INTRODUCTION

Epilepsy is a common neurological condition that affects approximately 1% of the adult population and 2% of children (Hauser and Hesdorffer, 1990) and includes a diverse group of seizure disorders that vary in their region of origin, age of onset, pathophysiological manifestations, and underlying mechanisms. Approximately half of all seizure disorders fall into the category of acquired epilepsy (AE), whereby an initial brain insult such as stroke, status epilepticus (SE), or head trauma results in a permanent neuronal plasticity change, which underlies the pathophysiology of these conditions (Hauser and Hesdorffer, 1990). The phase during which the brain undergoes maladaptive alterations in neuronal function following an insult, and ultimately results in the occurrence of spontaneous recurrent seizures (SRSs), is termed epileptogenesis (Delorenzo et al., 2005). Although the epilepsies as a group represent a complex subject, all of these disorders are associated with SRS discharges arising from the synchronous firing of a population of neurons due to a disruption in the balance between excitatory and inhibitory neuronal transmission (Lothman et al., 1991; Scharfman, 2007; Badawy et al., 2009a,b). In spite of a wide array of available anti-epileptic drug (AED) therapies, it is estimated that between 25 and 40% of newly diagnosed epilepsy patients will have seizures that are refractory to current treatments (Schmidt and Sillanpaa, 2012). The proceedings from a meeting of the International League Against Epilepsy/American Epilepsy Society set the framework for a number of future goals, one of which emphasized the need for the development of research strategies and model systems to elucidate novel therapeutic targets for seizure control (Wilcox et al., 2013).

The endocannabinoid system (ECS) plays an essential role in the brain through its regulation of many neuronal processes involved in both physiological and pathological conditions (Di Marzo et al., 1998; Alger, 2006; Mackie and Stella, 2006; Kano et al., 2009; Castillo et al., 2012). It is comprised of receptors that are acted upon by endogenous lipid ligands (endocannabinoids) and the enzymatic
machinery involved in their synthesis, uptake, and degradation. The brain cannabi-
noid type-1 (CB1) receptor is a G_i/o protein-coupled receptor (GPCR) and has been
identified as the primary mediator of the central effects of cannabinoids/endocan-
nabinoids (Devane et al., 1988; Matsuda et al., 1990; Howlett, 1995). The CB1
receptor is widely distributed throughout the brain and is one of the most abundant
GPCRs in the CNS (Herkenham et al., 1991; Egertova and Elphick, 2000). Unlike
classical neurotransmitters that are synthesized and maintained in vesicular
storage, the endogenous cannabinoids, arachidonylethanolamine (AEA) and
2-arachidonylglycerol (2-AG) (Devane et al., 1992; Mechoulam et al., 1995), are
synthesized “on demand” by the enzymes N-acyl phosphatidylethanolamine phos-
pholipase D (NAPE-PLD) and diacylglycerol lipases (DGL-α and DGL-β), respect-
ively (Bisogno et al., 2003; Okamoto et al., 2004), in response to sustained
neuronal depolarization and elevated intracellular Ca^{2+} levels (Kondo et al., 1998;
Stella and Piomelli, 2001). Both AEA and 2-AG cross the synapse in a retrograde
manner to act on presynaptic CB1 receptors followed by rapid carrier-mediated
reuptake (Di Marzo et al., 1994; Hillard et al., 1997; Piomelli et al., 1999) and
enzyme degradation by fatty acid amide hydrolase (FAAH) (Deutsch et al., 2002)
and monoacylglycerol lipase (MAGL) (Dinh et al., 2002), respectively. Activation
of presynaptic CB1 receptors results in responses that are mediated via a number of
effector systems that include inhibition of adenylate cyclase-dependent cAMP
accumulation and protein kinase A (PKA) activation, inhibition of voltage-gated Ca^{2+}
channels, activation of G protein-coupled inwardly-rectifying K^{+} (GIRK)
channels, and downstream activation of the mitogen-activated protein (MAP)
kinase pathway (Howlett et al., 2004). The primary functional role of the brain
ECS is the “on-demand” fine-tuning of synaptic transmission via regulation of pre-
synaptic neurotransmitter release mechanisms. Following presynaptic release of
neurotransmitters and subsequent postsynaptic membrane depolarization, endocan-
nabinoids are synthesized, traverse back over the synapse to activate presynaptic
CB1 receptors, and inhibit further release of neurotransmitter, a process that has
been termed either depolarization-induced suppression of inhibition (DSI) or
depolarization-induced suppression of excitation (DSE) when occurring at inhibi-
tory or excitatory synapses, respectively (Kano et al., 2009; Castillo et al., 2012).

Given that SRSs appear, and then cease, as a result of a transient dysregulation
of synaptic transmission within either an isolated population (focal seizures) or
broad region (generalized seizures) of neuronal networks, research efforts have
focused on understanding the potential role that the ECS function/dysfunction has
on epileptic seizure discharge. Additionally, although knowledge of the potential
therapeutic benefits of Cannabis sativa can be dated as far back as 5000 years, it
has only been in the last 25 years that an ever-developing understanding of the
brain ECS at the molecular and cellular levels, through a plethora of research
studies, has revealed potential therapeutic targets for the control of neuronal excit-
ability (Mechoulam and Parker, 2013).

This chapter will attempt to concisely present the amassed research findings
on the relationship between the brain ECS and the epileptic condition, and will be
organized into the following sections: (1) ECS regulation of excitatory neuronal synaptic transmission, (2) alterations in the ECS with seizures and epilepsy, and (3) therapeutic potential of modulating the ECS in seizures and epilepsy.

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**ECS REGULATES EXCITATORY NEURONAL SYNAPTIC TRANSMISSION**

Following the discovery of the CB₁ receptor (Matsuda et al., 1990) and its endogenous ligands AEA and 2-AG (Devane et al., 1992; Mechoulam et al., 1995; Sugiura et al., 1995), much research has been undertaken towards elucidating the mechanisms and the potential therapeutic role of targeting the ECS for the treatment of seizures and epilepsy (Pertwee, 2012; Hofmann and Frazier, 2013). In simplest terms, epileptic seizures are the result of an imbalance between excitatory glutamatergic and inhibitory GABAergic transmission resulting in synchronous hyperexcitable neuronal discharge (Lothman et al., 1991). Thus, it follows that control of seizure discharges would necessitate a need for the regulation of excitatory glutamatergic synaptic transmission. A paradox to the anticonvulsant properties of cannabinoids were the findings that CB₁ receptors are more abundantly expressed on axon terminals of inhibitory interneurons (Katona et al., 1999, 2000; Egertova and Elphick, 2000; Hajos et al., 2000), which preceded, in 2001, the first evidence for CB₁ receptor-dependent regulation of synaptic transmission via DSI of inhibitory postsynaptic potentials (IPSPs) (Ohno-Shosaku et al., 2001; Wilson and Nicoll, 2001, 2002). With the exception of findings in the cerebellum (Kreitzer and Regehr, 2001), there was little evidence at that time supporting the physiological existence of a CB₁ receptor-mediated regulation of excitatory glutamatergic synaptic transmission (i.e., DSE), although several studies using in vitro slice electrophysiology clearly demonstrated a role for cannabinoid-mediated suppression of glutamatergic synaptic transmission via a yet undetermined cannabinoid receptor (Szabo et al., 2000; Hajos et al., 2001; Hajos and Freund, 2002a,b). Thus, a physiological role for CB₁ receptor-dependent regulation of glutamatergic synaptic transmission had been the subject of rigorous debate, which has now been resolved by a number of pivotal studies over the last decade (Melis et al., 2004; Straiker and Mackie, 2005; Takahashi and Castillo, 2006; Katona et al., 2006; Kawamura et al., 2006; Kodirov et al., 2010; Xu et al., 2010; Peterfi et al., 2012). The endocannabinoid regulation of synaptic transmission is beyond the scope of this chapter, but several excellent reviews are available (e.g., Kano et al., 2009; Castillo et al., 2012).

The development of CB₁ receptor knockout (CB₁-KO) mice (Zimmer et al., 1999) allowed for the investigation of what role, or the lack thereof, the CB₁ receptor had in regulating different forms of synaptic transmission. Kano and colleagues demonstrated in hippocampal and cerebellar slice preparations from wild-type mice the presence of CB₁ receptor-dependent regulation of excitatory postsynaptic potentials (EPSPs), while tissue from CB₁-KOs was devoid of this activity.
Immunohistochemical staining for CB₁ receptors revealed weak signals that co-localized with the glutamatergic synaptic marker VGlut1 in wild-type mice that were absent in CB₁-KO mice. Additionally, electron microscopy (EM) analysis for CB₁ receptors within the stratum radiatum of the hippocampus revealed positive gold particles at asymmetric excitatory synapses. From these findings, they determined that the number of CB₁ receptors on hippocampal excitatory terminals was 10–20 times lower than what was observed on inhibitory terminals. Chen and colleagues demonstrated in hippocampal slices from wild-type and CB₁-KO mice that select hippocampal innervations of CA1 pyramidal neurons by the excitatory Schaffer-collaterals (SC) originating from CA3 onto proximal dendrites generated a form of long-term depression (LTD) that was dependent on DSE, while excitatory perforant path inputs originating from the entorhinal cortex on to distal dendrites generated a form of LTD that was independent of CB₁ receptor activation (Xu et al., 2010). These findings demonstrate the spatial complexity of ECS-mediated control of synaptic transmission at the cellular level, whereby excitatory inputs onto the proximal (within the stratum radiatum) or distal (within the stratum lacunosum-moleculare) dendritic fields of an individual CA1 pyramidal neuron can undergo CB₁ receptor-dependent or -independent LTD, respectively. Utilizing electrophysiological, immunohistochemical, and in situ hybridization analysis, Katona and colleagues evaluated the role of the endocannabinoid 2-AG towards mediating LTD in hippocampus via excitatory afferents on to either glutamatergic pyramidal cells or specific GABAergic interneurons (Peterfi et al., 2012). Their results suggest that during high levels of glutamatergic transmission, such as during seizure discharge, the 2-AG-mediated LTD on to glutamatergic pyramidal cells from CB₁ receptor containing excitatory afferents has a lower threshold for induction when compared to that of LTD of GABAergic interneurons from CB₁ receptor containing inhibitory afferents. Such a scenario would allow for EC-mediated fine-tuning of suppression of glutamatergic transmission while allowing for a maintained GABAergic inhibitory tone, which may act as an intrinsic mechanism to prevent the transformation of neuronal transmission from normal activity to a pathological state such as with epileptic seizures.

Another series of eloquent studies utilizing conditional mutant mice has demonstrated that CB₁ receptors located on asymmetric glutamatergic terminals play an essential role in the brain as a defense mechanism against glutamate-induced excitotoxicity (Marsicano et al., 2003; Domenici et al., 2006; Monory et al., 2006; Ruehle et al., 2013; Steindel et al., 2013). Lutz and colleagues developed a mutant mouse strain designated as CB₁\textsuperscript{CaMKII-Cre} that was devoid of CB₁ receptors selectively on forebrain principal neurons, while CB₁ receptor presence on inhibitory terminals remained intact (Marsicano et al., 2003). Following kainic acid (KA)-induced seizures, CB₁\textsuperscript{CaMKII-Cre} mice displayed a decreased seizure threshold, increased mortality, increased hippocampal cell death, and a lack of sensitivity to the protective effects of endocannabinoid uptake blockers or pro-convulsant actions of CB₁ receptor antagonists. Additionally, following
KA-induced seizures Lutz et al. (Ibid.) demonstrated $\text{CB}_1^{\text{CaMKII-Cre}}$ animals lacked $\text{CB}_1$ receptor-dependent induction of protective intracellular cascades, which included activation of the extracellular signal-regulated kinase (ERK) pathway and increased transcriptional expression of the immediate early genes (IEGs) $\text{c-fos, zif268}$, and the neurotrophin brain-derived neurotrophic factor (BDNF).

A second paper from this group (Monory et al., 2006) extended their work with mutant mice with the development of two additional strains with conditional deletion of $\text{CB}_1$ receptors on terminals of forebrain GABAergic interneurons (GABA-CB$_1^{-/-}$) or on principal glutamatergic neurons regionally limited to the cortical forebrain areas (Glu-CB$_1^{-/-}$), while preserving $\text{CB}_1$ receptors in subcortical and diencephalic regions of the forebrain. The GABA-CB$_1^{-/-}$ mice displayed comparable KA-induced seizure scores to wild-type controls. In the GABA-CB$_1^{-/-}$ mice, $\text{CB}_1$ receptor antibody staining revealed that the highest level of localization of $\text{CB}_1$ receptors at asymmetric glutamatergic terminals within the hippocampus was indicated by a defined and intense band within the inner third molecular layer of the dentate gyrus. Diffuse staining signal was also observed throughout the strata molecularis, radiatum, and oriens. Double in situ hybridization analysis of $\text{CB}_1$ receptors with VGluT1, a marker for glutamatergic terminals, demonstrated co-expression in mossy cell bodies within the hilar region of dentate gyrus and CA3 pyramidal cell bodies. The researchers concluded, by demonstrating a loss of $\text{CB}_1$ receptor-dependent suppression of EPSPs in Glu-CB$_1^{-/-}$ mice hippocampal slice, that virally induced and regionally select deletion of $\text{CB}_1$ receptors on glutamatergic terminals within the dentate gyrus of wild-type mice resulted in increased KA-induced seizure scores. Their findings indicate that $\text{CB}_1$ receptor-dependent regulation of glutamatergic afferents projecting from hilar mossy and CA3 pyramidal neurons on to dentate granule cell dendritic fields plays an essential role in the endocannabinoid-dependent protection against KA-induced seizures (Monory et al., 2006). A further discussion of the predominant role of $\text{CB}_1$ receptor-dependent regulation of glutamatergic transmission within the inner third molecular of the dentate gyrus in relation to findings in clinical and experimental epilepsy will be discussed later in this chapter.

Analysis of distribution and efficiency of $\text{CB}_1$ receptor agonist-stimulated [$^{35}\text{S}$]GTP$\gamma$S binding to measure $\text{CB}_1$ receptor–G protein coupling in the Glu-CB$_1$-KO and GABA-CB$_1$-KO animals revealed a comparable regional distribution to the above staining studies, and indicated that the functional efficiency of agonist-stimulated G protein signaling at $\text{CB}_1$ receptors localized to glutamatergic terminals was six-fold higher than that at $\text{CB}_1$ receptors on GABAergic terminals (Steindel et al., 2013). These findings are in agreement with an earlier study demonstrating variability in regional distribution and density of $\text{CB}_1$ receptor-stimulated G protein signaling and that the efficiency was inversely proportional to receptor density (Breivogel et al., 1997). Thus, although the level of $\text{CB}_1$ receptors on GABAergic terminals predominates over that on glutamatergic terminals, the increased efficiency of G protein signaling at the excitatory synapses would allow for a more sensitive
response to lower concentrations of agonists, which may explain the findings discussed above regarding differing sensitivities of CB₁ receptor-dependent induction of LTD at excitatory and inhibitory synapses (Peterfi et al., 2012). Utilization of the conditional CB₁-KO models has also elucidated a role for CB₁ receptor-dependent regulation of glutamatergic synaptic transmission towards suppressing cortical synchronous fast oscillations via a thalamocortical—striatonigral pathway, which would contribute to CB₁ receptor-dependent regulation of neuronal excitability and epileptic seizure discharge (Sales-Carbonell et al., 2013).

The above studies clearly establish a physiological role of the ECS towards the regulation of excitatory synaptic transmission via activation of CB₁ receptors on glutamatergic terminals. Furthermore, the evidence clearly demonstrates that this aspect of the ECS localized to excitatory synapses within corticolimbic brain regions and throughout network pathways involved in evoking high-frequency neuronal synchronous discharges is an essential defense mechanism in the brain that acts to protect against excessive glutamatergic excitatory transmission that occurs in pathophysiological states such as seizures and epilepsy. As an interesting side note, a recently discovered attribute of the select strain of the Amazonian rodent Proechimys, which is resistant to the development of SRSs following pilocarpine-induced SE, is that it has an overall higher level of hippocampal CB₁ receptor expression when compared to the expression patterns in the Wistar rat strain (Araujo et al., 2010).

ALTERATIONS IN THE ECS IN SEIZURES AND EPILEPSY

The discovery and cloning of the CB₁ and CB₂ receptors opened the gates for uncovering the components of the ECS (Niehaus et al., 2007; Piomelli, 2014). These discoveries were followed by the development of highly specific antibodies and pharmacological agents and ligands, which have allowed for ongoing research on roles that each component of the ECS has in both physiological and pathological processes. A number of studies, both experimental and clinical, have utilized these technologies to evaluate alterations in the brain ECS in association with seizures and epilepsy and allowed for a better understanding of the mechanisms that underlie the anticonvulsant/pro-convulsant properties of cannabinoid compounds.

REORGANIZATION OF CB₁ RECEPTOR EXPRESSION AND FUNCTION IN PILOCARPINE-INDUCED TEMPORAL LOBE EPILEPSY

Alterations in the ECS and brain CB₁ receptor expression and function have been demonstrated in a number of experimental models of seizures and epilepsy, which shed light on some of the mechanisms that underlie the regulatory role of the ECS in these pathologies. In rat or mouse, pilocarpine-induced SE is followed by a 2—4
week latency “seizure-free” phase of epileptogenesis during which time changes in neuronal plasticity culminate in a permanent state of altered neuronal hyperexcitability as evidenced by behavioral and electrographic SRSs, which are associated with many of the brain morphological and behavioral characteristics of human temporal lobe epilepsy (TLE) (Turski et al., 1983). Utilizing this model in the rat, DeLorenzo and colleagues were the first to demonstrate a CB1 receptor-dependent role for the ECS towards the tonic regulation of epileptic seizure frequency and duration, whereby the administration of the cannabimimetic WIN 55,212-2 (WIN) suppressed epileptic seizures, while specific blockade of CB1 receptors with SR141617A resulted in a pro-convulsant effect with an increase in seizure durations and frequencies reaching levels comparable to those seen in SE (Wallace et al., 2003) (Figure 6.1). Furthermore, levels of the endocannabinoid 2-AG were increased acutely following seizures, and epileptic animals displayed a long-lasting and permanent change in the distribution of hippocampal CB1 receptor expression.

Additional work in this same model extended upon by Falenski et al. (2007) demonstrated a selective reorganization of hippocampal CB1 receptor expression.

**FIGURE 6.1**

The effects of CB1 receptor modulation on epileptiform activity in control and epileptic rats. (A) Representative EEG recordings of control, epileptic, $S(-)$WIN55,212-, $[(+)\text{WIN}]$-treated (5 mg/kg i.p.) epileptic animals and $R(+(+)\text{WIN})$55,212-, $[(+\text{WIN})]$-treated (5 mg/kg i.p.) epileptic animals. + WIN treatment completely suppressed SRS activity in epileptic rats. (B) Antagonism of CB1 receptors by SR141716A (10 mg/kg i.p.) in epileptic rats caused increased seizure frequency and produced status epilepticus in some animals. The data represent EEG and behavioral seizures observed over the 1-h recording period for epileptic and epileptic + SR conditions. These recordings represent continuous EEG recordings from an epileptic rat 60 min before and 60 min after treatment with SR141716A. Arrows represent individual seizures. The control + SR representative EEG recording demonstrates that treatment of control (non-epileptic) animals with SR141716A did not produce seizure activity.

*Modified with permission from Wallace et al. (2003).*
evidenced by loss in the dentate gyrus inner third molecular layer and stratum pyramidale of CA1−CA3, and a concomitant increase throughout the strata molecularis, radiatum, and oriens of the dentate gyrus and CA1−CA3 regions (Figures 6.2 and 6.3A). Furthermore, the redistribution of hippocampal CB1 receptor expression levels in epileptic animals was linked to corresponding changes in hippocampal CB1 receptor binding and G protein coupling (Figure 6.2). During the latency phase in this rat model of TLE, which lasts for 2−4 weeks following the initial pilocarpine-induced insult of SE, the process of epileptogenesis ensues during which time hippocampal CB1 receptor expression is dramatically reduced within the first week, returning to near control levels by 2 weeks, and expressing the long-lasting pattern of reorganization by 4 weeks (Figure 6.2), at which time the animals start displaying SRSs (Falenski et al., 2009).

**FIGURE 6.2**
Alterations in hippocampal CB1 receptor expression, binding, and function in epileptic rats. (*Left panels*) Pseudo color enhanced staining for CB1 receptor using an N-terminus antibody demonstrates a redistribution of hippocampal receptor levels with marked and significant increases within the stratum oriens (SO) and stratum radiatum (SR), while levels within the CA1 stratum pyramidale (SP) and inner molecular layer (IML) of the dentate gyrus (DG) were significantly reduced when compared to control. Redistribution of hippocampal CB1 receptor expression in epileptic animals was mirrored by concomitant and significant changes in both [3H]-WIN receptor binding analysis (*middle panels*) and WIN-stimulated [35S]-GTPγS binding analysis (*right panels*).

*Modified with permission from Falenski et al. (2007).*
FIGURE 6.3
Alterations of CB₁ receptor expression in rodent and human temporal lobe epilepsy (TLE) hippocampus. (A) Immunohistochemical analysis using a C-terminus antibody to CB₁ receptor in TLE rat hippocampus showing a redistribution of CB₁ receptors with increases within the strata radiatum and oriens and dropout in staining within the CA1 stratum pyramidale and the inner third molecular layer of the dentate gyrus (arrows). (B) CB₁ receptor immunohistochemical staining with a C-terminus antibody showing a decrease within the IML in human sclerotic TLE hippocampus. The decrease in CB₁ receptors was confirmed to be occurring exclusively at asymmetric excitatory terminals by ultrastructural analysis (not shown). Scale bars, upper panels 500 μm, lower panels 100 μm. (C) Immunohistochemical analysis of TLE mouse dentate gyrus with an antibody specific for CB₁ receptor staining on symmetric inhibitory terminals showing marked increases within the stratum moleculare of the dentate gyrus DG with postsynaptic targets including both cell bodies and dendrites (arrows—upper and lower right panels, respectively). Scale bars, left panels 200 μm, middle panels 50 μm, right panels 1 μm. (D) Immunohistochemical analysis of sclerotic human TLE dentate gyrus with an antibody specific for CB₁ receptor staining on symmetric inhibitory terminals showing marked increases within the stratum moleculare of the dentate gyrus. Confocal laser scanning analysis revealed a significant increase in CB₁ receptor-positive fibers in epileptic samples (histogram), which was confirmed by ultrastructural analysis to exclusively occur at symmetric inhibitory terminals (lower-right panel). Scale bars, left and middle panels 50 μm, lower-right panel 0.5 μm.

(A) Modified with permission from Falenski et al. (2007). (B) Modified with permission from Ludanyi et al. (2008). (C) and (D) Modified with permission from Magloczky et al. (2010).
Additional work from the DeLorenzo lab using the rat pilocarpine model of TLE employed statistical parametric mapping (SPM), enabling the three-dimensional (3D) reconstruction of levels of whole brain CB₁ receptor binding and G protein coupling in epileptic rats (Figure 6.4). In addition to the earlier reorganization of CB₁ receptors observed within the hippocampus of epileptic animals, this work demonstrates long-lasting alterations in CB₁ receptor binding and G protein coupling throughout the forebrain with selective regional increases in striatum, cortex, and select nuclei of the thalamus (Sayers et al., 2012).

**Figure 6.4**
Statistical parametric mapping (SPM) reveals significant increases in both [³H]WIN binding (left panel) and WIN-stimulated [³⁵S]GTPγS binding (right panel) in discrete forebrain regions of epileptic animals when compared to control ($n = 6$ per group). Representative epileptic coronal, sagittal, and transverse images illustrate the regionally select increases in CB₁ receptor binding and WIN-stimulated [³⁵S]GTPγS binding as demonstrated by colored overlays (red to yellow) that correspond to levels of significance. Fr1-3, frontal cortex, areas 1–3; I, insular cortex; Cg1-2, cingulate gyrus, areas 1–2; CPu, caudate putamen; FL, forelimb cortex; S, septum; Par, parietal cortex; HL, hind limb cortex; LD, laterodorsal thalamic nucleus; VLM, ventrolateral/medial thalamic nuclei; Te, temporal cortex, hippocampal area CA3; DLG, dorsal lateral geniculate nucleus; VLG, ventral lateral geniculate nucleus; MG, medial geniculate nucleus; VPM/L, ventral posterolateral/medial thalamic nuclei; SnR, substantia nigra; PAG, periaqueductal gray; Cblm, cerebellum.

*Modified with permission from Sayers et al. (2012).*
The work from these three studies demonstrates a temporal reorganization of brain CB1 receptor expression and function alongside the development and maturation of SRS activity, which likely represents a compensatory mechanism evidenced by the CB1 receptor-dependent regulatory role the ECS has towards suppressing excessive seizure discharge in pilocarpine-induced TLE. Work from Freund and colleagues utilizing the pilocarpine mouse model found that within 2 hours following SE, a pronounced decrease in CB1 receptor staining was observed throughout the hippocampus and occurred on both symmetrical and asymmetrical terminals, while at days 1 and 3, levels for CB1 receptors returned to control and then slightly increased, respectively (Karlocai et al., 2011). By 1 month following SE, patterns for hippocampal CB1 receptor expression demonstrated an overall increase throughout the stratum moleculare and select increases around surviving sclerotic regions of CA1. The increases in CB1 receptor stain were confirmed by EM to occur on both inhibitory and excitatory terminals, with symmetric terminals displaying an increase in the number of receptors per terminal. Utilizing an antibody that specifically labeled CB1 receptors at inhibitory terminals, additional findings from this group demonstrated increases of CB1 receptors within the dentate gyrus stratum moleculare including a marked increase in the inner third molecular layer (IML) (Figure 6.3C), and also preservation of interneuronal somatic staining in the CA1 and dentate gyrus regions (Magloczky et al., 2010).

Studies in the pilocarpine mouse model carried out by Bhaskaran and Smith (2010a) demonstrated an increased frequency of EPSPs that was sensitive to suppression by CB1 receptor activation via decreasing release of glutamate from presynaptic terminals, while these observations were not present in control tissue. Western analysis revealed a significant increase in CB1 receptor protein levels in the dentate gyrus of epileptic animals, which likely underlies the increased sensitivity of the enhanced EPSPs to CB1 receptor agonists. In light of the above studies, several papers from Houser and colleagues demonstrated in pilocarpine-TLE mice that cholecystokinin/CB1 receptor-positive terminals innervating CA1 and the IML of the dentate gyrus were markedly reduced while increased innervations were observed on glutamatergic spines throughout the strata radiatum and oriens (Wyeth et al., 2010, 2012).

OTHER EXPERIMENTAL FINDINGS

In a model of febrile-induced seizures (HT) in P10 rat pups, Soltesz and colleagues utilized hippocampal slice electrophysiology to demonstrate that HT resulted in a long-lasting (5 weeks post-HT) increase in CB1 receptor-dependent DSI in CA1, as well as in the emergence of a novel CB1 receptor-dependent DSI in the dentate gyrus that was not detected in control slices (Chen et al., 2003). In association with the enhanced DSI findings, immunoblot, light, and EM analysis revealed that CB1 receptor protein levels were significantly increased throughout the hippocampus and shown to be selectively up-regulated throughout the.

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molecular layers on cholecystokinin (CCK)-positive terminals, which were the result of increased CB1 receptor levels and not newly sprouted processes. In rats, Bojnik et al. (2012) demonstrated an increase in anandamide-stimulated GTP\(\gamma\)S binding in hippocampal and cortical membranes occurring three hours following KA-induced seizures, with corresponding increases in mRNA levels for CB1 receptor and CRIP1a; CRIP1a is the cannabinoid receptor interacting protein that has been shown to modulate CB1 receptor activity (Niehaus et al., 2007). In a paper by Friedman and colleagues, juvenile rat pups (second to third week postnatal) demonstrated a dose-dependent effect of WIN against KA-induced seizures in that 0.5 and 1.0 mg/kg WIN showed protection both behaviorally and morphologically 3 days post-insult, while a higher dose of 5 mg/kg WIN displayed seizure scores and hippocampal neuronal cell loss comparable to findings in control KA animals (no WIN) (Rudenko et al., 2012). In agreement with the above studies, CB1 receptor staining was elevated in hippocampal stratum radiatum, stratum oriens, and the IML of the dentate gyrus in the control KA seizure group, while it was unchanged in animals that received 0.5 and 1.0 mg/kg WIN and decreased/down-regulated in the animals administered 5.0 mg/kg WIN, with or without KA (control no seizure), suggesting that juvenile rats may have an increased sensitivity to CB1 receptor agonist-induced receptor desensitization/down-regulation.

In a genetic model of absence seizures in the rat (WAG/Rij), it is suggested that an enhanced GABAergic tone in the ventrobasal thalamic nuclei (VBTN) contributes to the generation of spike-wave discharges (SWDs) within the thalamic—cortical—thalamic network, which underlie absence seizures. In situ hybridization and immunoblot analysis revealed significant decreases in CB1 receptor mRNA and protein levels in the reticular thalamic and the ventrobasal thalamic nuclei regions in symptomatic WAG/Rij rats, which may contribute to a decrease in DSI resulting in enhanced GABAergic tone in these regions (van Rijn et al., 2010).

Homer proteins are localized at the postsynaptic density where they act as a scaffolding network that couples to and regulates selected target proteins involved in many levels of synaptic plasticity, including protein components involved in endocannabinoid production. A small alternatively sliced isoform of the Homer family, Homer 1a (H1a), acts to modulate postsynaptic function through uncoupling/deregulating of target proteins from the Homer scaffolding network, and has been shown to have increased expression following a number of neuronal processes that include long-term potentiation (LTP) and increased levels of BDNF (Worley et al., 2007). Thayer and colleagues have demonstrated that transfection of an expression construct for H1a in hippocampal neuronal cultures acts to decrease metabotropic-induced suppression of excitation (MSE) and enhance DSE, both of which were dependent on CB1 receptor activation and likely attributed to H1a acting to decrease or increase 2-AG synthesis, respectively (Roloff et al., 2010). To test this effect, under more physiological conditions, they showed that addition of BDNF to hippocampal cultures resulted in a 32-fold increase in H1a mRNA expression and a significant suppression and enhancement of MSE and DSE, respectively. A second study in the same hippocampal culture
preparation demonstrated that epileptiform seizures induced by bicuculline +
4-aminopyridine (4-AP) resulted in a group I mGluR-dependent increase in
expression of H1a and decrease in MSE. Furthermore, 4 hours of bicuculline +
4-AP-induced epileptiform activity occluded mGluR5 activation-induced
IP3-sensitive Ca\(^{2+}\) mobilization. They speculate that H1a regulation of CB\(_1\)
receptor-mediated MSE and DSE may contribute to neuronal plasticity changes
and protective mechanisms, respectively (Li et al., 2012). In support of the above
findings, increased H1a expression following pilocarpine-induced status epilepti-
cus in the rat occurs and is thought to act as a countermeasure against neuronal
hyperexcitability (Cavarsan et al., 2012).

### ALTERATIONS IN THE ECS IN HUMAN TEMPORAL LOBE EPILEPSY

In clinical epilepsy research, PET scan imaging studies, evaluation of surgically
resected or postmortem hippocampal tissue, and analysis of cerebrospinal fluid
(CSF) indicate a number of alterations in the ECS in association with the patho-
physiological state of TLE in humans. Using the \([^{18}\text{F}]\)-MK9470-specific marker
for CB\(_1\) receptors, PET scan imaging in patients with mesial TLE with hippocam-
pal sclerosis revealed an increase in CB\(_1\) receptor availability in the ipsilateral
hemisphere of the TLE zone, as well as a decrease in the superior insular cortex
both ipsilateral and contralateral to the epileptic focus when compared to controls.
The increase in CB\(_1\) receptors was directly correlated to the number of seizures
and inversely correlated to seizure latency in the month prior to PET imaging
(Goffin et al., 2011). Analysis of CSF from patients with newly diagnosed TLE
who had not started AED therapy showed that levels for the endocannabinoid
anandamide were significantly elevated compared to controls, while no differ-
ences in levels for 2-AG were found (Romigi et al., 2010).

Utilizing quantitative polymerase chain reaction (qPCR), and immunohisto-
chemical and EM analysis of human surgically resected or postmortem tissue from
controls and patients with intractable TLE with either sclerotic or non-sclerotic hip-
 pocampal pathology, Katona and colleagues demonstrated a significant overall
decrease in CB\(_1\) receptor mRNA and protein levels in both sclerotic and non-
sclerotic hippocampal tissue, with the exception of a modest increase in receptor
staining in stratum oriens of the CA2—CA3 subfields in sclerotic samples (Ludanyi
et al., 2008). Staining analysis revealed a marked decrease in CB\(_1\) receptors within
the IML of the dentate gyrus (Figure 6.3B) and ultrastructural EM analysis
revealed that these losses in receptor levels within the IML occurred exclusively on
 glutamatergic terminals, while no change in receptor levels was observed on sym-
metric GABAergic terminals. Additionally, mRNA levels for the cannabinoid
receptor interacting protein (CRIP1a) and the 2-AG synthesizing enzyme DGL-\(\alpha\)
were significantly decreased in sclerotic tissue, while levels for DGL-\(\beta\), and for
NAPE-PLD and FAAH (anandamide synthesizing and degrading enzymes, respectively), and MAGL (2-AG degrading enzyme) showed no change from controls.

Freund and colleagues utilized an antibody that exclusively stained for CB₁ receptor on GABAergic terminals and compared expression patterns at the light and EM levels in human hippocampal tissue from control and patients with intractable TLE (Magloczky et al., 2010). In comparison to the loss of CB₁ receptors at glutamatergic terminals in the above paper, the results from this study demonstrated a significant increase in CB₁ receptor staining on inhibitory GABAergic terminals within the stratum molecularis of the dentate gyrus as well as in the interneuronal somata throughout the CA1 region and dentate gyrus (Figure 6.3D).

In agreement with findings in experimental models of seizure and epilepsy, the human data presented above demonstrate alterations in a number of components of the ECS as well as a reorganization of the hippocampal CB₁ receptor at inhibitory and excitatory terminals. Such a redistribution of receptors in human TLE hippocampus would result in a shift in the CB₁ receptor-dependent regulation of synaptic transmission that would increase at GABAergic (DSI) and decrease at glutamatergic (DSE) terminals, a scenario that would be expected to contribute to epileptic seizure initiation.

**DO ALTERATIONS IN CB₁ RECEPTORS WITHIN THE IML OF THE DENTATE GYRUS CONTRIBUTE TO TLE?**

In the hippocampus, the presence of an excitatory network termed “trisynaptic pathway” involves incoming excitatory projections (perforant path) from the entorhinal cortex onto the dentate granule cells, which then project terminals to innervate the CA3 pyramidal cells that send axons onto the CA1 pyramidal cells via the Schaffer collaterals. Ultimately, the CA1 pyramidal cells project to the subiculum, whereupon the main hippocampal output returns to the entorhinal cortex completing the loop (Figure 6.5). The hippocampus is a limbic structure that is primed to generate seizure discharges largely due to intrinsic properties of CA3 pyramidal neurons that have a low threshold for excitatory discharges due to their extensive interconnectivity with neighboring CA3 cells. Regulation of this intrinsic seizure-prone excitatory hippocampal circuit is, in part, mediated by dentate granule cells (McNamara, 1999). Observed in temporal lobe epilepsy, in both humans and animal models, is a rewiring of the hippocampal network as evidenced by a process termed “mossy fiber sprouting” (MFS), whereby dentate granule cell mossy fiber terminals are redirected back onto neighboring granule cell dendrites within the IML of the dentate gyrus (Figure 6.5) (Sutula et al., 1988; Represa et al., 1993; Okazaki et al., 1995). There has been substantial debate as to the functionality of MFS having either a cause or effect role in the generation of epileptic seizures, although recent data suggest the former.
FIGURE 6.5

Diagrams of hippocampal network and working model of altered endocannabinoid-mediated regulation of synaptic transmission within the IML of epileptic DG. (*Upper diagram*) The tri-synaptic hippocampal circuit begins with input from the entorhinal cortex (EC) onto the dentate gyrus (DG) and CA3 pyramidal cells via the perforant path (PP) consisting of both lateral (LPP) and medial (MPP) projections. Mossy fibers project from DG cells onto CA3 pyramidal cells, which then send projections via the Schaffer collateral (SC) and associated commissural (AC) pathways onto the ipsilateral and contralateral CA1 pyramidal cells, respectively. CA1 cells can also receive input from the PP and send their axonal projections to the subiculum (Sb), which then send the primary hippocampal output back to the EC. (*Lower diagrams*) Diagrams of proposed working model of alterations within the IML of the DG in TLE. Both experimental and clinical TLE are associated with loss of vulnerable hilar cells and a rewiring of DG mossy fibers projecting back onto DG cell dendrites within the IML. Additionally, TLE is associated with increased innervations of the IML by inhibitory symmetric terminals possibly arising from GABAergic basket cells within the DG. A shift of CB$_1$ receptor levels from excitatory asymmetric terminals to inhibitory symmetric terminals occurs in both experimental and clinical TLE. Such a change of CB$_1$ receptor distribution on presynaptic terminals within the IML would be expected to decrease DSE and increase DSI, resulting in an overall increase in excitatory transmission at this point within the epileptic hippocampal network.
Houser et al. (2012) have demonstrated with EM analysis of MFS in human TLE a dramatic increase in complexity of projecting terminals at asymmetric excitatory synapses, which present with ultrastructural characteristics indicative of functional synapses. Additionally, in TLE mice, induced expression of the activity-related markers Fos and phosphorylated-ERK in the dentate gyrus following spontaneous seizures indicates that the maladaptive MFS within the IML is functional and, thus, likely has a contributory role in the generation of epileptic seizures. As reviewed in the previous section, conditional knockout mice (GABA-CB1<sup>−/−</sup>) that expressed CB<sub>1</sub> receptor only on glutamatergic terminals that form asymmetric excitatory synapses demonstrated that the highest level of hippocampal expression was within the IML of the dentate gyrus (Monory et al., 2006). Findings reviewed above in human TLE hippocampus demonstrate a dramatic loss of CB<sub>1</sub> receptors on asymmetric excitatory terminals within the IML of the dentate gyrus (Figure 6.3B) (Ludanyi et al., 2008). In agreement with these clinical findings, a long-term dropout in CB<sub>1</sub> receptor expression within the IML has also been observed in the rat pilocarpine model of TLE, while receptor expression is increased in the strata radiatum and oriens (Figure 6.3A) (Falenski et al., 2007). In contrast, CB<sub>1</sub> receptor levels on GABAergic symmetric terminals were observed to be increased within the IML of the dentate gyrus in both human tissue and a mouse model of TLE (Figure 6.3C and D) (Magloczky et al., 2010). Thus, in the TLE hippocampus, a rewiring of dentate gyrus cell mossy fiber axon terminals within the IML may contribute to a state of hyperexcitability by undermining a regulatory circuit responsible for governing the intrinsic seizure-prone state of the hippocampal/CA3 network, while CB<sub>1</sub> receptor-dependent regulation of synaptic transmission is redistributed from glutamatergic terminals (decreased DSE) and increased at GABAergic terminals (increased DSI) within the IML of the epileptic dentate gyrus (Figure 6.5).

Although all of the neuroplasticity changes associated with epileptogenesis and the development of SRSs are most likely not exclusively mediated by alterations in the ECS alone, the combination of MFS, increased excitatory transmission, and the maladaptive shifting of CB<sub>1</sub> receptor-dependent regulation of synaptic transmission within IML of the dentate gyrus represents a “perfect storm” scenario, which may possibly contribute to a pathophysiological state that would favor initiation of SRS discharge. This scenario would support a pro-convulsant effect of CB<sub>1</sub> receptor agonism, which is in disagreement with a considerable amount of experimental findings demonstrating CB<sub>1</sub> receptor-dependent anticonvulsant effects. A resolution to this discrepancy could be the up-regulation of CB<sub>1</sub> receptors observed throughout the CA1–CA3 strata radiatum and oriens regions, which may act to suppress/block spreading seizure discharges originating upstream in the hippocampal circuit. Further studies to evaluate the functionality of CB<sub>1</sub> receptor-dependent regulation/dysregulation of synaptic transmission within the IML of the epileptic hippocampus are warranted.
THERAPEUTIC POTENTIAL OF MODULATING THE ECS IN SEIZURES AND EPILEPSY

THE PHYTOCANNABINOIDS

The use of *Cannabis sativa* medicinally can be historically dated as far back as the second millennium BC (Mechoulam and Parker, 2013). The first formal publication on the therapeutic potential of *Cannabis*, which included its anticonvulsant properties, was presented by William B. O’Shaughnessy in 1842 (O’Shaughnessey, 1842). A major breakthrough in *Cannabis* research was the isolation and synthesis of the major psychoactive constituent Δ⁹-tetrahydrocannabinol (Δ⁹-THC) by Mechoulam and Gaoni in the 1960s, which was the starting point in a new era of cannabinoid research towards elucidating the biological effects of the constituent compounds and ultimately revealing the existence of the ECS (Mechoulam and Gaoni, 1965). Of the 421 constituents found in *Cannabis sativa* (Turner et al., 1980), 80 fall within the classification of phytocannabinoids, of which Δ⁹-THC is primarily responsible for the central psychotropic effects via activation of CB₁ receptors (Izzo et al., 2009). Earlier studies found that Δ⁹-THC possessed anticonvulsant properties characteristic of certain classical AEDs as indicated by its effectiveness against both acute and epileptic seizures (Lemberger, 1980). The anticonvulsant activity of Δ⁹-THC has been demonstrated in the maximal electro shock (MES), audiogenic, and pentylentetrazole (PTZ) seizure tests, although adverse effects of psychotoxicity and CNS excitation (Consroe and Wolkin, 1977; Karler and Turkanis, 1981), as well as the neuronal hyperexcitability following withdrawal (Karler and Turkanis, 1980), limit the clinical efficacy of this cannabinoid in seizure disorders. Additionally, contrary to the suppressive effects of Δ⁹-THC in some models of seizure, proconvulsant effects were observed in rabbits (Martin and Consroe, 1976) and beagles (see commentary: Feeney, 1977). In light of the above experimental findings with Δ⁹-THC, several clinical reports have presented seizure exacerbation in patients with focal epilepsy following withdrawal from self-medicating with cannabinoids (Hegde et al., 2012) and an incidence of first-ever seizures in multiple sclerosis patients undergoing a trial with long-term administration of cannabinoid-based therapy for control of spasticity (Wade et al., 2006).

Another major phytocannabinoid present in *Cannabis sativa* is the nonpsychotropic constituent cannabidiol (CBD), which has shown substantial promise for its therapeutic potential in control of seizures and epilepsy. Earlier experimental studies in seizure models indicated that CBD demonstrated anticonvulsant activity against tonic–clonic-type seizures and enhanced the effect of the classical AED phenytoin in an audiogenic seizure model, while having a negative effect when co-administered with the AEDs clonazepam, chlordiazepoxide, and trimethadione (Consroe and Wolkin, 1977). Karler and Turkanis (1981) carried out a comparative evaluation of CBD, Δ⁹-THC, phenytoin, and ethosuximide in both MES and PTZ models of seizure and found that CBD demonstrated effective...
suppression of seizures, lacked neurotoxicity and the development of tolerance, and had potential therapeutic efficacy comparable to that of classical AEDs in controlling seizures in grand mal, cortical focal, and complex partial epilepsies. In light of these earlier findings, the only clinical trial for CBD as an antiepileptic agent was performed in 1980 by Mechoulam and colleagues in epileptic patients who had maintained little control of their disease with classical AEDs. The authors demonstrated a positive response to prolonged (4½ months) CBD treatment, with 50% of patients being seizure free and 38% having partial improvement (Cunha et al., 1980); none of the patients or healthy volunteers receiving CBD showed any adverse effect from the treatment regimen.

In recent years, a number of studies utilizing both *in vivo* and *in vitro* models of seizure and epilepsy have further confirmed a therapeutic potential for CBD (Wallace et al., 2001; Jones et al., 2010, 2012; Shirazi-zand et al., 2013) in addition to the non-psychototropic phytocannabinoids cannabidivarin (CBDV) (Hill et al., 2012a, 2013) and Δ⁹-tetrahydrocannabivarin (THCV) (Hill et al., 2010) as anticonvulsant agents, and have determined that their mechanism of action is unlikely via CB₁ receptor modulation, but may involve mechanisms regulating Ca²⁺ (Jones et al., 2010; Shirazi-zand et al., 2013). Analysis of CBD’s many pharmacological effects over the years has shed light on a number of underlying mechanisms that may contribute to its seizure suppressive properties and include antioxidant effects, and inhibition of AEA degradation and reuptake, as well as adenosine reuptake, regulation of mitochondrial-dependent Ca²⁺ homeostasis and inhibition of T-type Ca²⁺ channels, and direct modulation/activation of transient receptor potential (TRP) ion channels, G protein-coupled receptors 55 (GRP55), and serotonin 5HT₁A receptors (Hill et al., 2012b; Mechoulam et al., 2007). A paper presenting findings from a survey of parents of children with treatment-resistant epilepsies who had failed to respond, on average, to 12 different AEDs and chose to self-medicate with CBD-enriched cannabis-based medicines (CBMs), found that of 19 families that satisfied the study criteria, two (11%) were seizure free, eight (42%) had an 80% reduction of their seizures, and six (32%) had a 20–60% suppression of seizures as a result of implementing CBD-enriched CBM therapy (Porter and Jacobson, 2013). Although this somewhat subjective survey shows promise with CBD-based therapies in this population of refractory epilepsy, in conclusion the authors voice concern over possible risks to patients resulting from the lack of regulation and quality control in the production and distribution of available CBMs, and the need for objective clinical trials to thoroughly assess the therapeutic efficacy of CBD or other CBMs in seizure and epilepsy (Porter and Jacobson, 2013). This latter concern is further substantiated by Gloss and Vickrey (2012) that ran an extensive database and literature search of clinical studies, case reports, and meeting proceedings, as well as consulting directly with drug manufacturers on the use of CBMs for the treatment of epilepsy, which concluded that at this time no reliable determinations can be made on the use of CBMs for the clinical treatment of seizures and epilepsy, underscoring the need for properly designed clinical trials with sufficient power.
TARGETING CB₁ RECEPTORS IN SEIZURES AND EPILEPSY

There have been an ever-growing number of studies in experimental models of seizures and epilepsy demonstrating the anticonvulsant properties of cannabinoids (Lemberger, 1980; Karler and Turkanis, 1981), and it was not until the discovery of the CB₁ receptor, its endogenous ligands, and the enzymes involved in their synthesis, uptake, and degradation, that subsequent development of highly specific pharmacological agents to target the ECS allowed for elucidating the anticonvulsant potential of CB₁ receptor modulation. Wallace et al. (2001) were the first to demonstrate a CB₁ receptor-dependent anticonvulsant effect of Δ⁹-THC and WIN in the mouse MES seizure model of partial seizures with secondary generalization as evidenced by their anti-seizure actions being blocked with the antagonist SR141617. Additional work from this group in the MES model demonstrated anticonvulsant activity of anandamide and its metabolically stable analogue O-1812, as well as a significant decrease in MES-induced seizure threshold with SR141617 alone, indicating a role for the ECS towards regulating seizure discharge through a CB₁ receptor-dependent pathway (Wallace et al., 2002). An earlier study in the mouse MES model demonstrated that the endogenous fatty acid ethanolamide N-palmitoylethanolamide (PEA) was protective in this model of partial seizures (Lambert et al., 2001), which may be acting as an anticonvulsant indirectly through CB₁ receptors (Citraro et al., 2013b) and will be discussed in more detail below. In the maximal dentate activation rat model of limbic partial complex seizures (Stringer and Lothman, 1990), where an electrical stimulus to the dentate gyrus results in the onset of an after-discharge response, Sardo and colleagues showed that pre-administration of WIN resulted in an increase in latency to onset and a decrease in duration of after discharge (Rizzo et al., 2009). The suppressive effect of WIN could be blocked by AM251, indicating a CB₁ receptor-dependent mechanism, while AM251 administered alone had no effect on the parameters after discharge, suggesting the lack of an endogenous endocannabinoid tone in regulation of the MDA-induced response.

In the rat pilocarpine model of TLE, DeLorenzo and colleagues demonstrated that both Δ⁹-THC and WIN administration suppressed epileptic seizure activity while antagonism of CB₁ receptors with SR141617 resulted in increased durations and frequency of seizures reaching levels comparable to those found with clinical SE (Wallace et al., 2003) (Figure 6.1). These findings not only demonstrate the anticonvulsant properties of exogenous cannabinoids in this model of TLE, but also reveal a possible compensatory shift in CB₁ receptor-dependent endogenous tone that may be attributed to a redistribution of hippocampal CB₁ receptors observed in a number of studies discussed earlier in this chapter (Wallace et al., 2003; Falenski et al., 2007, 2009; Ludanyi et al., 2008; Magloczky et al., 2010; Karlocai et al., 2011; Sayers et al., 2012). A study by Bhaskaran and Smith (2010a) carried out an extensive electrophysiological analysis of excitatory synaptic transmission in hippocampal slices from control and pilocarpine-induced TLE mice, and demonstrated CB₁ receptor-dependent suppression of EPSP frequency in epilepsy slices that was
attributed to a suppression of miniature EPSPs indicating the presynaptic regulation of excitatory neurotransmitter release. Additionally, CB₁ receptor expression levels in the dentate gyrus region were shown to be elevated in epilepsy tissue, which was associated with a decrease in dentate gyrus stimulated-induced after-discharge threshold that could be blocked by anandamide and WIN in a CB₁ receptor-dependent fashion. In an amygdala kindling model in the rat, WIN (500 μg/rat, i.c.v.) was shown to significantly suppress after-discharge and seizure durations and increase latency of stage 4 kindled seizures (Naderi et al., 2012), while findings from Wendt et al. (2011) in a mouse kindling model of TLE indicated that WIN and the FAAH enzyme inhibitor URB597 had no anticonvulsant activity in the fully kindled state. The discrepancy between the outcomes of these two studies may result from differences in species, kindling paradigm, or route of administration. An additional finding from the above study in the mouse kindling model of TLE was that daily administration of WIN early on during the kindling (acquisition) phase of this model resulted in an increase in threshold for stimulus-induced seizure and a decrease in seizure severity when compared to kindled controls (no WIN) (Wendt et al., 2011), which is in agreement with findings from the 1970s and 1980s showing that a number of cannabinoids and levonantradol were ineffective at suppressing fully kindled seizures in rats and cats, but showed some promise for blocking the development of the kindling effect and also in the possible adjunctive therapy with classical AEDs (Wada et al., 1975; Corcoran et al., 1978; Ehlers et al., 1981).

In absence epilepsy, a hallmark characteristic of the EEG is SWDs, which are driven by a pathophysiological synchronization of activity within the thalamocortical circuit, which involves the ventrobasal thalamic nuclei, somatosensory cortex, and the reticular thalamic nucleus (Blumenfeld, 2005). In the WAG/Rij genetic rat model of absence epilepsy, which expresses spontaneous absence-like seizures, Ngomba and colleagues demonstrated significant decreases in CB₁ receptor protein levels within the reticular thalamic nucleus (RTN) and VBTN of symptomatic WAG/Rij rats, and administration of WIN was anticonvulsant via a CB₁ receptor-dependent mechanism (van Rijn et al., 2010). They propose the decrease in CB₁ receptor expression is in GABAergic interneurons localized within the RTN that project their terminals into the VBTN, and this loss in endocannabinoid-mediated presynaptic regulation of these inhibitory inputs contributes to an increased GABAergic tone within the VBTN, which has been shown to underlie SWD initiation by McCormick and Bal (1997). De Sarro and colleagues (LoVerme et al., 2005) have presented findings from two papers in the WAG/Rij model which indicate a role for PEA and endogenous activation of CB₁ receptors towards suppression of SWDs. In symptomatic WAG/Rij rats, SWD activity was suppressed by administration of AEA or PEA, this latter functioning through activation of the nuclear receptor peroxisome proliferator activated receptor-α (PPAR-α) or inhibition of FAAH resulting in increased AEA levels (Lambert and Di Marzo, 1999). The anticonvulsant effect of PEA could be blocked by antagonists for either CB₁ (SR141617) or PPAR-α (GW6471) receptors, while the AEA suppression of SWDs was only sensitive to CB₁ receptor
antagonism (Citraro et al., 2013b). These findings demonstrate that PEA can act to suppress SWDs in WAG/Rij rats, which likely occurs through either direct activation of PPAR-α or indirectly through CB1 receptor-dependent mechanisms. A second study in the WAG/Rij model from this group demonstrated that specifically localized stereotactic administration of the CB1 receptor agonists WIN or AEA within select targets throughout the thalamocortical circuit resulted in suppression of SWDs, while injection of the antagonist SR141617 exacerbated SWD activity only when administered into the ventroposteromedial thalamic nuclei (Citraro et al., 2013a). The above findings in the WAG/Rij rat model of absence epilepsy allow for a better understanding of how maladaptive alterations in the ECS may underlie SWD generation and may lead to future research studies to develop potential therapeutic strategies of targeting the ECS in this condition.

In addition to the findings of CB1 receptor-dependent anticonvulsant properties of cannabinoids in the mouse MES model reviewed above, a number of studies have demonstrated the seizure suppressing property of CB1 receptor activation in other acute seizure models. In PTZ induced seizures, a model for generalized absence and myoclonic seizures (White, 1997; Loscher, 2011), earlier studies found that the anticonvulsant potential of Δ9-THC was limited as a result of psychotoxicity and lethality (Lemberger, 1980; Karler and Turkanis, 1981). In more recent years, a number of studies have employed acute seizure models to demonstrate CB1 receptor-dependent anticonvulsant properties of select agonists, which include WIN, ACEA, and AEA in the PTZ model (Shafaroodi et al., 2004; Gholizadeh et al., 2007; Bahremand et al., 2009; Naderi et al., 2011, 2012; Andres-Mach et al., 2012; Vilela et al., 2013), ACEA in penicillin-induced focal seizures (Kozan et al., 2009; Cakil et al., 2011; Arslan et al., 2013), and WIN in KA-induced seizures (Rudenko et al., 2012). More in-depth discussions of the use of these models to evaluate CB1 receptor modulation of seizure activity in regard to CB1 receptor agonists as adjunctive AED therapy, and also the interaction of the ECS with other neuronal systems towards seizure, will be addressed further in this chapter.

Primary hippocampal neuronal cultures exposed to low-Mg$^{2+}$ conditions result in the initiation of high-frequency and unremitting burst activity similar to that in clinical SE, and replacement of Mg$^{2+}$ in the culture media following 3 hours of low-Mg$^{2+}$-induced SE results in the development of a permanent hyperexcitable state in the hippocampal cultures evidenced by the presence of spontaneous recurrent epileptiform discharges (SREDs) (Sombati and DeLorenzo, 1995). WIN has been demonstrated to have anticonvulsant activity by suppressing both low-Mg$^{2+}$-induced SE and SREDs in a CB1 receptor-dependent manner (Blair et al., 2006). Additionally, the endocannabinoids 2-AG and methanandamide blocked low-Mg$^{2+}$-induced SE-like activity in this hippocampal culture model in a concentration-dependent manner (Deshpande et al., 2007b). Furthermore, in the low-Mg$^{2+}$-induced SE model, WIN maintained anticonvulsant potency while the conventional AED agent lorazepam developed pharmacoresistance to its anticonvulsant activity (Deshpande et al., 2007a). The presence of a CB1 receptor-dependent endogenous tone towards the regulation of SRED activity following low-Mg$^{2+}$ treatment was evident in that
antagonism of the CB₁ receptor with either SR141617 or AM251 resulted in exacerbation of seizure activity, reaching high-frequency SE-like activity (Deshpande et al., 2007c). Low-Mg²⁺-induced SE in this preparation has been shown to result in a loss of Ca²⁺ homeostasis (Pal et al., 1999), which is required for the permanent expression of SRED activity in hippocampal cultures (DeLorenzo et al., 1998). Thayer and colleagues have demonstrated in a similar hippocampal culture preparation that CB₁ receptor agonists suppress glutamatergic-driven Ca²⁺ spikes through an inhibitory G protein-dependent blockade of presynaptic glutamate release (Shen et al., 1996). In view of these findings, it is likely that a primary mechanism underlying the anticonvulsant properties of CB₁ receptor activation in low-Mg²⁺-induced SE and SREDs in hippocampal cultures, as well as the tonic CB₁ receptor-dependent regulation of epileptiform activity, involves suppression of glutamatergic-driven Ca²⁺ spikes.

CB₁ RECEPTOR AGONISTS IN ADJUNCTIVE AED THERAPY

The above review of research findings, which support a role for the anticonvulsant properties of CB₁ receptor activation, are very convincing and underscore the function of the ECS towards the regulation of both physiological and pathophysiological neuronal synaptic transmission. Although there appears to be a great potential for cannabinoids as anticonvulsant agents, their therapeutic application for seizure control is limited by both the presence of psychotropic/psychotoxic effects and the development of pharmacological tolerance. Thus, a number of studies have researched the application of cannabinoids in adjunctive therapy strategies for seizure control. Two studies utilizing both the PTZ and MES mouse models by Czuczwar and colleagues have generated a number of papers on the adjunctive effect of cannabinoids with classical and second-generation AEDs (Luszczki et al., 2006, 2010). In two papers using the mouse MES model of partial seizures, the highly selective CB₁ receptor agonist arachidonyl-2′-chloroethylamide (ACEA) plus phenylmethylsulfonyl fluoride (PMSF) was evaluated alone or in combination with the AEDs valproate, carbamazepine, lamotrigine, oxcarbazepine, phenobarbital, phenytoin, and topiramate for protection against MES-induced seizures as well as for memory (step-through passive-avoidance task), muscle strength (grip test), motor impairment (chimney test), and brain and free plasma levels of AEDs. At the subeffective dose of ACEA (2.5 mg/kg) + PMSF (30 mg/kg), no changes in MES-induced seizure parameters were observed, while the combination of ACEA + PMSF with the AEDs resulted in an enhancement of both valproate and phenobarbital as indicated by a significant decrease in the ED₅₀ dose required for anticonvulsant activity without changes in adverse effects (strength and memory tests). Analysis of brain and free blood plasma levels of the AEDs revealed an increase in valproate and no change in phenobarbital, demonstrating a pharmacokinetic and pharmacodynamic effect, respectively, with the co-administration of ACEA + PMSF. Thus, the CB₁ receptor agonist ACEA may prove to be an effective adjunctive therapy in combination with phenobarbital for the treatment of clinical partial seizures.
Two additional studies in the MES model from this group evaluated the combinatorial effect of the cannabimimetic WIN on the anticonvulsant efficacy of both classical and second-generation AEDs (Luszczki et al., 2011b, 2013). In a dose–response effect of WIN alone in MES-induced seizures, 15 mg/kg showed some protection while the doses of 2.5, 5, and 10 mg/kg were subeffective; thus, the three lower doses in combination with the four classical AEDs phenytoin, phenobarbital, valproate, and carbamazepine were evaluated for protection against MES-induced seizures as well as for memory, muscle strength, motor impairment, and brain and free plasma levels of AEDs. At the 10 and 5 mg/kg doses, WIN significantly enhanced the anticonvulsant properties (decrease in ED50) of all AEDs, and valproate and carbamazepine, respectively, while having no effect on total brain levels of anti-seizure drugs. Yet, any combination of each AED with either the 5 or 10 mg/kg doses of WIN was not devoid of an increase in acute adverse effects as indicated by alterations in one or more tests (i.e., decrease in muscle strength and memory, or increase in motor impairment), thus occluding any potential for the clinical efficacy of WIN in combination with these AEDs in seizure control (Luszczki et al., 2011b). Conversely, in combination with the second-generation AEDs lamotrigine, oxcarbazepine, pregabalin, and topiramate, WIN 5 mg/kg significantly enhanced the anticonvulsant effects of all AEDs with the exception of oxcarbazepine, without altering total drug brain levels or acute adverse effects. Thus, the researchers conclude that at the lower dose of 5 mg/kg, WIN may have potential benefits in combination with select second-generation AEDs to enhance anticonvulsant properties without increasing the risk of acute adverse effects (Luszczki et al., 2013).

In the PTZ mouse model of generalized absence and myclonic seizures, two studies from the above group demonstrated similar findings that ACEA significantly enhanced the anticonvulsant activity of phenobarbital with no adverse effects (Andres-Mach et al., 2012), while WIN increased anticonvulsant efficacy of classical AEDs but showed no clinical potential due to adverse effects (Luszczki et al., 2011a). Naderi et al. (2008) evaluated the effects of the CB1 receptor agonist WIN, antagonist AM251, the endocannabinoid uptake inhibitor AM404, and the FAAH inhibitor URB597 on the anticonvulsant efficacy of diazepam (DZ) in the mouse MES model. Following determination of the ED50 for protection against MES seizures (equal to 0.43 mg/kg and 1.49 mg/kg for DZ and WIN alone, respectively), the authors demonstrated that a ratio of 3:1 (DZ:WIN) resulted in a synergistic enhancement of anticonvulsant activity, while the ratios of 1:1 and 1:3 resulted in an additive effect only. AM251 and AM404 showed no effects on MES seizures either alone or in combination with DZ. Administration of URB597 alone resulted in significant protection against MES seizures, while it resulted in antagonizing anticonvulsant efficacy when combined with DZ. The researchers conclude that the synergistic increase in protection against MES seizures with the 3:1 ratio of DZ and WIN may prove to be a potential therapeutic strategy and that the antagonistic effects of FAAH inhibition on DZ anticonvulsant efficacy was likely attributed to an increased endocannabinoid tone at GABAergic synapses.
TARGETING ENDOCANNABINOID DEGRADATION FOR SEIZURE CONTROL

Another strategy for exploiting the role of the ECS in the control of the neuronal hyperexcitability associated with seizures and epilepsy is the targeting of degradation mechanisms of AEA and 2-AG to increase endocannabinergic tone within the synapse. In the brain, the actions of the two endocannabinoids, following their on-demand synthesis and release, are rapidly terminated by the degradation enzymes FAAH and MAGL (Piomelli, 2014). Bahr and colleagues have presented findings from three studies using synthetic inhibitors specifically targeted to either FAAH or to both FAAH and MAGL in both in vitro organotypic hippocampal slice culture and in vivo rat models of KA-induced excitotoxicity and seizures (Karanian et al., 2007; Naidoo et al., 2011, 2012). The first two studies evaluated the irreversible and reversible FAAH inhibitors AM374 and AM5206, respectively. Both compounds demonstrated select and potent inhibition of FAAH as indicated by a 2.5 to 4.8-fold increase in brain AEA levels, activation of the MAPK/ERK signaling pathway, or select inhibition of FAAH over MAGL in a fluorometric assay. In KA-treated rats, both AM374 and AM5206 administration resulted in a significant decrease in seizure scores. Western immunoblot and histochemical analysis demonstrated that both AM374 and AM5206 also blocked KA-induced alterations in markers for neuronal degeneration and hippocampal pyramidal cell loss in rat and hippocampal slice cultures, respectively. The CB₁ antagonist AM251 blocked the protective effects of AM374 in the rat, with levels of pyramidal cell loss and alterations in neuronal degeneration markers equal to that of KA-treated alone controls, demonstrating a CB₁ receptor-dependent mechanism involved in the protection afforded FAAH inhibition (Karanian et al., 2007; Naidoo et al., 2011). A third study from this group compared the protective properties of the hydrolase inhibitor AM6701, which demonstrated equipotent inhibition of both FAAH and MAGL, with those of the compound AM6702, which demonstrated 44-fold higher selectivity for inhibition of FAAH over MAGL (Naidoo et al., 2012). Utilizing the in vivo and in vitro models (rat and hippocampal slice culture) and the same experimental strategy of the above studies, both compounds were protective against KA-induced seizures, hippocampal pyramidal cell loss, and excitotoxicity-induced changes in neuronal degeneration markers, although the dual inhibition of both FAAH and MAGL by AM6701 resulted in a higher overall protection than that achieved with the selective FAAH inhibitor AM6702. Additionally, behavioral tests demonstrated that dual inhibition of FAAH and MAGL afforded better protection than selective inhibition of FAAH against KA-induced decreases in performance of balance and coordination.

In acute PTZ-induced seizures in rat and mouse, Naderi et al. (2011, 2012) showed that inhibition of either FAAH or MAGL can confer protection against chemoconvulsant-induced seizures. Interestingly, the FAAH inhibitor URB597 showed protection in the PTZ-treated mouse when systemically administered via the intraperitoneal route, while i.c.v. administration in the rat yielded no protective effects suggesting a possible route of administration or species dependency for protection.
Contrary to the above studies demonstrating a protective/anticonvulsant effect of FAAH inhibition, Cravatt and colleagues (Clement et al., 2003) developed a genetic knockout mouse strain devoid of FAAH [FAAH(−/−)] and with over 10-fold higher levels of brain AEA, and found that these mice demonstrated increased seizure severity following KA or bicuculline. Pre-administration of exogenous AEA in FAAH(−/−) mice resulted in a further exacerbation of seizure activity and increased hippocampal cell loss following KA or bicuculline, which was shown to be CB₁ receptor dependent in bicuculline-treated animals. Several possibilities may explain the conflicting results of the above study with those demonstrating anticonvulsant properties of FAAH inhibition, which may include a compensatory shift of CB-dependent mechanisms favoring regulation of GABAergic synaptic transmission or a possible increase in AEA availability at the transient receptor potential vanilloid type 1 (TRPV1) receptors, which may evoke pro-convulsant actions in FAAH(−/−) mice, the latter of which will be further discussed below. Thus, targeting the endocannabinoid degradation machinery to enhance the anticonvulsant/protective effects of the ECS may prove an effective therapeutic strategy for the control of seizures and epilepsy. Yet, this approach should be considered carefully in that inhibition of both FAAH and MAGL is capable of increasing endocannabinergic tone to levels resulting in CB₁ receptor-dependent behavioral effects comparable to those of exogenously administered cannabinoids such as Δ⁹-THC (Long et al., 2009; Wise et al., 2012).

**CB₁ RECEPTOR ANTAGONISM AS A PROPHYLACTIC TREATMENT FOR SEIZURES AND EPILEPSY**

To this point in this chapter, the findings discussed primarily support a role for CB₁ receptor activation towards anticonvulsant activity, while the opposite is observed for antagonism of this pathway, as indicated by exacerbation of seizure discharges. However, there is evidence that supports a paradoxical strategy for targeting the ECS during the early stages of pathological alterations in neuronal physiology, whereby antagonism of CB₁ receptor may prevent the development of the epileptic phenotype (Armstrong et al., 2009). Soltesz and colleagues carried out electrophysiological analysis of hippocampal slices from rat pups that underwent hyperthermia-induced seizures (febrile seizures) and found an enhancement of DSI that resulted from an increase in the number of CB₁ receptors on terminals of CCK+ GABAergic interneurons (Chen et al., 2003). This maladaptive plasticity of the ECS with febrile seizures was persistent in that both the potentiation of DSI and increased CB₁ receptors on inhibitory terminals were still present 5 weeks following the hyperthermic insult. From these findings, the authors hypothesized that the CB₁ receptor-dependent enhancement of DSI may contribute to the neuronal hyperexcitability following febrile-induced seizures. A single administration of receptor agonist SR141617 either prior to or within 2 minutes following the hyperthermia treatment prevented the enhancement of DSI, up-regulation of CB₁ receptors, and the plasticity observed in febrile seizures.
receptor on GABAergic terminals, and the persistent neuronal hyperexcitability observed in this model of febrile seizures (Chen et al., 2007). Utilizing another brain insult model of long-lasting neuronal hyperexcitability, this same group demonstrated that a single administration of SR141617 within 2 minutes following lateral fluid percussion-induced traumatic brain injury (TBI) in P20 rats prevented the persistent increase in seizure susceptibility associated with this model of brain insult (Echegoyen et al., 2009).

In the DHPG (type I metabotropic receptor agonist) model of epileptiform activity in hippocampal slices, Karr et al. (2010) demonstrated that co-incubation of SR141617 with DHPG blocked the induction of LTD/decrease release probability as well as a shift to epileptiform activity. They propose that DHPG results in a CB1 receptor-dependent suppression of synaptic transmission and changes in membrane excitability leading ultimately to network synchronization. SR141617 had no effect in hippocampal slices with established DHPG-induced epileptiform activity, suggesting that the time window for the CB1 receptor-dependent contribution to, or prophylactic blockade of, neuronal hyperexcitability takes place during the early acquisition phase in this model.

Dudek et al. (2010) evaluated the effect of SR141617 administration in an adult rat model of KA-induced TLE and found that CB1 receptor antagonism immediately following the first KA-induced seizure had no effect on the development of TLE. They concluded that the discrepancy between their findings and those of the Soltesz study may be due to differences of developmental stage, degree of neuronal injury/death, or model differences.

The above studies suggest that the efficacy of utilizing CB1 receptor antagonist in a prophylactic manner to inhibit maladaptive changes in neuronal plasticity leading to a hyperexcitable state shows promise, and may be dependent on several factors that include timing of administration, developmental stage, or the mode of neuronal insult, which may result in either an increased excitatory/excitotoxic synaptic transmission (DSE) or alter inhibitory mechanisms (DSI) driving the pathophysiological network synchronization.

Another strategy for implementing antagonism of CB1 receptors for the control of seizures is supported by experimental findings that have been shown in the opiate (Crain and Shen, 1995; Wang et al., 2005) and endocannabinoid (Paquette et al., 2007) systems, wherein ultra-low doses of antagonists at G protein-coupled receptors can both enhance pharmacological efficacy and block the development of pharmacological tolerance to agonist exposure. The underlying mechanism of this phenomenon is believed to involve the antagonists blocking a small subpopulation of stimulatory Gs protein-coupled receptors that have been shown to mediate the paradoxical (stimulatory) effects with both opioid (Wang et al., 2005) and CB1 receptors (Glass and Felder, 1997; Felder et al., 1998; Hampson et al., 2000; Chen et al., 2010). Thus, this strategy has been investigated in the mouse models of PTZ-induced myoclonic (PTZ i.v. infusion) and grand mal (PTZ systemic administration) seizures, whereby ultra-low doses (pico-/nanogram/kg ranges) of the CB1 receptor antagonist AM251 resulted in an increase in the anticonvulsant potency of
the CB₁ receptor agonist ACEA 100—100,000 fold (Gholizadeh et al., 2007). The therapeutic approach of augmenting the anticonvulsant efficacy of CB₁ receptor agonists via adjunctive treatment with ultra-low doses of antagonists may allow for the development of novel therapeutic strategies that can benefit from maintaining CB₁ receptor-dependent seizure control with lower dose regimens of cannabinoids without the unwanted psychotropic effects or the development of tolerance.

**ECS INTERACTION WITH OTHER BRAIN SYSTEMS IN SEIZURE AND EPILEPSY**

In addition to control of glutamatergic and GABAergic transmission, the ECS interacts at multiple levels with other neuronal signaling systems, which include the nitrergic, opioidergic, noradrenergic, dopaminergic, serotonergic, and cholinergic (Carney et al., 2009; Kano et al., 2009; Welch, 2009; Carvalho et al., 2010; Howlett et al., 2010; Castillo et al., 2012; Ohno-Shosaku et al., 2012). Additionally, many of these signaling systems/transmitters can modulate endocannabinergic tone via binding to their respective Gₙ/₁₁ protein-coupled receptors (Katona and Freund, 2012) and the CB₁ receptor is capable of interacting with an array of associated proteins (Howlett et al., 2010), as well as undergoing heterodimerization with other GPCRs (Rozenfeld et al., 2012) to increase the range of endocannabinoid-mediated physiological/pathophysiological responses. Thus, a number of studies have investigated the role of cross-system interactions with the ECS in regard to regulation/dysregulation of neuronal hyperexcitability and seizure discharge. In the mouse model of PTZ-induced seizure, increase in release of nitric oxide (NO) with L-arginine administration enhanced the anticonvulsant efficacy of the CB₁ receptor agonist ACEA, while inhibition of the nitric oxide synthesizing enzyme (NOS) with either N⁴-G-nitro-L-arginine methyl ester (L-NAME) or 7-nitroindazole (7-NI) resulted in a decrease in anticonvulsant efficacy of ACEA. Additionally, inhibition of NOS enhanced the pro-convulsant effect of the CB₁ receptor antagonist AM251 (Bahremand et al., 2009). Thus, activation of the NO pathway results in a synergistic interaction with the ECS in control of seizure discharge. Using the same experimental design and seizure model, this same group demonstrated that co-administration of the α₂-adrenoreceptor agonist clonidine (0.1 and 0.5 mg/kg), which alone displayed marginal anticonvulsant properties at higher doses (1.0 and 5.0 mg/kg), and the antagonist yohimbine, resulted in suppressing and enhancing the anticonvulsant effect of ACEA, respectively. Additionally, low doses of clonidine resulted in increasing the pro-convulsant effect of CB₁ receptor antagonism with AM251 (Shafarooodi et al., 2013).

In the rat model of PTZ-induced seizures, Naderi et al. (2011) demonstrated that i.c.v. administration of either WIN or the GABA_A agonist isoguvacine (IGN) alone produced anticonvulsant effects, while their combined administration resulted in no anticonvulsant activity. The FAAH inhibitor URB597 alone had no effect on PTZ-induced seizures, but acted to block the anticonvulsant effect
of IGN. The researchers hypothesized that IGN may be canceling out an inhibitory feedback mechanism (self-inhibition) of GABAergic interneurons that requires CB₁ receptor-dependent induction of DSI. This inhibitory feedback mechanism of GABAergic interneurons has been shown to occur in layer V of the rat neocortex, where they project on to the dendrites of glutamatergic pyramidal neurons and regulate the intrinsic excitability of this network (Bacci et al., 2004).

In a hippocampal culture preparation that displays high-frequency spike neuronal discharge activity following withdrawal from the cannabimimetic WIN (1 μM, 24 h), Deshpande et al. (2011) demonstrated that a profound agonist-induced down-regulation of CB₁ receptors was associated with a suppression of GABAergic transmission as a result of decreased GABAₐ receptor channel number, and probably contributed to the observed increase in neuronal excitability in this preparation.

Messer and Levine (2012) utilized a mouse hippocampal slice preparation to demonstrate that the anticonvulsant efficacy of CB₁ receptor agonism was dependent on the conditions used to evoke epileptiform activity, wherein WIN was effective at suppressing low-Mg⁺⁺⁺-induced seizures while ineffective with low-Mg⁺⁺⁺/high K⁺-evoked seizures. The presynaptic GABAₐ receptor antagonist baclofen partially restored WIN’s ability to suppress seizures in the low-Mg⁺⁺⁺/high K⁺ conditions, which suggested that an interaction between presynaptic GPCRs may contribute to their observed results. They conclude that such opposing interactions between CB₁ and GABAₐ receptors towards the control of excitatory synaptic transmission may underlie the paradoxical effects that cannabinoids produce in different models of epilepsy.

An increasing amount of evidence supporting an interaction or “cross-talk” between the opioid and endocannabinoid systems has been generated, which is especially true in regard to systems involved in cognition, reward, dependence, and tolerance (Fattore et al., 2004; Robledo et al., 2008). Additionally, synergistic or antagonistic interactions between the opioid and endocannabinoid systems may be targeted in the development of more efficacious pharmacotherapy in pain management (Welch, 2009) or drug addiction and relapse (Fattore et al., 2007), respectively. The findings from several studies suggest that such approaches may also hold true in the control of seizures. In PTZ-induced clonic seizures in the mouse, the CB₁ receptor agonist ACPA has anticonvulsant activity, whereas the opioid agonist morphine demonstrates biphasic activity being anticonvulsant at low doses (0.5–1.0 mg/kg) and pro-convulsant at higher doses (30 mg/kg). The “cross-talk” between these systems was evident in that CB₁ (AM251) and opioid (naltrexone) receptor antagonists blocked the anticonvulsant activity of low-dose morphine and ACPA, respectively, while the combination of the two agonists was synergistic towards seizure control. Interestingly, the pro-convulsant effect of high-dose morphine (30 mg/kg) was blocked by CB₁ receptor antagonism, suggesting an additional level of interaction between the opioid and endocannabinoid systems in seizure control (Shafaroodi et al., 2004). A more recent study from the same group took the strategy of using an ultra-low dose of receptor antagonists, discussed in the section above, to further evaluate interactions between these two systems in the
mouse PTZ seizure model. Co-administration of an ultra-low dose of the opioid antagonist naltrexone (1–500 pg/kg) significantly potentiated the anticonvulsant effect of the CB1 receptor agonist ACEA, as indicated by a 10–100-fold increase in efficacy. The synergy between naltrexone and ACEA was still dependent on CB1 receptor activation in that the addition of AM251 blocked the anticonvulsant effect (Bahremand et al., 2008). Thus, the above studies suggest that drawing on the interactions between the opioid and endocannabinoid systems may allow for the development of novel therapeutic strategies in seizure control.

The endovanilloid and endocannabinoid systems cross paths in that the endocannabinoid AEA is a full agonist at the TRPV1 channel, a non-selective cation channel that has been termed the “capsaicin receptor” due to its responsiveness to the constituent that is present in hot chili peppers, and that has been characterized to regulate nociception on peptidergic sensory neurons induced by either thermal or chemical stimuli. Additionally, TRPV1 has been demonstrated to reside in the CNS within cortical, cerebellar, olfactory, midbrain, and hindbrain regions (Starowicz et al., 2007).

Two studies a potential role for TRPV1 activation in ECS-mediated regulation/dysregulation of acute and epileptic seizure discharge. Bhaskaran and Smith (2010b) carried out electrophysiological studies in dentate granule cells of hippocampal slices from TLE mice and showed that application of the TRPV1 agonist capsaicin significantly increased EPSP frequency, while having no effect in control slices. Additionally, in TLE slices, AEA was either pro-convulsant or anticonvulsant against increased EPSP frequency when co-incubated with specific antagonists for either CB1 receptor or TRPV1, respectively, and immunoblot analysis revealed a significant increase in TRPV1 levels in the epileptic dentate gyrus. In the mouse model of acute PTZ-induced seizures, Manna and Umathe (2012) demonstrated that i.c.v. administered AEA showed biphasic properties being anti-convulsant at low doses (10, 20, or 40 μg/mouse) and pro-convulsant at high doses (80 or 100 μg/mouse), while TRPV1 antagonism with capsazepine enhanced the seizure suppressive effect of AEA even at the high doses. Dose-dependent increases in endogenous AEA tone with either AM404 or URB597 demonstrated comparable biphasic effects on PTZ seizures to exogenous AEA, as well as enhancement in their protective effects with TRPV1 antagonism at all doses studied. The authors concluded by demonstrating that either TRPV1 agonism (capsaicin) or antagonism (capsazepine) alone resulted in exacerbation of or protection against PTZ-induced seizures, respectively.

Thus, the above studies indicate that either high doses of exogenous AEA or increases of endogenous AEA to levels that would “spill over” to activate the TRPV1 channel would produce pro-convulsant effects, and may explain some of the paradoxical effects of cannabinoids in different seizure models. Interestingly, the increase in seizure susceptibility observed in the abovementioned study in FAAH knockout mice with a greater than 10-fold increase in brain AEA levels (Clement et al., 2003) could be the result of TRPV1 channel activation. In light of these findings, the approach of enhancing endocannabinoid tone, especially that of AEA, as a strategy for suppressing excessive neuronal excitability should be carefully considered.
CANNABINOID-BASED MEDICINES: IMPLICATIONS FOR THEIR PROLONGED USE IN CLINICAL SEIZURES AND EPILEPSY

Evidence put forth in the previous sections supports that the ECS performs as an essential regulator of neuronal synaptic transmission and functions as an unending defense mechanism against excitotoxicity in the brain primarily via activation of CB₁ receptors on asymmetric excitatory terminals. Additionally, over the last decade, the findings from many experimental studies employing a variety of in vivo and in vitro models of seizures and epilepsy clearly indicate that either exogenous cannabinoids or increasing endogenous endocannabinoid tone can suppress seizure activity through activation of presynaptic CB₁ receptors. Although anecdotal data have suggested beneficial effects in the use of CBMs for their anticonvulsant properties in human seizures and epilepsies, to date there is limited scientific evidence suggesting clinical efficacy for this approach. A telephone survey of 136 patients under management by a Canadian tertiary care epilepsy center obtained data on individual levels of marijuana use in association with the state of their clinical condition, as well as each patient’s perception and knowledge of the possible risks/benefits of self-medicating with marijuana (Gross et al., 2004). Although 41% of the surveyed patients had some knowledge of a potential risk/benefit of marijuana use with epilepsy, there were 28 (21%) epileptic patients who were active marijuana users, of which 68% and 54% perceived that self-medication improved epileptic seizure severity and frequency, respectively. The overall findings suggest that marijuana use by epileptics is higher than that of the general population, and is independent of factors such as age, gender, and employment status. In regard to the state of their clinical condition, patients with longer duration of disease and increased seizure frequency were more likely to self-medicate with marijuana, which, although inconclusive, may result from either a tendency to search out alternative treatments for their condition or a possible causal relationship between level of marijuana use and increased seizure frequency (Gross et al., 2004).

A more recent review of cannabinoid use in clinical epilepsy carried out an extensive search of six databases and found that no reliable conclusions can be made regarding the clinical efficacy of cannabinoid use for the management of epilepsy (Gloss and Vickrey, 2012), although a follow-up commentary generally agreed with their findings, but suggested that the non-psychotropic cannabinoid CBD may prove to have some beneficial effects in the treatment of seizure disorders (Miller, 2013).

The subject of CBMs for the treatment of epilepsy and other ailments has gained substantial momentum and positive attention over the last decade, which has led to a number of states within the USA approving their use. Interestingly, there have been isolated case reports that suggest the contrary following prolonged administration of CBMs. In one paper presenting two separate cases of patients with a history of cannabis use, one epileptic patient who was self-medicating with Cannabis was admitted to the epilepsy monitoring unit and
experienced an increase in seizure frequency upon withdrawal from Cannabis (Hegde et al., 2012). The second case involved an undiagnosed patient who had experienced amnesic episodes with loss of consciousness and unexplained injuries over a 2-year period and had a 40-year history of smoking six to eight marijuana cigarettes per day; upon admission to the epilepsy monitoring unit (EMU) and cessation of cannabis use, within 24 hours the patient developed SE consisting of five seizures in a 12-hour period, which were brought under control with anticonvulsant therapy. The patient stated that his experience in the EMU was similar to the amnesic episodes experienced over the previous 2 years (Hegde et al., 2012).

In a clinical trial evaluating long-term use of a CBM (Sativex®) for the treatment of spasticity associated with multiple sclerosis, treatment resulted in subjective symptomatic relief in a majority of patients, although of the 137 patients in the study, four experienced first-ever seizures (Wade et al., 2006). The researchers concluded that this treatment regimen shows promise for symptomatic relief in multiple sclerosis, but further evaluation of the effects of long-term CBM on seizure threshold is warranted.

Prolonged exposure to cannabinoids results in the development of tolerance as indicated by a progressive decrease in their pharmacological efficacy (Martin et al., 2004; Gonzalez et al., 2005), which, in the CNS, is primarily attributed to CB1 receptor desensitization or down-regulation (Sim-Selley, 2003). The development of tolerance in humans, following chronic and heavy cannabis smoking or ingesting high doses of cannabinoids, has been demonstrated both behaviorally (Gorelick et al., 2013) and at the cellular level with a down-regulation of CB1 receptors (Villares, 2007; Hirvonen et al., 2012; Ceccarini et al., 2013). Additionally, upon cessation of using cannabinoids, heavy users who have become dependent on cannabis as defined in the DSM-IV (American Psychiatric Association, 2000) experience a withdrawal syndrome further confirming an adaptation of the ECS in humans with chronic cannabinoid exposure. With the current availability of many strains of Cannabis sativa and cannabinoid-containing edibles that contain potent levels of Δ9-THC, there has been a substantial increase in the number of adolescents and adults being treated for cannabis dependence and withdrawal in the last 10–15 years (Budney and Hughes, 2006). The concern in regard to the current chapter is that unmanaged self-treatment with CBMs by epileptics may lead to the development of tolerance as a result of CB1 receptor adaptation, a scenario that may result in exacerbation of the epileptic condition upon withdrawal. Experimental findings in an in vitro model of epileptiform discharges in hippocampal neuronal cultures demonstrated an acute CB1 receptor-dependent anticonvulsant effect of WIN (Blair et al., 2006), while prolonged exposure resulted in increase in seizure frequency, which was directly correlated to concentration of WIN and level of agonist-induced CB1 receptor down-regulation (Blair et al., 2009). Findings from both in vivo and in vitro preparations of epileptiform activity have demonstrated that antagonism of CB1 receptors with SR141617 results in increased severity of seizure discharge, further confirming the essential role that an intact CB1
receptor pathway has in the management of seizure discharge in the epileptic phenotype (Blair et al., 2009; Deshpande et al., 2007c, 2011; Wallace et al., 2002, 2003). This effect has also been observed in an isolated clinical case in a patient with a history of generalized idiopathic epilepsy who had been seizure free for 20 years, and experienced the recurrence of partial seizures following the initiation of rimonabant therapy for the treatment of obesity; following termination of rimonabant treatment, the seizure activity ceased (Braakman et al., 2009). The development of novel therapeutic strategies aimed at modulating the ECS shows much potential in the management of seizures and epilepsy, as well as treating other neurological disorders, although in light of the above findings, consideration should be made regarding the effects of prolonged cannabinoid exposure and subsequent adaptations of the ECS, which may result in undermining its intrinsic defense properties in the brain.

**CONCLUSION AND FUTURE DIRECTIONS**

The complexity and sensitivity of the brain ECS underlies one of its essential functions to maintain an on-demand fine-tuning of brain-network communication, and is evident in its ability to regulate neuronal transmission both temporally and spatially throughout the CNS. It is within these attributes that the ECS plays a fundamental role in mediating neuronal processes, which underlie learning and memory, anxiety, depression, addiction, appetite and feeding, pain, neuronal excitability, and protection (Kano et al., 2009). Thus, in a pathophysiological state whereby neuronal mechanisms become compromised and hyperexcitability ensues, the ECS is triggered to intervene resulting in the on-demand synthesis and release of lipid-derived transmitters that act to rein in overzealous neuronal discharges much like an operator’s response to a runaway stagecoach. In this sense, the ECS functions in a suppressive manner and could be thought of as the “emergency brakes” of the brain. The intent of this chapter in the previous sections was to briefly summarize the enormous amount of progress made in experimental research over the last two decades towards understanding the brain ECS and more specifically present findings that clearly demonstrate maladaptive changes that occur with this system in association with seizures and epilepsy, and how these alterations may be contributing in either a compensatory or causative manner towards the expression of these neuropathological conditions. To this end, much experimental work has focused on targeting select aspects of the brain ECS as a means for developing novel therapeutic strategies for the treatment of seizure disorders.

The findings reviewed in the previous sections from a multitude of experimental *in vivo* and *in vitro* models of seizure and epilepsy provide clear evidence that activation of presynaptic CB1 receptor suppresses epileptiform activity. The actions of many experimental cannabimimetics, as well as the primary natural constituent of *Cannabis sativa* Δ⁹-THC, produce both their anticonvulsant and psychototropic/
psychotoxic effects via a CB₁ receptor-dependent mechanism. Thus, there has been a tremendous amount of research focused on the therapeutic potential for exogenous CB₁ receptor agonists for the treatment of seizure disorders and other neurological maladies. Drawbacks for the exclusive use of exogenously administered cannabinoid compounds as long-term anticonvulsant agents include their ubiquitous and non-specific CB₁ receptor agonism throughout the CNS and psychotropic/psychotoxic effects, and the development of pharmacological tolerance. A potential therapeutic strategy for exploiting the anticonvulsant activity of cannabinoids has been in their application in adjunctive therapy with classical AEDs. These experimental findings indicate that this combined therapeutic approach may allow for a significant lowering of the effective doses needed of each agent for seizure control, thus resulting in the potential benefit of decreasing dose-dependent adverse effects.

Another adjunctive approach has been investigated by taking advantage of the “cross-talk” between the ECS and the opioid system for seizure control, whereby initial experimental findings demonstrate that the combined pharmacological interventions of these two systems can result in a greater than 100-fold increase in efficacy for each individual agent. The above adjunctive approaches may hold greater promise for utilizing CB₁ receptor agonists for the treatment of seizures and epilepsy. Contrary to a CB₁ receptor agonism approach to seizure suppression, other evidence suggests that ECS-mediated regulation of synaptic transmission immediately following a brain insult may contribute to the development of maladaptive plasticity changes that ultimately result in recurrent seizure discharge. To this end, a single administration of CB₁ receptor antagonists immediately following a brain insult has been shown to act in a prophylactic manner by blocking the development of the pathophysiology that underlies the epileptic phenotype (Chen et al., 2007; Echegoyen et al., 2009).

A more plausible approach to targeting the ECS for seizure control is underscored by the unquestionable evidence from many experimental studies that in the neuropathophysiologica1 state of recurrent seizure activity, there is an increase in tone of the endogenous “defensive” response of on-demand synthesis and release of the endocannabinoids AEA or 2-AG, which act to restrict further seizure discharge via their actions at presynaptic CB₁ receptors. Thus, the use of highly specific pharmacological inhibitors of select metabolic enzymes (FAAH and MAGL) or reuptake mechanisms (Piomelli, 2014), would act to prolong the intrinsic response of the ECS to seizure discharge. Targeting these specific aspects of the ECS may allow for enhancing the “defensive” response to hyper-synchronous neuronal discharges that are specifically confined both temporally and spatially, and may be effective in treating a wide spectrum of epilepsy disorders wherein seizure activities arise from regionally diverse origins throughout the complex neuronal-circuitry pathways within the brain.

Evidence suggesting a role for the ECS in regulation of seizure discharge through CB₁ receptor-independent pathways is supported by findings demonstrating anticonvulsant properties of the non-psychotropic phytocannabinoids CBD, CBDV, and THCV, which are devoid of activity at CB₁ receptors. From this
group, CBD has been the most extensively studied in seizure and epilepsy research and has been evaluated in a clinical trial, which indicated its promising therapeutic potential as an anticonvulsant (Cunha et al., 1980). Additionally, some anecdotal findings indicate that select populations of epileptic patients may benefit from using CBMs enriched in CBD for symptomatic control of their disease (Porter and Jacobson, 2013). The cellular mechanism(s) involved in the seizure suppressive properties of this group of phytocannabinoids is still unclear; however, a number of physiological effects of CBD have been elucidated, which may eventually contribute to a better understanding of their anticonvulsant effects and interactions they have with the ECS. Although CBD shows virtually no activity at CB1 receptors, there is evidence that it modulates a number of other associated components of the ECS, which allows us to segue into a brief discussion of potential future discoveries of ECS-mediated control of neuronal excitability. CBD has been shown to regulate a number of systems in association with endocannabinoid signaling, which include the TRP family of protein channels, GRP55 and 5HT1A receptors (Hill et al., 2012b; Mechoulam et al., 2007). Recent discoveries have given support to the possible existence of non-CB1/CB2 receptors as indicated by the assembly of a list of potential candidate proteins that may act as a “CB3” receptor. Future research endeavors will more than likely elucidate additional ECS-associated systems, which may allow for a more thorough understanding of its regulation of both physiological and pathophysiological neuronal processes. Further discussion on this subject is beyond the scope of this chapter, although a consortium of international investigators has published an extensive review on potential cannabinoid receptors and ligands “beyond” CB1 and CB2 receptors (Pertwee et al., 2010).

This chapter would not be complete without briefly mentioning the involvement of the brain ECS in regulating the physiological processes of another “non-neuronal” cell type with a gargantuan presence within the CNS called glial cells. Experimental studies have presented evidence that establishes a presence, in some cases activity dependent, of CB1, CB2, and CB-like receptors on microglia, astrocytes, and astrocytoma brain cells, and that activation of these receptors is involved in controlling pro-inflammatory processes within these glial cell types (Stella, 2010). Inflammatory processes are thought to play an essential role in the process of epileptogenesis following an initial brain injury, which may allow a time window for implementation of prophylactic anti-inflammatory therapeutics to block the development of the pathophysiology that ultimately results in the epileptic phenotype (Vezzani et al., 2013). Thus, targeting ECS-mediated regulation of pro-inflammatory responses of glial cells immediately following a brain insult may allow for a novel therapeutic strategy towards the prevention of recurrent seizure disorders. Experimental findings have demonstrated a role for CB1 receptor-dependent regulation of astroglial cells on the formation of working memory, suggesting the involvement of a CB1 receptor—glial cell pathway in the synaptically-driven process of LTP (Han et al., 2012). More pertinent to this chapter were findings in an in vitro model of 4-AP-induced epileptiform activity in hippocampal slice cultures that demonstrated that activation
of CB₁ receptor on astrocytes was shown to result in a Ca²⁺-dependent increase in epileptiform activity, which was most likely attributed to increased release of glutamate (Coiret et al., 2012). Thus, endocannabinoid-mediated control of glial cell function during pathophysiological conditions in the brain may open avenues for the development of novel therapeutic strategies for the treatment and possibly prevention of the epileptic condition.

The medicinal use of Cannabis to modulate the ECS to treat seizures has been occurring, without any knowledge of its existence, for many hundreds of years, being first noted in the 15th century in Baghdad to treat the epileptic son of a caliphate counselor (Mechoulam, 1986). Since its discovery over the past 20 years, the importance of the ECS’s essential role in mediating a multitude of physiological processes throughout many organ systems is made evident by the ever-increasing number of experimental publications within the scientific literature. As a result of exceptional studies in basic neuroscience research, great strides have been made towards a better understanding of the complex nature of the way in which the brain ECS can be rapidly summoned for the on-demand synthesis and release of the ECs AEA and 2-AG to regulate both physiological and pathophysiological neuronal activity. Substantial evidence from studies in both in vivo and in vitro models of seizure and epilepsy clearly demonstrates that the ECS acts, in both a phasic and tonic manner, to control hyperexcitable neuronal activity primarily via CB₁ receptor activation, and alterations occur within this endogenous system likely to support compensatory mechanisms. Basic scientific research studies that target different components of the brain ECS with specific pharmacological agents have shown great promise towards the development of novel therapeutic strategies for the control and possible prevention of epileptic seizure disorders.

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