δ^9-tetrahydrocannabinol attenuated spontaneous activity expressed as Percentage of inhibition. Animals treated with 30 and 70 mg/kg Δ^9-tetrahydrocannabinol exhibited significantly less spontaneous activity than vehicle animals ($P \leq 0.01$, ANOVA, Tukey Test). (C) Dose response of Δ^9-tetrahydrocannabinol-induced hypothermia, measured by rectal temperature, shows that animals treated with 10, 30 and 70 mg/kg Δ^9-tetrahydrocannabinol had significantly lower body temperature compared to vehicle ($P \leq 0.05$ ANOVA, Tukey Test).

Δ^9-tetrahydrocannabinol in each of the behavioral parameters measured following a 2-h pretreatment, the time point at which maximum anticonvulsant effects were observed. Our data showed that significant levels of analgesia (ED_{50} = 11.7 mg/kg i.p.) ($P \leq 0.05$ ANOVA, Tukey Test) and a significant decrease in spontaneous activity (ED_{50} = 47 mg/kg i.p.) ($P \leq 0.01$ ANOVA, Tukey Test) were experienced at 30 and 70 mg/kg i.p. Δ^9-tetrahydrocannabinol. Significant hypothermia (ED_{50} = 16.6 mg/kg i.p.) was produced with 10, 30 and 70 mg/kg i.p. Δ^9-tetrahydrocannabinol ($p \leq 0.05$ ANOVA, Tukey Test).

4. Discussion

Studies by Karler and others demonstrated that Δ^9-tetrahydrocannabinol and cannabidiol were anticonvulsant (Karler et al., 1973, 1974; Consroe et al., 1982). These results raised the possibility that cannabinoid CB_1 receptor activation may mediate the anticonvulsant effect of cannabinoids. However, direct evidence that cannabinoid anticonvulsant effects are mediated by cannabinoid CB_1 receptor activation was not provided in these research efforts. The studies in this report provide direct evidence that, like the classic tetrad of cannabinoid behaviors, the anticonvulsant activity of Δ^9-tetrahydrocannabinol and WIN 55,212-2 in the maximal electroshock model is mediated by cannabinoid CB_1 receptor activation. From these data, we concluded that Δ^9-tetrahydrocannabinol and WIN 55,212-2, compounds that were anticonvulsant and bind the cannabinoid CB_1 receptor, lost their anticonvulsant activity when animals were pretreated with the selective cannabinoid CB_1 receptor antagonist SR141716A. Evidence that SR141716A blocks the ability of Δ^9-tetrahydrocannabinol and WIN 55,212-2 to prevent tonic hind limb extension in the maximal electroshock model indicates the involvement of the cannabinoid CB_1 receptor. Conversely, the anticonvulsant activity of cannabidiol, a compound that binds the cannabinoid CB_1 receptor extremely weakly, did not lose its protective activity when treated with SR141716A. Therefore, the anticonvulsant effects of Δ^9-tetrahydrocannabinol and WIN 55,212-2 are cannabinoid CB_1 receptor-mediated, while cannabidiol's protective effect is not. The results from these studies extend and support the original observations by Karler and others (Karler et al., 1973, 1974; Consroe et al., 1977) and indicate the involvement at the cannabinoid CB_1 receptor in mediating the anticonvulsant cannabinoid effects.

Prior to this investigation, WIN 55,212-2 had not been studied in the in vivo seizure models. However, it was shown to be effective in several in vitro seizure models. WIN 55,212-2 was shown to be anticonvulsant against seizures produced in the low-Mg^{2+} neuronal culture model of status epilepticus (Shen et al., 1996) and in stimulus-induced epileptiform discharges in the hippocampal slice preparation (Ameri and Simmet, 2000), effects that were blocked by SR141716A perfusion. Our finding that WIN 55,212-2 was protective in this model substantially expands the relevancy of existing in vitro data on this compound.

The anticonvulsant effects of cannabidiol were not mediated by cannabinoid CB_1 receptor activation. Anticonvulsant activity of cannabidiol was not inhibited by SR141716A under conditions where this cannabinoid CB_1 receptor antagonist completely blocked the anticonvulsant activity of Δ^9-tetrahydrocannabinol and WIN 55,212-2. This finding is consistent with data showing that although cannabidiol is anticonvulsant, it does not bind the cannabinoid CB_1 receptor with reasonable affinity (Thomas et al., 1998) nor does it evoke the classic tetrad of cannabinoid-induced behaviors. The anticonvulsant mechanism of cannabidiol remains unknown, but has been hypothesized to involve activation of γ aminobutyric acid(ergic) systems (Consroe et al., 1982).

It has been suggested that the mechanisms by which Δ^9-tetrahydrocannabinol and WIN 55,212-2 decrease hyperexcitability in in vitro models involve cannabinoid CB_1 receptor-modulated ion channels. Extensive molecular and pharmacological studies have shown that agonist binding to the cannabinoid CB_1 receptor activates an inhibitory G-protein, leading to decreased production of 3′, 5′ Cyclic adenosine monophosphate and thus reduced activity of the enzyme protein kinase A (Howlett, 1985; Felder et al., 1995; Hampson et al., 1995), known to modulate the activity of several ion channels. These G-protein-mediated effects on ion channels are pertussis-toxin sensitive (Matsuda et al., 1990; Mackie et al., 1995; Pan et al., 1996) and appear to alter permeability to multiple neuronal voltage-gated ion channels. These include voltage-gated Ca^{2+} channels, the G-protein coupled inward rectifier K^+ current (Mackie et al., 1995) and the A-type K^+ current (Deadwyler et al., 1993, 1995). The cannabinoid CB_1 receptor-mediated increases in rectifier and A-type K^+ currents serve to stabilize neuronal membrane potential, making the cell less likely to manifest seizure activity. In addition, cannabinoid CB_1 receptor activation produces a decrease in N and P/Q type voltage-gated Ca^{2+} currents (Pan et al., 1996). The subsequent reduction in presynaptic intracellular Ca^{2+} load causes a decrease in Ca^{2+}-dependent neurotransmitter release (Ishac et al., 1996; Gifford and Ashby, 1996; Katona et al., 1999), most notably of the neurotransmitter glutamate (Shen et al., 1996; Shen and Thayer, 1999; Kim and Thayer, 2000). Glutamate is the primary excitatory neurotransmitter of the central nervous system and elevated levels have been found in human epileptogenic foci (Leach et al., 1986). An attenuation of glutamate release would theoretically prevent seizure spread via synaptic transmission from an epileptic focus to the rest of the brain.

The abolition of hind limb extension following the administration of a convulsive current in maximal electroshock indicates that the anticonvulsant drug mechanism

impedes seizure spread. Drugs that are successful in suppressing maximal electroshock evoked hind limb extension are generally effective in treating generalized tonic–clonic and partial seizures. The prototype drug representing this classification is phenytoin. Δ^9-Tetrahydrocannabinol, WIN 55,212-2, and cannabidiol were as effective as phenytoin, used as a positive control in these studies, indicating that these cannabinoid compounds are potent anticonvulsants (Fig. 1). It is unlikely that the mechanism underlying cannabinoid anticonvulsant activity involves a general sedating effect resulting in suppression of motor activity and, therefore, impairment of tonic–clonic seizures. Animals are partially sedated at doses of Δ^9-tetrahydrocannabinol that produce suppression of hind limb extension. However, these animals still manifest all the motor activity characteristic of the clonic phase of seizure in the presence of sedative levels of Δ^9-tetrahydrocannabinol. In addition, post-shock animals treated with cannabinoid drugs run away immediately following cessation of clonus. This behavior indicates that the anticonvulsant activity of cannabinoid drugs is not simply due to sedation and impairment of locomotor activity. Sedation and impairment of locomotor activity does not necessarily confer anticonvulsant activity. For example, opiates are highly sedating but are not anticonvulsant in maximal electroshock. Cannabinoid compounds also produce hypothermia. However, it is not likely that this hypothermic effect is involved in the anticonvulsant action. Lowering animal body temperature to the level produced by cannabinoid drugs is not protective in the maximal electroshock model (Karler et al., 1974).

The ability to develop cannabinoids that have anticonvulsant effects, but have less psychoactive effects, may be useful in the clinical treatment of epilepsy. Unfortunately, the psychoactive side effects of Δ^9-tetrahydrocannabinol and WIN 55,212-2 limit their actual therapeutic utility. As our behavioral data reflects, at anticonvulsant doses, Δ^9-tetrahydrocannabinol produced a significant decrease in spontaneous activity, a psychoactive side effect. Likewise, cannabinoid-induced disruption of short-term memory via inhibition of long-term potentiation and long-term depression in the hippocampus could be detrimental in terms of patient function and compliance. Nevertheless, these data provide strong evidence that the anticonvulsant activity of Δ^9-tetrahydrocannabinol and WIN 55,212-2 are cannabinoid CB_1 receptor-mediated, whereas the protective activity of cannabidiol is not. These data further call into question the role cannabinoid CB_1 receptors play in the brain's ability to modulate synaptic activity, suggesting that perhaps a malfunctioning of the endogenous cannabinoid system contributes to the pathophysiology of epilepsy.

It is well-established that the hippocampus is a major region in the brain for modulating seizure activity and is especially sensitive to the development of recurrent seizure discharge or epilepsy. The high number of cannabinoid CB_1 receptors in hippocampus (Herkenham et al., 1990; Matsuda et al., 1990; Tsou et al., 1998) further implicate a role for cannabinoid CB_1 receptors and their endogenous ligands, anandamide and 2-arachidonylglycerol, in modulating excitability of the hippocampus. These data strongly suggest a role for the cannabinoid CB_1 receptor in modulating intrinsic neuronal excitability.

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