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Marijuana, endocannabinoids, and epilepsy: potential and challenges for improved therapeutic intervention

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Abstract

Phytocannabinoids isolated from the cannabis plant have broad potential in medicine that has been well recognized for many centuries. It is presumed that these lipid soluble signaling molecules exert their effects in both the central and peripheral nervous system in large part through direct interaction with metabotropic cannabinoid receptors. These same receptors are also targeted by a variety of endogenous cannabinoids including 2-arachidonoyl glycerol and anandamide. Significant effort over the last decade has produced an enormous advance in our understanding of both the cellular and the synaptic physiology of endogenous lipid signaling systems. This increase in knowledge has left us better prepared to carefully evaluate the potential for both natural and synthetic cannabinoids in the treatment of a variety of neurological disorders. In the case of epilepsy, long standing interest in therapeutic approaches that target endogenous cannabinoid signaling systems are, for the most part, not well justified by available clinical data from human epileptics. Nevertheless, basic science experiments have clearly indicated a key role for endogenous cannabinoid signaling systems in moment to moment regulation of neuronal excitability. Further it has become clear that these systems can both alter and be altered by epileptiform activity in a wide range of in vitro and in vivo models of epilepsy. Collectively these observations suggest clear potential for effective therapeutic modulation of endogenous cannabinoid signaling systems in the treatment of human epilepsy, and in fact, further highlight key obstacles that would need to be addressed to reach that goal.

Introduction

Smoke from the dried leaves of the cannabis plant (marijuana) provides an effective delivery system for approximately 80 distinct phytocannabinoids, and many other chemicals (Turner *et al.*, 1980; Radwan *et al.*, 2009). Of these, Δ^9-tetrohydrocannabinal (Δ^9-THC), first isolated in 1964 (Gaoni & Mechoulam, 1964), is believed to be the primary psychoactive ingredient. Indeed, many of the diverse effects of marijuana on human cognition and perception are thought to depend critically on action of Δ9-THC at metabotropic cannabinoid type I receptors (CB1Rs). These receptors are broadly expressed in the CNS, are almost always expressed presynaptically, are coupled to $G_{i/o}$, and when activated are strongly implicated in short and/or long term inhibition of synaptic transmission.

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There is significant interest in the idea that Δ^9-THC and/or other phytocannabinoids from the cannabis plant, acting on some aspect of the endogenous cannabinoid system, could have potential value as an antiepileptic agent. Indeed, potential as an antiepileptic is just one of many possible therapeutic uses of so called medical marijuana. Inhalation of marijuana smoke in humans has also been noted to have potential application as an appetite stimulant, an antiemetic, a muscle relaxant, and as a potent analgesic, among other possibilities (for review see Ben Amar, 2006; Hazekamp & Grotenhermen, 2010). Although personal use of marijuana in any context in the United States continues to violate federal law, an interesting combination of anecdotal evidence, scientific data, and public interest have successfully advanced medical marijuana laws in 16 states and in the District of Columbia. In many ways it is not surprising that the list of potential medicinal benefits of marijuana is so long given the complex combination of cannabinoids and other substances found in the cannabis plant, combined with what we are increasingly realizing is a broad and complex role for cannabinoid systems in the regulation of neuronal activity. However, it is important to note that direct scientific data supporting therapeutic use of marijuana smoke is stronger with respect to some applications than others.

In the case of epilepsy, there is actually very limited direct scientific data supporting the use of smoked marijuana or oral cannabinoids in humans as antiepileptic agents. In fact, there is only one small clinical trial (Cunha *et al.*, 1980), one largely epidemiological study (Ng *et al.*, 1990), and a handful of case reports (e.g. see Consroe *et al.*, 1975; Ellison *et al.*, 1990) that support the idea that smoked marijuana can have antiepileptic effects. However, there is also at least one conflicting case report indicating proconvulsant effects (Keeler & Reifler, 1967), and there has not been clear consensus between a small number of studies that have attempted to quantify incidence of voluntary marijuana use, and subjective impressions of effectiveness, in human epileptics (e.g. see Gordon & Devinsky, 2001; Zagnoni & Albano, 2002; Gross *et al.*, 2004). Further, as will be noted in greater detail below, various studies in animal models have indicated both anticonvulsant and proconvulsant effects. Overall, it might be argued that what is called for is a large-scale, well-controlled, double-blind randomized trial that rigorously tests the effects of smoked marijuana in a large population of human epileptics. However, continuing legal complexities, significant concerns about psychoactive properties, some potential for addiction liability, and the sheer complexity of the chemical exposure that results from smoking marijuana all represent notable obstacles to initiation of such a study. Thus a significant challenge in the science of cannabis, as it relates to multiple potential disorders including epilepsy, is to isolate more specific interventions that can be used to more selectively target specific mechanisms of therapeutic interest.

In that regard, it is important to note that at the present time the best evidence that the endogenous cannabinoid system is an appropriate, and indeed an exciting potential target for development of better antiepileptic therapies does not come from the limited scientific data on marijuana use in humans noted above. Instead, it comes from relatively recent insights produced by basic science research on endocannabinoid systems. Indeed, in the last ten years in particular, basic science experiments have provided a wealth of new information about the molecular mechanisms of endocannabinoid signaling, and have convincingly exposed a central role for these systems in the moment to moment regulation of cortical excitability. Thus, this review will attempt to concisely highlight specific basic scientific evidence strongly implicating endocannabinoid systems in both the development and potential treatment of epilepsy, while also highlighting several significant obstacles that currently still complicate effective therapeutic manipulation of this system.

The (basic science) case for the endocannabinoid system as a therapeutic target for the treatment of epilepsy

The endocannabinoid system

In order to consider recent experiments specifically implicating endocannabinoid systems in the development and/or treatment of epilepsy, it is first necessary to at least briefly review some of the major insights that have been gained about the nature of the endocannabinoid signaling system itself. In the simplest sense, the endocannabinoid system is made of the receptors for endocannabinoids, the ligands that activate them, and a variety of enzymes, transporters, and other proteins involved in the synthesis, mobilization, and uptake of those ligands.

To date, there are two widely recognized cannabinoid receptors, and two primary endogenous ligands. The primary receptors are known as the cannabinoid type I receptor (CB1R) and the cannabinoid type II receptor (CB2R). Both of these receptors are classic metabotropic receptors coupled to $G_{i/o}$. However, the CB2R is predominately expressed in the immune system (Berdyshev, 2000), and has very limited expression in the CNS . Although there are some intriguing reports of specific CB2R effects in the CNS (e.g. see Xi *et al.*, 2011), the vast majority of known central effects of cannabinoids are CB2R independent. As such, the CB2R will not be covered further here. By contrast, the CB1R is highly expressed in CNS, is found almost exclusively in presynaptic locations, and when activated is directly implicated in inhibition of synaptic transmission, typically through action on voltage gated Ca^{2+} and/or K^{+} channels (Hampson *et al.*, 1995; Mackie *et al.*, 1995; Twitchell *et al.*, 1997; Schweitzer, 2000).

While the CB1R is responsible for the vast majority of the currently known effects of cannabinoids in the CNS, it is worth noting that additional cannabinoid receptors may exist. GPR55 in particular has been noted to be activated by some cannabinoid ligands, including (high concentrations of) Δ^{9}-THC (Ryberg *et al.*, 2007). However, there are conflicting reports regarding its responsiveness to endogenous cannabinoids (Ryberg *et al.*, 2007; Ross, 2009; Yin *et al.*, 2009), and additional work will be necessary to reach a consensus on how it is best classified (Moriconi *et al.*, 2010). Overall, while there is significant potential and interest in further identifying and characterizing novel cannabinoid receptors, this area of research is still developing, and falls largely outside the scope of the current review (for addtional information see Mackie & Stella, 2006; Kano *et al.*, 2009; Kreitzer & Stella, 2009).

The two primary ligands for CB1Rs in the CNS are 2-arachidonoylglycerol (2-AG) and N-arachidonoylethanolamide (anandamide or AEA). Each of these ligands is a fatty acid derivative that is widely available in the CNS, although 2-AG is present with a significantly higher basal concentration (Sugiura *et al.*, 1995; Stella *et al.*, 1997). 2-AG is produced in large part through the action of diacylglycerol lipase (DAGL) which catalyzes the production of 2-AG from diacylglycerol. Availability of diacylglycerol for this reaction depends heavily on the activity of phospholipase C (PLC), which produces diacylglycerol via hydrolysis of specific membrane phospholipids. By contrast, a primary pathway for production of AEA involves hydrolysis of *N*-arachidonoyl phatidylethanolamine (*N*-arachidonoyl PE) by *N*-acylphosphatidylethanolamine-hydrolyzing phospholipase D (NAPE-PLD), a reaction that produces both AEA and phosphatidic acid. The availability of by *N*-arachidonoyl PE for this reaction depends on the action of *N*-acyltransferase (NAT), a highly calcium dependent enzyme, which produces *N*-arachidonoyl PE from phosphatidylethanolamine.

Finally, it should be noted that there is significant evidence that carrier mediated transport systems are involved in clearing endocannabinoids from the extracellular space (Di Marzo *et al.*, 1994; Hillard *et al.*, 1997; Piomelli *et al.*, 1999), and that subsequent enzymatic degradation of endocannabinoids can proceed through either hydrolysis or oxidation. Specifically, monoacylglycerol lipase (MAGL) preferentially hydrolyzes 2-AG (Dinh *et al.*, 2002) while fatty acid amide hydrolase (FAAH) promotes hydrolysis of AEA (Deutsch *et al.*, 2002). Alternatively, oxidation of endocannabinoids is catalyzed by cyclooxygenase and related enzymes (Yates & Barker, 2009). For additional and more detailed review of the precise molecular mechanisms that underlie endocannabinoid mediated signaling please see (Piomelli, 2003; Sugiura *et al.*, 2006a; Sugiura *et al.*, 2006b; Okamoto *et al.*, 2007; Kano *et al.*, 2009).

Overall, it is our contention that a compelling case can be made that the endocannabinoid system as briefly described above does indeed represent a compelling target for development of future antiepileptic therapies. Although to date there is minimal direct clinical evidence in humans, the case can be made based on three broad types of evidence that have been provided by basic science experiments over the last ten years. These include: 1) Evidence that the endocannabinoid system is intimately involved in the regulation of cortical excitability, 2) Evidence that the endocannabinoid system is altered in epilepsy or by epileptic seizures, 3) Evidence that modulation of the endocannabinoid system can alter seizure activity or change the development of epileptogenesis in various in vitro and in vivo models. Each of these will be considered in turn in the sections below.

Cannabinoids and regulation of cortical excitability

Activation of presynaptic CB1Rs by endogenous ligands is believed to occur largely through retrograde transmission. Indeed, in 2001, endogenous cannabinoids were identified as retrograde messengers in a form of short term synaptic plasticity known as depolarization induced suppression of inhibition (DSI). In this form of plasticity, originally identified in CA1 pyramidal cells (Pitler & Alger, 1992) and cerebellar Purkinje cells (Llano *et al.*, 1991), depolarization of a postsynaptic cell results in transient reduction in the amplitude of either spontaneous or evoked inhibitory postsynaptic currents. As such, DSI represents a mechanism by which depolarization of a postsynaptic cell may effectively modulate the release probability of some of its own afferents. A crucial role for endocannabinoids in DSI is indicated by the fact that DSI is blocked by CB1R antagonists, is occluded by a CB1R agonists, and is absent in $CB1R^{-/-}$ animals (Kreitzer & Regehr, 2001a; Ohno-Shosaku *et al.*, 2001; Wilson & Nicoll, 2001; Yoshida *et al.*, 2002).

After initial descriptions of endocannabinoid mediated and CB1R dependent DSI in hippocampus and cerebellum, it quickly became clear that this form of synaptic plasticity is prevalent in many areas of the CNS including the dentate gyrus (Isokawa & Alger, 2005; Hofmann *et al.*, 2006; Howard *et al.*, 2007), basal ganglia (Wallmichrath & Szabo, 2002; Engler *et al.*, 2006; Narushima *et al.*, 2006), cerebral cortex (Trettel & Levine, 2003; Fortin *et al.*, 2004; Bodor *et al.*, 2005), hypothalamus (Di *et al.*, 2003; Jo *et al.*, 2005), amygdala (Zhu & Lovinger, 2005), and brain stem (Mukhtarov *et al.*, 2005), among others. Further, it is now clear that endocannabinoids can be released from postsynaptic neurons not only due to depolarization or activity dependent calcium influx, but also in response to activation of specific metabotropic receptors, or due to the combination of the two. Indeed, specific mechanisms of endocannabinoid release appear to mobilize cannabinoids with distinct temporal, and likely spatial, parameters that can promote a wide range of effects on synaptic transmission. For example, activation of metabotropic glutamate receptors drives endocannabinoid mediated long term depression of GABAergic transmission in many areas where there is DSI (for review see Lovinger, 2008; Kano *et al.*, 2009), while activation of muscarinic acetylcholine receptors facilitates depolarization induced release of

endocannabinoids in several areas (Ohno-Shosaku *et al.*, 2003; Fukudome *et al.*, 2004; Hashimotodani *et al.*, 2005; Hofmann *et al.*, 2006).

Interestingly, for several years the bulk of new information about endocannabinoid mediated effects on synaptic transmission focused heavily on either short or long-term inhibition of GABAergic transmission. If activation of CB1Rs exclusively modulated release from inhibitory terminals, complex network effects would likely be required to explain net antiepileptic effects putatively associated with smoking marijuana. Thus, it is essential to emphasize that it has now become abundantly clear that CB1Rs are also expressed on a many glutamatergic terminals, and that CB1R dependent inhibition of excitatory transmission is also widespread in the CNS. Specifically, depolarization induced suppression of excitation (DSE) has now been observed in the cerebellum, hippocampus, hypothalamus, ventral tegmental area, and amygdala, among other areas (e.g. see Kreitzer & Regehr, 2001b; Ohno-Shosaku *et al.*, 2002; Melis *et al.*, 2004a; Melis *et al.*, 2004b; Di *et al.*, 2005a; Di *et al.*, 2005b; Domenici *et al.*, 2006; Hofmann *et al.*, 2008). Further, as will be reviewed in more detail below, in some cases CB1Rs on glutamatergic terminals have been directly implicated modulation of seizure threshold (Marsicano *et al.*, 2003; Monory *et al.*, 2006).

Thus, over the last ten years basic science experiments have revealed an enormous wealth of endocannabinoid mediated and CB1R dependent synaptic phenomena in the CNS that collectively produce a wide range of short-term, long-term, and even metaplastic phenomena at numerous inhibitory and excitatory synapses throughout the CNS. While this summary has been necessarily brief, a primary point in the context of this review is that any system so deeply ingrained in moment to moment regulation of cortical excitability meets any reasonable initial criteria for further consideration as a potential target for antiepileptic therapies. For recent and more extensive review please see (Lovinger, 2008; Kano *et al.*, 2009).

Epilepsy induced changes in the endocannabinoid system

Expression of CB1Rs in epilepsy—Following the cloning of the CB1R and the production of CB1R antibodies, scientists could begin investigating whether there were changes to the expression of CB1Rs both during epileptogenesis and after recurrent seizures. However, there are several different versions of the CB1R antibody, with those targeting short sequences of amino acids on the C-terminal end of the protein generally being notably more effective at revealing CB1R expression on glutamatergic terminals (Fukudome *et al.*, 2004; Katona *et al.*, 2006; Monory *et al.*, 2006). In initial studies using the pilocarpine model of epilepsy, the DeLorenzo laboratory discovered that following induction of epilepsy there was an increase in CB1R receptor expression one year after the initial induction of status epilepticus (Wallace *et al.*, 2003). In agreement with these results, the febrile seizure model also demonstrated an increase in CB1R expression using a long C-terminal antibody which was blocked with a CB1R antagonist. Electron microscopy and immunocytochemistry failed to elucidate an increase in the number of CB1R expressing axons suggesting this result was not due to sprouting of CB1R positive axons (Chen *et al.*, 2003; Chen *et al.*, 2007). The results from these labs suggest that increased expression of CB1R at GABAergic interneurons could increase excitability and potentially play a role in epileptogenesis.

Using human control and epileptic tissue, the Katona lab investigated changes to the expression of CB1R in the hippocampus. Brain homogenates containing the dentate gyrus, all CA subfields, and a small part of the subiculum at the CA1 border were analyzed using quantitative real-time PCR and these results displayed a reduction in CB1R mRNA levels. In addition, a down-regulation of CB1R was observed in stratum radiatum of all CA

subfields and stratum moleculare in all epileptic tissue and in stratum oriens and stratum pyramidale of sclerotic epileptic tissue using a short C-terminal antibody for immunocytochemistry. Further analysis of the inner molecular layer using electron microscopy revealed a decrease in CB1R expression ratio that was specific to glutamatergic synapses with no change to the ratio at GABAergic synapses. In support of this result, immunostaining showed a decrease in the density of mossy cells, whose axons synapse onto granule cell dendrites in this layer, with no change to the density of interneurons in the dentate gyrus (Ludanyi *et al.*, 2008). This fits well with other research showing a loss of mossy cells, which are also believed to express presynaptic CB1Rs, in epileptic models (Buckmaster & Jongen-Relo, 1999; Ratzliff *et al.*, 2002; Sloviter *et al.*, 2003; Chiu & Castillo, 2008). Overall, the results from this research tend to implicate decreases in CB1R expression at glutamatergic synapses in the etiology of epilepsy, although there may also be a role for loss of CB1R positive excitatory terminals associated with the death of mossy cells. While this research would at first glance appear to be contradictory to the observations of increased CB1R expression discussed above, they did find increased CB1R expression in stratum oriens of some sclerotic tissue (Ludanyi *et al.*, 2008).

Indeed, using a long C-terminal antibody in human epileptic tissue it was demonstrated that there was actually an increase in CB1R expression at GABAergic synapses in the dentate molecular layer supporting the earlier findings in animal models of epilepsy (Magloczky *et al.*, 2010). In addition, work done by the DeLorenzo lab on the pilocarpine model in rats found an up regulation of CB1Rs in stratum radiatum, stratum lacunosum-moleculare, and stratum oriens. However, they also found a down regulation of CB1Rs in CA1 and CA3 pyramidal cell layers and the inner molecular layer of the dentate gyrus which were not entirely due to cell loss. Interestingly, they found the same results regardless of whether they were using a short C-terminal or longer N-terminal antibody to CB1 (Falenski *et al.*, 2007; Falenski *et al.*, 2009). Further investigations examined how CB1R expression changes during epileptogenesis and they found a biphasic expression of this receptor with an initial decrease in CB1R expression throughout the hippocampus. At about one month post status epilepticus increases in CB1Rs were observed in stratum radiatum, stratum lacunosum-moleculare, and stratum oriens while remaining decreased in the CA1 and CA3 pyramidal cell layers and the inner molecular layer of the dentate gyrus (Falenski *et al.*, 2009). However, there is also recent research using showing an overall decrease in CB1R expression in strata pyramidal, oriens, and radiatum in area CA1 due to the loss of CCK positive boutons which typically express CB1R (Wyeth *et al.*, 2010). This study used a somewhat unusual C-terminal antibody (see also Nyiri *et al.*, 2005) that nevertheless was noted as potentially selective for GABAergic terminals due to possible interaction with cannabinoid receptor-interacting protein CRIP1a (Katona *et al.*, 2006; Niehaus *et al.*, 2007). Finally, recent work done by the Smith lab on the pilocarpine model of epilepsy demonstrated a change in the sensitivity of glutamatergic afferents to cannabinoids. The research indicates that the newly sprouted mossy fiber axons now express CB1Rs and this is confirmed with increased expression of CB1Rs in a Western blot of tissue including the molecular and the granule cell layers (Bhaskaran & Smith, 2010a). This is interesting in part because when compared to previous results regarding decreased expression of CB1Rs at mossy cell axon terminals (Monory *et al.*, 2006), it would seem to suggest that it may be possible to get differential regulation of CB1Rs at distinct glutamatergic terminals.

Overall, the current research demonstrates obvious changes to CB1R expression in epilepsy. While there isn't complete agreement, current data broadly suggest the possibility that CB1R expression tends to be up-regulated at GABAergic synapses and down-regulated at glutamatergic synapses in epilepsy, with mossy fiber terminals being a potential exception. Overall, this model is noteworthy in that both of these changes to CB1R expression could plausibly contribute to reduced seizure threshold in epileptic tissue.

Production of endogenous cannabinoids in epilepsy—Besides changes to the expression of the CB1R receptor, changes to the production and breakdown of endogenous cannabinoids could have profound effects on excitability in the hippocampus and provide potential therapeutic avenues. In the pilocarpine model of epilepsy there is evidence for an increase in the amount of 2-AG produced (Wallace *et al.*, 2003). This is supported by data demonstrating an increase in seizure frequency following application of a CB1R antagonist in both the pilocarpine model and the culture model of acquired epilepsy suggesting an increase in cannabinoid tone (Wallace *et al.*, 2003; Deshpande *et al.*, 2007b). There is also data showing a significant increase in the levels of anandamide twenty minutes after the injection of kainic acid (Marsicano *et al.*, 2003). While these data would imply that following seizures there is an increase in cannabinoid production other research has proven contradictory. In human epileptic tissue there was no apparent change to the expression or activity of NAPE-PLD and FAAH, which are important for the production and breakdown of anandamide respectively, when compared to controls (Steffens *et al.*, 2005; Ludanyi *et al.*, 2008). However, in newly diagnosed epilepsy patients there was significantly less anandamide in the cerebrospinal fluid compared to controls (Romigi *et al.*, 2010). In addition, there was a significant decrease in sclerotic epileptic tissue (but not non-sclerotic) of DGL-α, the enzyme responsible for the production of 2-AG, while the enzyme that breaks down 2-AG, MAGL, trended down in sclerotic tissue (Ludanyi *et al.*, 2008). Finally, in the febrile seizure model there was no detected increase in endogenous cannabinoid levels as tested using HPLC and also no changes to the activity of MGL or FAAH (Chen *et al.*, 2003). Overall, there is much less known about changes to the endogenous cannabinoid system and much more discrepancy in the results compared to changes to CB1R expression. It will be important to determine whether more or less endogenous cannabinoids are produced to create good therapeutic strategies. For example, if there was a decrease in the production of endogenous cannabinoids then receptor based therapeutic interventions might not be effective due to persistent shortage of agonists. In addition, it will be interesting to investigate how endogenous cannabinoid levels change through the course of epileptogenesis. Research has shown that prolonged exposure to WIN55,212-2, a CB1R agonist, can cause down-regulation of the CB1R at both glutamatergic and GABAergic synapses in the culture model of acquired epilepsy (Blair *et al.*, 2009). Perhaps an epilepsy inducing insult increases the production of endogenous cannabinoids resulting in the decrease in CB1R expression seen early on in the pilocarpine model of epilepsy.

Altered cannabinoid mediated plasticity in epilepsy—With obvious changes to both the expression of CB1Rs and production of endogenous cannabinoids, it is also imperative to understand how this translates into changes in cannabinoid mediated neuronal signaling. In general, this has been a highly understudied area; however, there is one publication that has begun to address the question of how epilepsy changes cannabinoid signaling. In the febrile seizure model, as discussed earlier, there is an increase in the expression of CB1Rs. This manifests into an increase in the amplitude and duration of DSI one week later and was still present at 11 weeks. Their data also indicates the enhanced DSI is due to the up-regulation of CB1Rs and not due to changes in endogenous cannabinoid production or breakdown. Interestingly, this change was specific to the GABAergic signaling as there was no obvious change to DSE following febrile seizures (Chen *et al.*, 2003). The intriguing findings by this laboratory demonstrate the need for additional investigations into alterations to the endogenous cannabinoid signaling system in epilepsy. Further research is needed to determine changes, or lack thereof, to DSI and DSE in other areas such as CA3 (Hofmann *et al.*, 2008) and the dentate gyrus (Isokawa & Alger, 2005; Hofmann *et al.*, 2006; Chiu & Castillo, 2008). There is also literature suggesting a role for the cannabinoid signaling system in long term potentiation (Carlson *et al.*, 2002) and long term depression (Chevaleyre & Castillo, 2003, 2004; Chevaleyre *et al.*, 2007; Edwards *et al.*,

2008; Heifets *et al.*, 2008; Nahir *et al.*, 2010) which should also be investigated following the induction of epilepsy. Finally, cannabinoids have been shown to affect GABAergic network activity (Hajos *et al.*, 2000; Reich *et al.*, 2005; Echegoyen *et al.*, 2009) and understanding changes to this could provide insight into the altered excitability in the hippocampus leading to epileptic seizures.

Effects of cannabinoids in in vitro and in vivo models of epilepsy

Although clinical data testing cannabinoids as antiepileptic agents in humans remains limited, cannabinoid receptor agonists and antagonists have been directly tested for effects on various aspects of epileptiform activity in a great many in vitro and in vivo models of epilepsy.

For example, one of the earlier studies to specifically implicate the CB1Rs in anticonvulsant action of cannabinoids used an electroshock model of a secondarily generalized seizure. In this model, a corneal shock is used to produce hind limb extension in rodents. Intraperitoneal injection of either WIN55,212-2 or Δ^9-THC produces dose dependent inhibition of this phenomenon in a manner that can be prevented by prior injection of a CB1R antagonist (Wallace *et al.*, 2001). Additional work revealed that a CB1R antagonist alone can reduce seizure threshold, suggesting a possible role for cannabinoid tone (Wallace *et al.*, 2002). This later result seems consistent with a recent case study indicating that rimonabant produced partial seizures in an epileptic patient that had been seizure free for many years (Braakman *et al.*, 2009).Further, in 2003 (Wallace *et al.*) reported that treatment of rats with either Δ^9-THC or the synthetic CB1R agonist WIN55212-2 essentially eliminated seizures in a pilocarpine model of epilepsy. Notably, these compounds were more effective at preventing seizures in pilocarpine treated rats than two conventional antiepileptics, phenytoin and phenobarbital. Conversely, application of a CB1R antagonist increased both seizure duration and frequency in epileptic but not in control animals. Finally, these authors reported increased expression of CB1R in hippocampal membranes (see also Falenski *et al.*, 2007; Falenski *et al.*, 2009), and increased levels of 2-AG during seizures.

Later work with the pilocarpine model revealed that WIN55,212-2, AEA, and 2-AG reduced the frequency of both spontaneous and miniature excitatory postsynaptic currents, but only in epileptic tissue. WIN55,212-2 was also shown to inhibit bursts of EPSCs observed in granule cells secondary to photolysis of glutamate in a CB1R dependent manner, while population discharges produced by antidromic activation of mossy fibers were similarly effected (Bhaskaran & Smith, 2010a). These data suggested the possibility that sprouted mossy fibers may have heightened sensitivity to cannabinoid agonists in this model. Additional work from this group, also noted that AEA can enhance glutamate release in the pilocarpine model in the presence of a CB1R antagonist via apparent activation of TRPV1 receptors. Consistent with this observation, the TRPV1 agonist capsaicin enhanced miniature and spontaneous frequency of excitatory postsynaptic currents only in mice that developed epilepsy (Bhaskaran & Smith, 2010b).

Additional intriguing effects of cannabinoids have been noted in an in vitro models of acquired epilepsy and status epilepticus as observed in hippocampal neuronal culture. For example, in 2006 (Blair *et al.*) used this preparation to demonstrate that low μM concentrations of WIN55,212-2 can produce CB1R dependent anticonvulsant effects against both spontaneous and recurrent epileptiform discharges, and against status epilepticus induced by application external solution containing low concentrations of magnesium. Again, in this preparation, cannabinoid agonists were more effective than conventional antiepileptics phenytoin and pentobarbital. Additional work with this model showed that 2-AG and methanandamide also produce dose and CB1R dependent inhibition of status

epilepticus as produced by low magnesium (Deshpande *et al.*, 2007a), that application of a CB1R antagonist alone will produce status epilepticus in cultures that display spontaneous recurrent epileptiform discharges one day after a three hour exposure to low magnesium, but not in control cultures (Deshpande *et al.*, 2007b), and that prolonged exposure of magnesium treated cultures to WIN55,212-2 will not only down regulate CB1R expression and negate its anticonvulsant effects, but indeed will produce a dose dependent increase in the frequency of spontaneous recurrent epileptiform discharges (Blair *et al.*, 2009). These results simultaneously reinforce some aspects of the findings with the pilocarpine model, and yet also begin to highlight some of the difficulties that will likely complicate any straightforward use of cannabinoid receptor agonists as therapeutic agents in human epilepsy.

Recent years have also brought interesting results regarding the effects of cannabinoid receptor agonists on threshold for seizures as induced in mice by injection of the GABA antagonist pentylenetetrazole (PTZ). Specifically, acute administration of ACPA increased threshold for PTZ induced seizures while administration of the CB1R antagonist AM251 effectively decreased seizure threshold and reversed the anticonvulsant effect of ACPA (Shafaroodi *et al.*, 2004). Intriguingly, this study also reported that the opioid receptor antagonist naltrexone produced an additive effect with low doses of AM251 in blocking the anticonvulsant action of ACPA. Interestingly later work with this model indicated that extremely low doses of AM251 actually potentiated rather than blocked anticonvulsant action of another CB1R agonist ACEA (Gholizadeh *et al.*, 2007), and that similar effects could be produced by L-arginine, a nitric oxide precursor (Bahremand *et al.*, 2009).

While the data above make a compelling case for the potential to manipulate epileptiform activity through modulation of cannabinoid mediated signaling, it is important to highlight two additional lines of evidence that may be of particular interest in considering the potential of endocannabinoids as antiepileptic agents. The first is based on work with febrile (fever induced) seizures. In this model of epilepsy long lasting increases in inhibitory synaptic transmission, CB1R expression, and DSI are all induced, along with long term limbic hyperexcitability, by brief exposure to high core body temperatures at postnatal day ten (Chen *et al.*, 1999; Chen *et al.*, 2003). Unexpectedly, later work found that a novel tetanus induced potentiation of DSI could be occluded by prior febrile seizures, and that the primary effects of febrile seizures themselves could also be prevented by application of a CB1R antagonist (generally considered a proconvulsant agent) during the initial insult (Chen *et al.*, 2007; commentary by Lutz & Monory, 2008). Consistent with this idea, the same group has more recently shown that a single application of a CB1R antagonist can, if delivered rapidly after an insult, also prevent long term development of hyperexcitability in a model of epilepsy based on fluid percussion injury of the head (Echegoyen *et al.*, 2009). Although sufficiently rapid delivery post insult may not always be an easy therapeutic goal to reach, these findings convincingly open up very intriguing and novel ideas about largely untested potential for prophylactic therapeutic interventions human epilepsies that may effectively short circuit epileptogenesis with brief, but precisely timed, inhibition of CB1R dependent signaling (Armstrong *et al.*, 2009). Nevertheless, it is important to note that a recent study, using a different model of epilepsy, has failed to show similar efficacy of a cannabinoid antagonist delivered early during an insult in disrupting epileptogenesis (Dudek *et al.*, 2010).

Finally, it is important to cover fascinating results about the role of cannabinoids and their receptors in the development of kainic acid (KA) induced seizures. In this model of epilepsy, systemic application of kainic acid drives excitatory synaptic activity in hippocampal circuits resulting in short term excitotoxic cell death and longer term hyperexcitability (Ben-Ari & Cossart, 2000). In 2003, (Marsicano *et al.*) demonstrated that

$CB1R^{-/-}$ mice were more susceptible to KA induced seizures than wild type littermates, and further that this heightened sensitivity was maintained in mice where the CB1R was selectively removed from all principal forebrain neurons, but not from associated interneurons. This result suggested that activation of CB1Rs on excitatory neurons was essential to protect from KA induced seizures, however it could not provide a definitive answer as to whether activation of CB1Rs on GABAergic neurons was also required. A subsequent study did reveal a likely role for brain derived neurotropic factor CB1R dependent protection from KA induced seizures (Khaspekov *et al.*, 2004). Importantly, these authors went on several years later to develop additional conditional knockouts of the CB1R that were selective to cortical glutamatergic or GABAergic neurons (Monory *et al.*, 2006). In this study it became clear that selective removal of CB1 from glutamatergic neurons produced a dramatic reduction in CB1R expression in the inner molecular layer of the dentate gyrus that was ultimately associated with hilar mossy cells. Further, this loss of CB1Rs only in glutamatergic neurons, like the conditional knockouts in principal neurons before, continued to dramatically increase the susceptibility of mice to KA induced seizures, while strikingly, selective removal of CB1Rs from GABAergic neurons did not. Overall, these selective knockout animals continue to be one of the more elegant tools developed to study the role of cannabinoid receptors in epileptogenesis, and these papers continue to provide an excellent demonstration of a specific case in which selective activation of CB1Rs on glutamatergic rather than GABAergic terminals is required for antiepileptic action of endogenous cannabinoids.

Overall summary, potential obstacles, and future directions—In the sections above we have attempted to provide evidence, drawn largely from basic science experiments conducted over that last decade, that the endocannabinoid system has significant potential as a target for future development of antiepileptic therapies. That argument is based on three main contentions: Endocannabinoid mediated systems are intimately involved in moment to moment regulation of neuronal excitability throughout many areas of the CNS. Significant aspects of endogenous cannabinoid signaling systems are altered in a wide range of epileptic conditions. External modulation of cannabinoid mediated systems can prevent or modulate important aspects of epileptiform activity in a wide range of in vitro and in vivo models. We believe these broad points can be made without any hesitation or reservation; however evidence presented here also clearly highlights multiple obstacles to effective therapeutic manipulation the endogenous cannabinoid system. For example, current options for therapeutic delivery of lipophilic CB1R selective compounds does not allow for precise timing, rapid delivery, rapid removal, or selective targeting (to brain area or to specific type of CB1R positive terminal). Further, endocannabinoid systems themselves are not stable in the face of chronic application of low concentrations of CB1R agonists or antagonists, or throughout the process of epileptogenesis. In sharp contrast, significant evidence confirms that endogenous cannabinoid systems are capable of producing rapid, time limited, space limited, on-demand, delivery of specific endogenous ligands to specific types of CB1R positive terminals in specific parts of the brain. Further, it seems clear that all of these features are likely essential to normal maintenance of the proper balance between excitation and inhibition in cortical networks.

While many of these problems are indeed substantial, there are still a number of avenues through which better therapeutic outcomes may ultimately be obtained. As noted above, one intriguing strategy involves short circuiting the process of epileptogenesis by briefly inhibiting cannabinoid systems during, or very shortly after, extreme insults (Armstrong *et al.*, 2009). Another perhaps more obvious approach is to place more emphasis on development of therapeutic interventions that target on-demand aspects of the endocannabinoid system. In other words, strategies that enhance the ability to release cannabinoids when and where needed, or to prolong the activity of endogenous

cannabinoids only where naturally released, may hold greater promise for effectively enhancing the brains own strategies for regulation of excitability. Consistent with this goal, three separate studies have recently and independently found that 2-AG as synthesized by DAGLα is likely essential to many fundamental forms of endocannabinoid mediated signaling in the CNS (Gao *et al.*, 2010; Tanimura *et al.*, 2010; Yoshino *et al.*, 2011). Similarly, selective enzyme inhibitors and uptake blockers have become available in recent years, although it generally remains to be seen whether endogenous cannabinoid systems will be more stable in the presence of these compounds than they are in the continued presence of exogenous agonists or antagonists. Other strategies of potential interest include developing methods to selectively target cannabinoid receptors based on tissue or time of expression (Pertwee, 2009), focusing on allosteric modulation of cannabinoid receptors by novel compounds (e.g. see Ross, 2007), or targeting more subtle CB1R independent effects of endogenous, synthetic, and phytocannabinoids. In this last regard it is worth noting that Δ^9-THC has been noted to be an effective GABA uptake blocker in some preparations (Maneuf *et al.*, 1996; Coull *et al.*, 1997), that WIN55,212-2 and AEA have recently been shown to facilitate action potential independent synaptic transmission via a CB1R independent mechanism (Sang *et al.*, 2010; Hofmann *et al.*, 2011), and that cannabidiol (a prominent phytocannabinoid with minimal psychoactive properties and poor affinity for CB1Rs) has shown significant potential for CB1R independent antiepileptic effects (Cortesi & Fusar-Poli, 2007; Jones *et al.*, 2010). Overall, it is clear that significant additional effort and time will be required to develop appropriately selective therapeutic interventions for epilepsy that more precisely target specific aspects of endogenous cannabinoid systems, however appropriate tools for ever more selective manipulation of brain systems continue to develop, and thus basic science should continue to strive to provide detailed mechanistic insights that will be appropriate to guide the use of future therapeutic tools.

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