

Evidence for a physiological role of endocannabinoids in the modulation of seizure threshold and severity

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Abstract

The anticonvulsant effect of cannabinoids has been shown to be mediated through activation of the cannabinoid CB₁ receptor. This study was initiated to evaluate the effects of endogenously occurring cannabinoids (endocannabinoids) on seizure severity and threshold. The anticonvulsant effect of the endocannabinoid, arachidonyl ethanolamine (anandamide), was evaluated in the maximal electroshock seizure model using male CF-1 mice and was found to be a fully efficacious anticonvulsant (ED₅₀ = 50 mg/kg i.p.). The metabolically stable analog of anandamide, (*R*)-(20-cyano-16,16-dimethyl-docosa-*cis*-5,8,11,14-tetraenoyl)-1'-hydroxy-2'-propylamine (O-1812), was also determined to be a potent anticonvulsant in the maximal electroshock model (ED₅₀ = 1.5 mg/kg i.p.). Furthermore, pretreatment with the cannabinoid CB₁ receptor specific antagonist *N*-(piperidin-1-yl-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride (SR141716A) completely abolished the anticonvulsant effect of anandamide as well as O-1812 ($P \leq 0.01$, Fisher exact test), indicating a cannabinoid CB₁ receptor-mediated anticonvulsant mechanism for both endocannabinoid compounds. Additionally, the influence of cannabinoid CB₁ receptor endogenous tone on maximal seizure threshold was assessed using SR141716A alone. Our data show that SR141716A (10 mg/kg i.p.) significantly reduced maximal seizure threshold (CC₅₀ = 14.27 mA) compared to vehicle-treated animals (CC₅₀ = 17.57 mA) (potency ratio = 1.23, lower confidence limit = 1.06, upper confidence limit = 1.43), indicating the presence of an endogenous cannabinoid tone that modulates seizure activity. These data demonstrate that anandamide and its analog, O-1812, are anticonvulsant in a whole animal model and further implicate the cannabinoid CB₁ receptor as a major endogenous site of seizure modulation.

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1. Introduction

Epilepsy is one of the most common neurological conditions and is characterized by spontaneously recurrent seizures (Hauser and Hesdorffer, 1990). Understanding the pathophysiology of seizure initiation and termination would have important implications for our ability to treat seizure disorders and for the potential development of novel anticonvulsant agents. Previous research by our laboratory and others has demonstrated that cannabinoid compounds are

anticonvulsant against seizures produced by maximal electroshock, a whole animal model of seizure initiation and spread that is widely used to evaluate anticonvulsant compounds (Karler et al., 1974; Wallace et al., 2001). We further demonstrated that this cannabinoid anticonvulsant effect was cannabinoid CB₁ receptor-dependent (Wallace et al., 2001). The next logical step is to evaluate the role of endogenous cannabinoids and cannabinoid CB₁ receptor activation in modulating seizure severity and threshold.

The cannabinoid CB₁ receptor is the most abundant G-protein-coupled receptor in the mammalian brain. CB₁ is the primary site of action for the illicit drug marijuana (Adams and Martin, 1996). However, the physiological function of this receptor has yet to be fully characterized. Arachidonyl ethanolamine, or anandamide, is an endogenous ligand for the cannabinoid CB₁ receptor (Devane et al., 1992). Orig-

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inally isolated from porcine brain, it is reported to be synthesized on-demand in a calcium-dependent manner (Di Marzo et al., 1994) and accumulates following excitotoxic neuronal injury (Hansen et al., 2001). Recent evidence suggests that the endogenous cannabinoid system plays a protective role in disorders of the central nervous system (CNS), particularly those associated with neuronal hyperexcitability and excitotoxic neurotransmitter release (Aboud et al., 2001; Baker et al., 2001; Van der Stelt et al., 2001).

Anandamide, like other cannabinoids, has been shown to dampen epileptiform activity elicited in hippocampal brain slice preparations (Ameri et al., 1999); however, endogenous cannabinoids have not been evaluated for anticonvulsant activity in the whole animal. This study was initiated to evaluate the anticonvulsant effects of anandamide and the anandamide analog, (*R*)-(20-cyano-16,16-dimethyldocosan-5,8,11,14-tetraenyl)-1'-hydroxy-2'-propylamine (O-1812), on seizure threshold and severity in the maximal electroshock model. These results demonstrate that a cannabinoid compound endogenously synthesized in brain is anticonvulsant. Using the maximal electroshock seizure model and the cannabinoid CB₁ receptor specific antagonist, *N*-(piperidin-1-yl-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamidehydrochloride (SR141716A), we also investigated the endogenous role of cannabinoid CB₁ receptor activation in modulating seizure threshold. The data indicate that anandamide is an endogenous cannabinoid that inhibits CNS excitability and regulates seizure activity by activation of the cannabinoid CB₁ receptor.

2. Methods

All experiments were conducted using 20- to 28-day-old male CF-1 mice, weighing at minimum of 30 g. Experiments were conducted between 1:00 and 5:00 p.m. and in accordance with University animal use protocols. Animals were maintained on a 12-h light/dark cycle (lights on at 7:00 a.m.) with food and water ad libitum. All drugs were dissolved in a vehicle consisting of 5% ethanol, 5% Emulphor (Rhone-Poulenc, France), and 90% normal (0.9%) saline. SR141716A was obtained from the National Institutes of Drug Abuse (Bethesda, MD). Anandamide and O-1812 were obtained from Organix (Woburn, MA). Each animal was used only once.

2.1. Maximal electroshock procedure

Maximal electroshock was administered via corneal electrodes. Immediately prior to shock, a drop of electrolyte solution containing lidocaine (2% lidocaine in 0.9% saline) was placed in the animal's eyes to decrease pain and improve electrode contact. Shock amperage was designated as the current required to produce tonic hind limb extension in greater than 97% of control animals. Maximal electro-

shock was produced using a 50-mA current for 0.2 s with a pulse train of 60 Hz (Electroshock unit fabricated by Bioengineering Department, Virginia Commonwealth University). The shock produced in this protocol was previously determined to be approximately threefold above maximal seizure threshold (see Methods: maximal seizure threshold). Complete suppression of hind limb extension was considered the positive measure of anticonvulsant activity. Data were expressed in terms of percent protection (% protection); specifically, the percentage of animals at a particular dose not displaying hind limb extension after maximal electroshock. Because data were quantal, probit analysis was used to calculate the ED₅₀ of each compound with 95% confidence limits. ED₅₀ was defined as the drug dose at which 50% of the total animals failed to exhibit hind limb extension. The Fisher exact test was used to test statistical significance where appropriate. Dose–response curves were generated using Microsoft Excel in conjunction with Origin 6.0 software (Microcal Software, Northampton, MA).

2.2. Time course of anandamide and O-1812 anticonvulsant effects

For the evaluation of anandamide's anticonvulsant time course, animals were given intraperitoneal (i.p.) injections of phenylmethylsulfonyl fluoride (Sigma-Aldrich) (30 mg/kg i.p.) or vehicle 10 min prior to the anandamide injection. Phenylmethylsulfonyl fluoride was administered to limit activity of the fatty-acid amidohydrolase enzyme, known to rapidly metabolize anandamide. The dose of phenylmethylsulfonyl fluoride was shown in previous research to be inactive in behavioral tests (Compton and Martin, 1997). A fixed dose of anandamide (300 mg/kg i.p.) was injected and then, maximal electroshock was administered 10, 15, 20, 60, or 120 min later. At each time point, the anticonvulsant effect was evaluated for drug-related anticonvulsant activity (*n* = 5 animals per time point). The anticonvulsant time course for O-1812 was determined in the absence of phenylmethylsulfonyl fluoride by treating animals with a 5 mg/kg i.p. dose of the drug with electroshock administered at 10, 20, 40, 60, and 120 min post-injection time points.

2.3. Dose–response of anandamide's anticonvulsant effect

Animals were pretreated with either phenylmethylsulfonyl fluoride (30 mg/kg i.p.) or vehicle. Ten minutes following phenylmethylsulfonyl fluoride or vehicle injections, animals were treated with various doses of anandamide. Twenty minutes following this injection, the time point at which anandamide was the most efficacious (Fig. 1), maximal electroshock was administered as described (*n* = 8–10 animals per dose of anandamide). The dose–response relationship of O-1812 was produced by treating animals with an i.p. injection of the compound followed by maximal electroshock 20 min later, the time point of greatest efficacy.

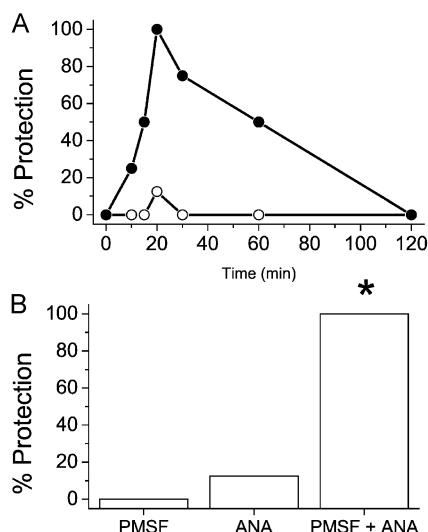


Fig. 1. Anticonvulsant activity of anandamide in maximal electroshock seizure model. (A) Time course of seizure protection by anandamide alone (300 mg/kg i.p., open circles) and in the presence of 30 mg/kg phenylmethylsulfonyl fluoride (closed circles). (B) Seizure protection of phenylmethylsulfonyl fluoride (30 mg/kg i.p., 0%), anandamide (ANA, 300 mg/kg i.p., 12.5%), and anandamide (300 mg/kg i.p.) in the presence of phenylmethylsulfonyl fluoride (30 mg/kg i.p., PMSF + ANA, 100%) 20 min post-injection. * $P \leq 0.01$, Fisher exact test vs. PMSF and ANA.

2.4. SR141716A antagonism of anandamide and O-1812 anticonvulsant activity

To test for cannabinoid CB_1 receptor-mediated anticonvulsant activity of anandamide, an i.p. dose of SR141716A (10 mg/kg i.p.) or vehicle was administered 10 min prior to the phenylmethylsulfonyl fluoride injection. For O-1812, CB_1 -mediated anticonvulsant activity was determined by preinjecting animals with SR141716A (10 or 15 mg/kg i.p.) 10 min prior to the agonist. To avoid nonreceptor-mediated, nonspecific effects of the drugs, the ED_{84} anticonvulsant doses of anandamide and O-1812 were used in these experiments ($n=8-14$ animals per treatment group).

2.5. Maximal seizure threshold procedure

Maximal seizure threshold was defined as the minimum current required to elicit a hind limb tonic extensor seizure, representing maximal neuronal discharge of the entire cerebrospinal axis (Swinyard, 1972). For evaluation of maximal seizure threshold, animals were given a fixed i.p. dose of SR141716A (10 mg/kg i.p.) or vehicle. Thirty minutes later, animals were given various current levels of electroshock (duration of 0.2 s with a pulse train of 60 Hz). The relationship between electrical current and percent of animals exhibiting tonic hind limb extension was analyzed for treatment-related shifts. The percentage of animals displaying tonic hind limb extension per current dose was expressed as % tonic ($n=7-11$ animals per current dose). Probit analysis was used to calculate the

CC_{50} of each compound with 95% confidence limits because of the quantal nature of the data (Litchfield and Wilcoxon, 1949). Convulsive current $_{50}$ (CC_{50}) was defined as the current dose at which 50% of the animals within a treatment group exhibited hind limb extension. Fisher exact test was used to test for statistical significance where appropriate.

3. Results

3.1. Anandamide is anticonvulsant in the maximal electroshock seizure model

Using an i.p. dose of 300 mg/kg, the time course for anandamide's anticonvulsant activity was determined with and without phenylmethylsulfonyl fluoride pretreatment. Fig. 1A shows that anandamide's effect peaked at 20 min regardless of whether phenylmethylsulfonyl fluoride is present. This phenylmethylsulfonyl fluoride treatment increased anandamide's potency about 10-fold and extended its duration of action to greater than 1 h. The impact of a behaviorally inactive dose of phenylmethylsulfonyl fluoride on anandamide anticonvulsant efficacy is illustrated in Fig. 1B. Phenylmethylsulfonyl fluoride and anandamide combined had a significant anticonvulsant effect compared to phenylmethylsulfonyl fluoride alone (Fisher exact test, $P \leq 0.01$). Furthermore, phenylmethylsulfonyl fluoride treatment significantly increased the efficacy of anandamide from 12.5% to 100% (Fig. 1B) (Fisher exact test, $P \leq 0.05$). These results demonstrate the rapid metabolic breakdown of anandamide and the need to use phenylmethylsulfonyl fluoride to reliably observe anandamide's effects.

The full dose-response relationship of anandamide anticonvulsant activity at 20 min post anandamide injection, its most efficacious time point, was determined in the presence of phenylmethylsulfonyl fluoride (30 mg/kg i.p.) in the maximal electroshock model (Fig. 2A). Anandamide was fully efficacious with complete protection achieved with a 100 mg/kg dose. The ED_{50} for anandamide in this experiment was 50 mg/kg i.p. (lower C.L. = 33, upper C.L. = 66). However, a dose of anandamide (300 mg/kg i.p.) that was 100% anticonvulsant in the presence of phenylmethylsulfonyl fluoride reached only 12.5% anticonvulsant activity in the absence of phenylmethylsulfonyl fluoride (Fig. 1A), again indicating that inhibition of the fatty-acid amidohydrolase enzyme by phenylmethylsulfonyl fluoride is required for anandamide to reach full efficacy. Phenylmethylsulfonyl fluoride and vehicle showed no anticonvulsant activity in maximal electroshock.

3.2. Anandamide's anticonvulsant effect is mediated by cannabinoid CB_1 receptor activation

A pretreatment dose of the cannabinoid CB_1 receptor antagonist, SR141716A (10 mg/kg i.p.), was used to deter-

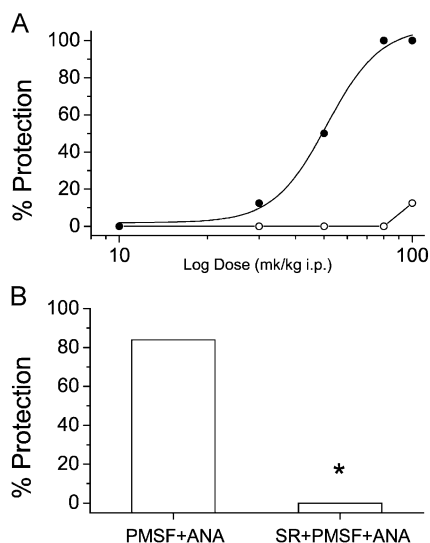


Fig. 2. Log dose–response curve of the anticonvulsant activity of anandamide in the presence of phenylmethylsulfonyl fluoride in the maximal electroshock seizure model. (A) Log dose–response relationship of anandamide alone (open circles) and anandamide in the presence of phenylmethylsulfonyl fluoride (30 mg/kg i.p., closed circles). (B) The effect of SR141716A (SR, 10 mg/kg i.p.) on the seizure protection of anandamide in the presence of phenylmethylsulfonyl fluoride (ANA, ED_{84} dose of 67 mg/kg i.p.). Note the significant reduction in seizure protection in the presence of SR141716A. * $P \leq 0.01$, Fisher exact test.

mine if anandamide's anticonvulsant effect in the maximal electroshock model is mediated via the cannabinoid CB_1 receptor. This dose of SR141716A was previously shown by our laboratory to completely abolish anticonvulsant activity of Δ^9 -tetrahydrocannabinol and (*R*)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone (WIN55,212-2) (Wallace et al., 2001). SR141716A completely blocked the activity at the ED_{84} dose (67 mg/kg i.p.) of anandamide, reducing its anticonvulsant efficacy from 84% to 0% (Fisher exact test, $P \leq 0.01$). These results indicate that the anticonvulsant property of anandamide is mediated via activation of the cannabinoid CB_1 receptor (Fig. 2B).

3.3. O-1812 is a potent anticonvulsant via a cannabinoid CB_1 receptor-mediated mechanism

The chemical structure of O-1812 is displayed in Fig. 3. The time course for O-1812's anticonvulsant activity (5 mg/

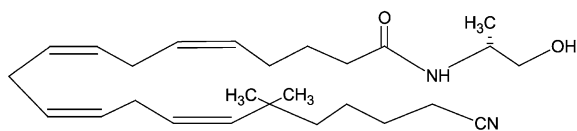


Fig. 3. Chemical structure of the metabolically stable anandamide analog, O-1812.

kg i.p.), determined in the absence of phenylmethylsulfonyl fluoride, was similar to that of anandamide in that both compounds displayed peak anticonvulsant efficacy when electroshock was administered 20 min post-injection (Fig. 4A). The dose–response relationship of O-1812 was examined at the 20 min time point (Fig. 4B) and demonstrated that the anandamide analog was a very potent anticonvulsant in the maximal electroshock seizure model ($ED_{50} = 1.5$ mg/kg i.p., lower C.L. = 1.0, upper C.L. = 2.0). The anticonvulsant activity of this compound was shown to be mediated via cannabinoid CB_1 receptor activation because like anandamide, pretreatment with the cannabinoid CB_1 receptor antagonist, SR141716A, at 10 mg/kg i.p. significantly reduced O-1812 anticonvulsant action ($P \leq 0.05$, Fisher exact test), and a 15 mg/kg i.p. dose of SR141716A completely abolished the anticonvulsant effect ($P \leq 0.01$, Fisher exact test) (Fig. 4C).

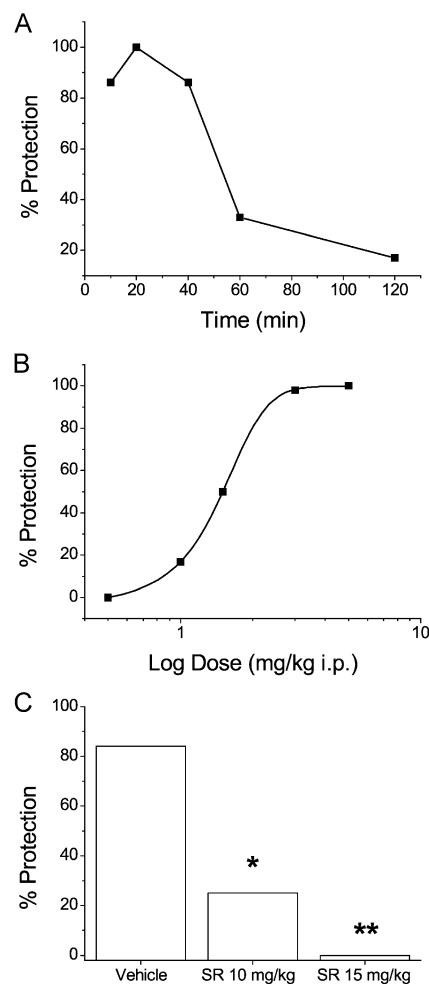


Fig. 4. Anticonvulsant activity of O-1812 in maximal electroshock. (A) Time course of seizure protection by O-1812 evaluated at 10, 20, 40, 60, and 120 min. (B) Log dose–response relationship of O-1812 anticonvulsant activity. (C) The effect of SR141716A pretreatment (SR 10, 10 mg/kg i.p.) (SR 15, 15 mg/kg i.p.) on the anticonvulsant efficacy of O-1812 (ED_{84} dose of 2.1 mg/kg i.p.). * $P \leq 0.05$ and ** $P \leq 0.01$, Fisher exact test.

3.4. Blockade of endogenous tone of the CB₁ receptor decreased maximal seizure threshold

If endogenous cannabinoid CB₁ receptor activation was playing a role in regulating seizure activity, then the CB₁ receptor inhibitor SR141716A would be expected to lower maximal seizure threshold. Fig. 5A illustrates that maximal seizure threshold was lowered when endogenous activity of the cannabinoid CB₁ receptor was blocked by SR141716A (10 mg/kg i.p.). Maximal seizure threshold of SR141716A-treated animals was significantly lower (CC_{50} = 14.27 mA) than vehicle-treated animals (CC_{50} = 17.57 mA) (Litchfield and Wilcoxon potency ratio = 1.23, lower confidence limit = 1.06, upper confidence limit = 1.43). The CC_{50} s of vehicle- and SR141716A-treated animals are compared in Fig. 5B. SR141716A-treated animals showed a significantly reduced seizure threshold (Litchfield and Wilcoxon potency ratio = 1.23, lower confidence limit = 1.06, upper confidence limit = 1.43). These results indicate that decreased function of the endogenous cannabinoid system increases the brain's

vulnerability to tonic–clonic seizures produced by electroshock.

4. Discussion

The results of this study for the first time demonstrate that anandamide, an endogenous cannabinoid, is a potent anticonvulsant in a whole animal model. The data also demonstrate that this anticonvulsant effect is mediated by activation of the cannabinoid CB₁ receptor. Furthermore, the reduction of maximal seizure threshold by SR141716A provides evidence for an endogenous cannabinoid tone that modulates the brain's excitability and, therefore, the potential to manifest seizures by altering convulsive threshold.

Anandamide is an eicosanoid that belongs to a class of fatty-acid derivatives of *N*-arachidonyl-phosphatidylethanolamine. The compound is reported to be synthesized “on-demand” by phospholipase-D in a depolarization and calcium-dependent manner (Di Marzo et al., 1994). Elevated intracellular calcium accompanies seizure activity (Limbrick et al., 2001). The depolarization and calcium-dependent synthesis of these compounds, therefore, suggests that the endogenous cannabinoid system plays a compensatory role in dampening seizure activity. Moreover, high concentrations of anandamide are detected in hippocampus (Felder et al., 1996), an area with high cannabinoid CB₁ receptor expression (Herkenham, 1991). The hippocampus is known to be a major brain region involved in epileptogenesis and seizure disorders (Lothman et al., 1991). Thus, endocannabinoids are likely to play an important role in modulating seizure threshold and severity.

Anandamide and other cannabinoids mediate their effects by binding to cannabinoid CB₁ and CB₂ receptors (Howlett, 1995). However, it is unlikely that the cannabinoid CB₂ receptor mediates the anticonvulsant effect of anandamide because this receptor is not present in brain. Furthermore, anandamide is inefficient at stimulating cannabinoid CB₂ receptor-dependent responses and may, in fact, act as an antagonist at this site (Facci et al., 1995; Bayewitch et al., 1996). Moreover, SR141716A has been shown to be selective for the cannabinoid CB₁ receptor, with negligible binding at cannabinoid CB₂ (Showalter et al., 1996). Anandamide can also bind vanilloid receptors (VR₁) that are found in brain (Szallasi and Di Marzo, 2000). However, it is unlikely that the vanilloid VR₁ receptor is anandamide's anticonvulsant site of action because the selective cannabinoid CB₁ receptor antagonist SR141716A, which has no affinity for vanilloid VR₁ receptors, completely blocks the anticonvulsant effect of anandamide. Furthermore, the O-1812 analog of anandamide is much more effective at the cannabinoid CB₁ site than either the cannabinoid CB₂ or vanilloid VR₁ receptors (Di Marzo et al., 2001). Thus, our data strongly implicate the cannabinoid CB₁ receptor as the mechanistic site of action mediating the anticonvulsant effects of endocannabinoids.

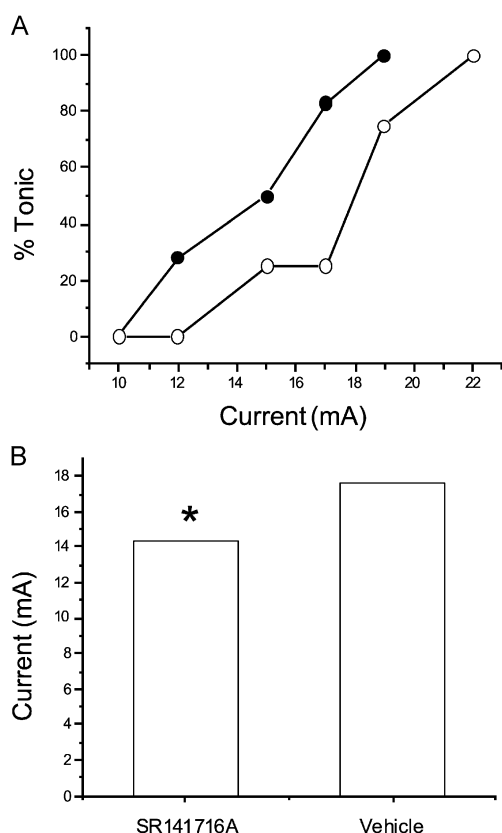


Fig. 5. The effect of SR141716A and vehicle on maximal seizure threshold. (A) Relationship between current magnitude and tonic hind limb seizure in the presence of SR141716A (10 mg/kg, closed circles) and vehicle (open circles). Percentage tonic defined as the number of animals displaying tonic hind limb extension. (B) CC_{50} for SR141716A (14.27 mA) is significantly lower (Litchfield and Wilcoxon potency ratio = 1.23, lower confidence limit = 1.06, upper confidence limit = 1.43) than vehicle (CC_{50} = 17.57 mA). * P < 0.05, Fisher exact test.

There is evidence that anandamide action at the cannabinoid CB₁ receptor is terminated when anandamide is rapidly taken up by a selective transporter into the presynaptic cell (Piomelli et al., 1999). The other inactivation pathway involves a fatty-acid amidohydrolase enzyme that hydrolyzes anandamide to arachidonic acid and ethanolamine (Giang and Cravatt, 1997). Because of the rapidity of this hydrolytic step, anandamide has a short duration of action, a factor that makes it difficult to study the physiological effects of this compound. Phenylmethylsulfonyl fluoride has been shown to inhibit the breakdown of anandamide (Compton and Martin, 1997), such that exogenous application of the compound is possible without rapid inactivation through enzymatic cleavage. The dose of phenylmethylsulfonyl fluoride used in our studies was shown in other laboratories to produce the minimum amount of behavioral activity with maximum enzyme inhibition (Compton and Martin, 1997). Although phenylmethylsulfonyl fluoride showed no intrinsic anticonvulsant activity, nonspecific actions of the inhibitor cannot be ruled out. Therefore, we chose to evaluate the metabolically stable anandamide analog, O-1812, because in previous *in vivo* studies, it was highly potent in the absence of phenylmethylsulfonyl fluoride. O-1812 is derived from the parent compound 1'-dimethylpentyl-2'-methyl-arachidonylethanolamine with a cyano group on the C-20 atom. This compound was determined in previous studies to have an affinity for the cannabinoid CB₁ receptor that is 580-fold and 1000-fold greater affinities for vanilloid VR₁ receptors or cannabinoid CB₂ receptors, respectively (Di Marzo et al., 2001), making it ideal for the study of endogenous cannabinoid-mediated anticonvulsant activity at the cannabinoid CB₁ receptor. Likewise, in the aforementioned study, O-1812 activity in the cannabinoid-induced "tetrad" of behaviors was not enhanced by pretreatment with phenylmethylsulfonyl fluoride, suggesting that it is not a substrate for the fatty-acid amidohydrolase enzyme. O-1812 was shown in this previous study to have weak inhibitory effects on the selective anandamide membrane transporter, an effect that may increase levels of anandamide and, therefore, indirectly amplify the anticonvulsant effect (Di Marzo et al., 2001). As in behavioral studies (Di Marzo et al., 2001), O-1812 was found to be very potent as an anticonvulsant. These results support the notion that the anticonvulsant effects of anandamide in the presence of phenylmethylsulfonyl fluoride are due to the parent compound.

In mice, not all of the classic cannabinoid-induced behaviors such as analgesia, hypothermia, catalepsy, and immobility that are produced by anandamide are blocked by SR141716A (Adams et al., 1998). In rats, however, anandamide-induced behaviors are blocked by SR141716A (Costa et al., 1999). Interestingly, the anticonvulsant effect of O-1812 was also blocked by SR141716A in a dose-dependent manner. Therefore, it is significant that the anticonvulsant effects of these drugs are completely abol-

ished by the CB₁ receptor antagonist, indicating a highly receptor specific anticonvulsant mechanism.

A likely mechanism for anandamide and O-1812's anticonvulsant properties involves activation of a cannabinoid CB₁-dependent G_i protein that leads to inhibition of the adenylate cyclase enzyme (Howlett et al., 1989) and, therefore, decreased activity of protein kinase A. Initiation of this second messenger cascade culminates in reduced neuronal intracellular calcium load through N and P/Q type channels and, therefore, diminished presynaptic neurotransmitter release (Mackie and Hille, 1992). A reduction in the release of the excitatory neurotransmitter glutamate has been shown to accompany activation of the cannabinoid CB₁ receptor (Shen et al., 1996). A cannabinoid CB₁-mediated reduction of neurotransmitter release would be beneficial because excitotoxic levels of glutamate are found in epileptic tissue (Leach et al., 1986). Cannabinoid CB₁ receptor activation has also been shown to increase A-type K⁺ channel permeability, serving to stabilize membrane potential during neuronal burst-firing (Deadwyler et al., 1993). Additionally, cannabinoids may also increase inhibitory tone that may serve as an anticonvulsant mechanism. For example, γ -aminobutyric acid reuptake in the globus pallidus is inhibited by cannabinoids (Sieradzan et al., 2001). Δ^9 -Tetrahydrocannabinol and synthetic cannabinoid compounds have been shown to activate the receptor and produce these downstream effects with marked enantioselectivity, further indication of specific, receptor-mediated effects. Further studies are needed to evaluate these mechanisms and their role in mediating anandamide's anticonvulsant effect.

This study provides direct evidence for a physiological role of endocannabinoids in modulating seizure threshold and severity. In addition, these data further establish the cannabinoid CB₁ receptor and the endogenous cannabinoid system as a potential treatment target for the control of epilepsy. Additional studies investigating the role of this system in epilepsy are clearly warranted.

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