Role of Endocannabinoid Signaling in Anxiety and Depression

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Contents

Abstract Cannabinoid receptors and their endogenous ligands are located throughout the limbic, or "emotional," brain, where they modulate synaptic neurotransmission. Converging preclinical and clinical data suggest a role for endogenous cannabinoid signaling in the modulation of anxiety and depression. Augmentation

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of endocannabinoid signaling (ECS) has anxiolytic effects, whereas blockade or genetic deletion of CB_1 receptors has anxiogenic properties. Augmentation of ECS also appears to have anti-depressant actions, and in some assays blockade and genetic deletion of CB_1 receptors produces depressive phenotypes. These data provide evidence that ECS serves in an anxiolytic, and possibly anti-depressant, role. These data suggest novel approaches to treatment of affective disorders which could include enhancement of endogenous cannabinoid signaling, and warrant cautious use of CB_1 receptor antagonists in patients with pre-existing affective disorders.

Keywords Cannabis • Fatty acid amide hydrolase • Post-traumatic stress disorder • Marijuana • Anandamide • Cannabinoid

Abbreviations

2AG	2-Arachidonoylglycerol
5-HT	5-Hydroxytryptamine, serotonin
ACC	Anterior cingulate cortex
AEA	Anandamide
BLA	Basolateral amygdala
CCK	Cholecystokinin
CUS	Chronic exposure to an unpredictable and variable set of stressors
ECS	Endocannabinoid signaling
ECT	Electroconvulsive therapy
FAAH	Fatty acid amide hydrolase
HPA	Hypothalamus–pituitary–adrenal
KO	Knockout
PFC	Prefrontal cortex
PTSD	Post-traumatic stress disorder
PVN	Paraventricular nucleus
SSRI	Selective serotonin re-uptake inhibitors

1 Human Studies Suggesting a Role for Endocannabinoid Signaling in Anxiety

Cannabis has been used for centuries for a variety of recreational and medicinal purposes. The primary psychoactive chemical in cannabis, Δ^9-tetrahydrocannabinol (THC), is a partial agonist of the CB_1 cannabinoid receptor (Breivogel et al. 1998). The most commonly cited reasons for continued recreational cannabis use are relaxation and reduction in tension (Reilly et al. 1998; Schofield et al. 2006; Thomas

1993). Paradoxically, the most commonly cited reasons for discontinuation of cannabis use are increased anxiety and panic reactions (Reilly et al. 1998; Szuster et al. 1988). Modulation of anxiety reactions by cannabis appears to be complex in that both dose and environmental context can modulate these effects. Subjects under "experimenter harassment" were more likely to experience anxiety reactions under the influence of cannabis than those in neutral environments (Gregg et al. 1976). Since the subjective effects of cannabis are mediated via the CB_1 receptor (Huestis et al. 2001), these data suggest a role for endocannabinoid signaling (ECS) in the regulation of anxiety.

A CB_1 receptor antagonist, rimonabant (also named Acomplia, SR141716 and SR141716A) has been developed and used in humans for the treatment of obesity, diabetes and dyslipidemia (Van Gaal et al. 2008). Psychiatric adverse effects, including anxiety, were cited as reasons for discontinuation by patients taking rimonabant significantly more than those taking placebo (Van Gaal et al. 2008), although objective measures of anxiety were not significantly increased in patients taking rimonabant (Scheen et al. 2006). A recent meta-analysis pooling data from four large clinical trials indicated that subjects taking rimonabant had a significantly greater increase in anxiety symptoms while taking the drug than patients taking placebo (Christensen et al. 2007). Therefore, human experience with a cannabinoid receptor agonist (THC) and antagonist (rimonabant) support the hypothesis that ECS regulates anxiety in humans and suggest that activation of the CB_1 receptor by endocannabinoids could produce anxiolytic effects.

Support for an inverse relationship between ECS and anxiety in humans also comes from a recent study of serum endocannabinoids in women with depression (Hill et al. 2008). In this study, the severity of anxiety experienced by women with major depression was inversely correlated with serum content of *N*-arachidonylethanolamine (AEA). Although very little is known about the source or potential target of circulating endocannabinoids, these data suggest that some of the somatic manifestations of anxiety could be related to reduced ECS.

2 Animal Studies Indicating a Role for ECS in Anxiety

2.1 Effects of CB_1 Receptor Blockade and Genetic Deletion on Unconditioned Anxiety Behaviors

A commonly used and well-validated test of unconditioned anxiety in rodents is the elevated plus-maze. This is an exploration-based test that utilizes the innate fear of open spaces exhibited by rodents. The maze measures the proportion of time rodents spend in well-lit "open" arms, compared to darker "closed" arms. A drug-induced increase in the proportion of time spent in the open arms is suggestive of an anxiolytic effect, whereas an increase in time spent in closed

arms is suggestive of an anxiogenic effect. An anxiogenic effect of rimonabant has been demonstrated using an elevated plus-maze test in rats (Navarro et al. 1997) and mice (Arevalo et al. 2001; Patel and Hillard 2006). A second CB_1 receptor antagonist, AM251, a structural analog of rimonabant, also shows anxiogenic effects in rodents in the elevated plus-maze (Haller et al. 2004b; Patel and Hillard 2006). Rimonabant exhibits an anxiogenic profile in the defensive-withdrawal (Navarro et al. 1997) and ultrasonic vocalization tests (McGregor et al. 1996) as well.

In contrast to these findings, other studies have demonstrated either no effect (Bortolato et al. 2006; Kathuria et al. 2003) or an anxiolytic effect of rimonabant (Degroot and Nomikos 2004; Griebel et al. 2005; Rodgers et al. 2003). In the studies in which no effect was seen, relatively low doses of the antagonists were used (Bortolato et al. 2006; Kathuria et al. 2003). Dose-dependent anxiolytic effects of rimonabant were seen in the elevated plus-maze and Vogel conflict test in mice (Griebel et al. 2005). Furthermore, using a design in which rodents were tested twice, rimonabant had no effect in the elevated plus-maze during the first trial, but produced an anxiolytic effect during the second exposure (Rodgers et al. 2003). Interestingly, rimonabant produced anxiolytic effects in CB_1 receptor knockout (KO) mice, leading Haller et al. to suggest its anxiolytic actions are mediated via non-CB_1-dependent mechanisms (Haller et al. 2002). These authors did not observe anxiogenic effects of AM251 in CB_1 receptor KO mice, and concluded that AM251 does not share the non-receptor effect of rimonabant (Haller et al. 2004a). Anxiolytic effects of rimonabant have also been demonstrated in the shock-probe burying test, although this effect could be due to the effect of the drug to enhanced memory function, rather than direct effects of unconditioned anxiety per se (Degroot and Nomikos 2004).

Administration of rimonabant results in activation of brain regions involved in the generation of fear and anxiety. Systemic administration of rimonabant increased Fos expression, a marker of neuronal activity, within the central amygdala, bed nucleus of the stria terminalis, hypothalamus and brainstem (Alonso et al. 1999; Patel et al. 2005b; Rodriguez de Fonseca et al. 1997). These studies further support the hypothesis that ECS is an endogenous anxiolytic system that dampens neuronal activity within brain regions critical for the generation of fear and anxiety responses.

CB_1 receptor KO mice exhibit increased anxiety-like behaviors in the elevated plus-maze (Haller et al. 2002, 2004a, b), and in the light–dark exploration model in young mice only (Maccarrone et al. 2002). Interestingly, these effects appear to be more prominent under environmentally stressful conditions (Haller et al. 2004a; Maccarrone et al. 2002). In particular, in a high light condition, which is considered stressful since rodents are nocturnal and have impaired vision under this condition, CB_1 receptor KO mice exhibit an anxiogenic phenotype; while under low light conditions, this phenotypic difference is absent (Haller et al. 2004a). This finding may explain why some studies have failed to detect an anxiogenic phenotype in CB_1 receptor KO mice (Marsicano et al. 2002). In addition to direct anxiogenic behaviors, CB_1 receptor KO mice display impaired behavioral responses

to non-cannabinoid anxiolytics including benzodiazepines and buspirone (Uriguen et al. 2004).

2.2 *Effects of Pharmacological and Genetic Augmentation of ECS on Unconditioned Anxiety Behaviors*

ECS occurs when synaptic concentrations of the endocannabinoids AEA and/or 2-arachidonoylglycerol (2AG) are increased through either increased synthesis or decreased catabolism. In particular, fatty acid amide hydrolase (FAAH) is a well-characterized enzyme that hydrolyzes and inactivates AEA and other *N*-acylethanolamines (Ho and Hillard 2005). Pharmacologic inhibition or genetic deletion results in significant increases in brain AEA but not 2AG content (Cravatt et al. 1996; Kathuria et al. 2003; Patel et al. 2005a). Systemic administration of a highly efficacious inhibitor of FAAH, URB597, produced anxiolytic effects in the elevated zero-maze (a slight modification of the elevated plus-maze described above) and in the ultrasonic vocalization test in rats (Kathuria et al. 2003). This effect was accompanied by an increase in brain AEA concentrations and blocked by the CB_1 receptor antagonist rimonabant (Kathuria et al. 2003). These data suggest that increased CB_1 receptor signaling by AEA produces anxiolytic behavioral effects that can be enhanced by pharmacological blockade of FAAH. This effect of URB597 has been replicated in mice using the elevated plus-maze (Moreira et al. 2008; Patel and Hillard 2006) and in rats using the light–dark box test (Scherma et al. 2008). FAAH KO mice also exhibit an anxiolytic phenotype in the elevated plus-maze and light–dark box test (Moreira et al. 2008; Naidu et al. 2007); effects that are blocked by pretreatment with rimonabant (Moreira et al. 2008). Taken together, these data support the hypothesis that the ECS in rodents provides an anxiolytic tone that can be enhanced if AEA-mediated signaling is increased. The role of 2AG in this system is not known.

These findings are consistent with data showing that exogenous administration of low doses of direct-acting CB_1 receptor agonists also produce anxiolytic effects in rodents (Patel and Hillard 2006; Scherma et al. 2008). However, unlike direct CB_1 receptor agonists that display anxiogenic effects at higher doses, FAAH inhibitors exhibit only dose-dependent anxiolytic effects without anxiogenic effects at high doses (Kathuria et al. 2003; Patel and Hillard 2006). These data suggest that the spatio-temporal properties of ECS are maintained by FAAH inhibition, in contrast to global CB_1 activation by direct agonists, and that this property of FAAH inhibitors subserves their uniphasic, anxiolytic properties. In other words, global CB_1 receptor activation can result in both decreased and increased anxiety, but the evidence using both inhibition of FAAH and CB_1 receptor antagonism indicate that the anxiogenic "pool" of CB_1 receptors is not endogenously active. We suggested earlier that the functional pools are anatomically distinct (Patel and Hillard 2006), a suggestion that is supported by a recent study using region-selective, virally mediated up-regulation

of FAAH. Parolaro and co-workers showed that increasing FAAH expression within the prefrontal cortex (PFC) caused a reduction in AEA concentrations and an increase in anxiety behaviors in the elevated plus-maze (Rubino et al. 2008b). These data confirm a role for ECS in the regulation of anxiety behaviors and suggest that the anatomical site of this ECS function includes the PFC.

However, another explanation for the difference in the effects on anxiety between FAAH inhibition and direct CB_1 receptor agonists is that the inhibition of FAAH increases levels of non-cannabinoid, fatty acid ethanolamides (NAEs) as well as AEA (Cravatt et al. 2001). Since the anxiolytic effects of FAAH inhibitors can be blocked by CB_1 receptor antagonists (Kathuria et al. 2003; Moreira et al. 2008), it can be concluded that CB_1 receptor activation is required for the anti-anxiety efficacy of FAAH inhibition. However, these data do not address the question of whether other NAEs contribute to the efficacy as well. In other words, it is not known whether CB_1 receptor activation is sufficient for the anxiolytic efficacy of FAAH inhibition.

In addition to inhibition of FAAH, inhibitors of endocannabinoid transport have also demonstrated anxiolytic properties. AM404 is an arachidonic acid analog that inhibits uptake of both AEA (Beltramo et al. 1997) and 2AG (Beltramo and Piomelli 2000), inhibits FAAH activity (Jarrahian et al. 2000), and increases brain AEA concentrations (Bortolato et al. 2006). Several studies have demonstrated that systemic administration of AM404 produces anxiolytic effects in the elevated plus-maze, defensive withdrawal test, and social isolation test (Bortolato et al. 2006; Patel and Hillard 2006). These effects are blocked by the CB_1 receptor antagonist rimonabant, consistent with the hypothesis that indirect activation of the ECS can produce anxiolytic effects (Bortolato et al. 2006). However, in another study in which drugs were administered into the periaqueductal gray of rats, AEA produced anxiolytic effects that were enhanced by AM404, but alone AM404 was not anxiolytic (Moreira et al. 2007).

2.3 Effects of CB_1 Receptor Deletion and Pharmacological Blockade on Conditioned Anxiety Behaviors

Conditioned, or "learned," fear is a model for certain types of anxiety disorders including post-traumatic stress disorder (PTSD). In this paradigm, a temporal contingency is established between environmental cues such as an auditory tone or a specific environmental context, i.e., "cage type," and an aversive stimulus such as an electric shock. After single or repeated "paired" presentations of these two stimuli, the environmental cues presented alone can elicit an innate, conditioned fear response such as freezing, and signs of sympathetic nervous system activation. After "conditioned" fear responses to cue presentation are established, presentation of environmental cues in the absence of the aversive stimulus causes a gradual extinction of conditioned fear responses.

Two different conditioning paradigms, context and tone, have been used to examine the role of ECS in the acquisition of conditioned fear responses. Several studies have shown no effect of either CB_1 receptor genetic deletion or pharmacological blockade on the acquisition of contextual or tonal fear conditioning (Marsicano et al. 2002; Suzuki et al. 2004). However, a recent study utilizing a multiple-trial acquisition model found enhanced acquisition of conditioned fear responses in trace and delayed fear conditioning paradigms, which are hippocampus- and amygdala-dependent, respectively (Reich et al. 2008). These data suggest that ECS could impair acquisition of conditioned anxiety responses under specific conditions.

It has been conclusively demonstrated that both pharmacological and genetic inhibition of CB_1 receptors impair the extinction of both contextual and tonal conditioned anxiety responses (Kamprath et al. 2006; Marsicano et al. 2002; Reich et al. 2008; Suzuki et al. 2004). Impaired extinction of aversive associative learning has also been demonstrated using fear-potentiated startle and passive avoidance protocols (Chhatwal et al. 2005), but not an appetitively motivated instrumental responding paradigm (Niyuhire et al. 2007).

Data from a novel paradigm that attempts to separate the associative and non-associative components of conditioned fear responses suggest that impairments in extinction observed in CB_1 receptor KO mice are due to deficits in habituation, the non-associative component of extinction (Kamprath et al. 2006). In this paradigm, presentation of the tone stimulus used in fear conditioning paradigms (preceded by a sensitizing shock) results in freezing behavior that habituates over repeated presentations; this represents a non-associative component of extinction of conditioned fear behavior. Mice lacking CB_1 receptors do not show habituation of these innate fear responses after repeated tone presentation. These authors suggest that the impairments in extinction of conditioned fear behavior observed in CB_1 receptor KO mice and after CB_1 receptor blockade are a result of an impaired "habituation component" of the extinction process (Kamprath et al. 2006). This suggestion is consistent with a growing body of literature supporting a role of the ECS in habituation of the behavioral and endocrine responses to stress (Patel and Hillard 2008).

2.4 Effects of ECS Augmentation on Conditioned Anxiety Behaviors

Similarly to unconditioned anxiety, insight into the role of ECS in conditioned anxiety comes from studies in which CB_1 receptor signaling is activated using low doses of agonists. For example, the CB_1 receptor agonist WIN55212-2 impairs acquisition of context-, but not tone-, conditioned anxiety responses (Pamplona and Takahashi 2006) and low doses of WIN55212-2 facilitate extinction of conditioned anxiety responses in a contextual fear-conditioning paradigm (Pamplona et al. 2006). Similarly, the indirect agonist, AM404, impairs extinction of fear-potentiated startle responses (Chhatwal et al. 2005), and FAAH KO mice exhibit enhanced

extinction of an aversively motivated, spatial memory task (Varvel et al. 2007). This appears selective for aversively motivated over appetitively motivated learning (Holter et al. 2005).

Taken together, data in animal models of unconditioned and conditioned anxiety support the hypothesis that activation or enhancement of ECS can produce a reduction in anxiety in rodents. This function of the ECS appears to be tonically "on" or easily activated since treatment of rodents in mildly aversive environments with CB_1 receptor antagonists enhances anxiety behaviors. It is likely that changes in CB_1 receptor activation can regulate anxiety in multiple brain regions and through multiple mechanisms (discussed further below). High doses of direct CB_1 receptor agonists can be anxiogenic, which parallels the human experience in which cannabis use can be both anxiolytic and anxiogenic. However, the lack of anxiogenic effects by FAAH inhibitors and the nearly consistent finding that CB_1 receptor blockade is monophasically anxiogenic support the hypothesis that the predominant effect of endogenous CB_1 receptor activation is a reduction in anxiety.

3 Neural Mechanisms Underlying Endocannabinoid Modulation of Anxiety

The neural mechanisms by which ECS affects anxiety are not well understood, yet several mechanisms at the systems, synaptic, and molecular level can be posited based on available data. The majority of available data indicate that ECS has anxiolytic properties in both conditioned and unconditioned anxiety models, and that these effects are more active during states of stress or high arousal (Haller et al. 2004a). The anxiolytic effects of ECS are mimicked by low doses of direct CB_1 receptor agonists (Patel and Hillard 2006); thus data exploiting this phenomenon can be used to increased our understanding of the neural mechanisms subserving the anxiolytic actions of the ECS system.

At the systems level, microinjections of low doses of the direct CB_1 agonist THC into the PFC (Rubino et al. 2008a), ventral hippocampus (Rubino et al. 2008a), and dorsal periaqueductal gray area (Moreira et al. 2007) exert anxiolytic effects in the elevated plus-maze. These effects are blocked by the CB_1 receptor antagonist AM251 (Moreira et al. 2007; Rubino et al. 2008b). Pharmacological inhibition of FAAH within the PFC produces CB_1-receptor-dependent anxiolytic effects, and over-expression of FAAH (which reduces local AEA levels) causes an anxiogenic effect in the elevated plus-maze (Rubino et al. 2008b). In contrast to the PFC and hippocampus, very low doses of THC produce only anxiogenic effects when administered into the basolateral amygdala (BLA); this was also dependent upon CB_1 receptor activation (Rubino et al. 2008a). These data suggest that the PFC and hippocampus are likely anatomical sites of action that subserve the anxiolytic effects of ECS. More specifically, the balance of ECS in favor of an increase in

the PFC and/or hippocampus and reduced signaling in the amygdala could be required for maximal anxiolytic effects.

With regard to endocannabinoid facilitation of extinction of conditioned fear responses, direct administration of CB_1 agonists into the lateral amygdala impairs fear memories by blocking reconsolidation in a fear-potentiated startle model (Lin et al. 2006). These data suggest that ECS in the amygdala during presentation of conditioned cues impairs reconsolidation of fear memories, and thus facilitates extinction of conditioned fear responses. Thus, in contrast to unconditioned anxiety responses (which are enhanced by CB_1 receptor activation in the amygdala), impairments in conditioned anxiety responses are observed after amygdalar CB_1 receptor activation. These data suggest a complex and potentially divergent role for amygdalar ECS in the modulation of conditioned vs. unconditioned anxiety behaviors.

At the synaptic level, activation of CB_1 receptors inhibits glutamatergic inputs to principal neurons in the cortex, hippocampus and BLA (Hashimotodani et al. 2007). In addition, CB_1 receptor activation inhibits GABA release from a subpopulation of cholecystokinin (CCK)-expressing interneurons that form perisomatic (and some dendritic) contacts with hippocampal principal neurons; however, this effect is only operative when the firing rates of these interneurons is low (Foldy et al. 2007). Haller and co-workers suggest that the anxiolytic effects of ECS are mediated via inhibition of GABAergic transmission within the hippocampus (Haller et al. 2007). This suggestion is based on data demonstrating an anxiolytic effect of WIN55212-2 in CD-1 mice, in which this compound was significantly more efficacious at inhibiting hippocampal GABAergic than glutamatergic transmission. By contrast, WIN55212-2 produced an anxiogenic effect and affected GABAergic and glutamatergic transmission equally in rats. In addition, AM251 blocked the anxiogenic effect of WIN55212-2 in mice, and blocked the effect of this compound on GABAergic transmission, but not glutamatergic transmission. These data led the authors to conclude that WIN55212-2 produced anxiolytic effects via inhibition of GABAergic transmission within the hippocampus. These pharmacologic studies led to the further suggestion that the anxiogenic effect of WIN55212-2 in rats is mediated by inhibition of glutamatergic transmission. These data provide an interesting hypothesis that requires further experimental evidence; particularly important will be studies using mouse models in which CB_1 receptors on either glutamatergic or GABAergic terminals have been selectively abolished (Monory et al. 2006).

A synaptic mechanism subserving endocannabinoid facilitation of extinction of conditioned fear responses has also been proposed (Lafenetre et al. 2007). These authors incorporate the ability of endocannabinoids to modulate both GABAergic and glutamatergic transmission within the amygdala in their model. They suggest that under basal conditions ECS is not active in the amygdala; a conclusion that is supported by c-Fos studies from our laboratory (Patel et al. 2005b). After tonal fear conditioning, presentation of the tone alone increases ECS in the BLA, which has been demonstrated experimentally (Marsicano et al. 2002). This increase in ECS inhibits GABAergic transmission, which results in dis-inhibition of BLA projection

neurons and facilitation of a "no fear" pathway mediated by activation of inhibitory neurons within intercalated cell masses. These neurons provide feed-forward inhibition onto central amygdala neurons, which are output neurons of the amygdala and activate conditioned behavioral and physiological responses. These authors also suggest that ECS signaling could decrease glutamatergic transmission in a "fear" pathway that transmits directly from the BLA to the central amygdala. Such depotentiation of the conditioned "fear" pathway could represent a synaptic mechanism for the habituation component of extinction of conditioned fear. The mechanisms that would segregate ECS into GABAergic and glutamatergic signaling in the "no fear" and "fear" pathways, respectively, remain to be determined.

Although the above data provide anatomical and synaptic insights into the mechanisms subserving the anxiolytic effects of ECS, they do not alone explain the context-dependent effects. Specifically, the anxiogenic effects of CB_1 receptor deletions or blockade are more robust under stress or high arousal (Haller et al. 2004a), suggesting increased ECS counteracts the anxiety produced by environmental stress. These observations suggest that exposure to the fear-evoking or stressful context results in an increase in endocannabinoid release. A potential explanation could involve the neuropeptide CCK, which is expressed by CB_1-receptor-positive, GABAergic interneurons. CCK is released under times of stress and high arousal (Nevo et al. 1996), and activation of CCK_2 receptors appears to result in endocannabinoid release from hippocampal principal neurons, based on the effects of AM251 (Foldy et al. 2007). These endocannabinoids can then activate receptors on GABAergic interneurons to produce anxiolytic effects as suggested above. This hypothesis remains to be experimentally tested.

At the molecular level, anxiolytic effects of low doses of CB_1 receptor agonists are associated with increased CREB expression within the PFC and hippocampus (Rubino et al. 2007). This increase was associated with an increase in ERK activation in the PFC, and a decrease in CAMKII (a kinase that inhibits CREB activation) within the hippocampus. In addition, anxiolytic doses of THC inhibited plus-maze exposure-induced Fos expression with the PFC and amygdala (Rubino et al. 2007). Behaviorally, the anxiolytic effects of low doses of THC are blocked by a mu-opioid receptor antagonist (Berrendero and Maldonado 2002), and a $5HT_{1A}$ serotonin receptor antagonist (Marco et al. 2004); the anxiolytic effects of AM404 are also blocked by a 5-HT_{1A} antagonist (Marco et al. 2004). These data suggest a role for opioid and serotonin receptors in the anxiolytic effects of ECS.

In the case of conditioned fear modulation, roles for ERK and calcineurin have been demonstrated. In response to conditioned tone presentation, CB_1 receptor KO mice exhibit relatively increased freezing behavior as a consequence of impaired extinction (Marsicano et al. 2002). These mice also exhibited decreased tone-induced phosphorylation of ERK and calcineurin expression in the BLA and PFC, while showing increased expression of these two proteins in the central amygdala (Cannich et al. 2004). CB_1 receptor KO mice also showed increased p-AKT in the BLA and dorsal hippocampus in response to conditioned tone presentation compared to wild-type mice (Cannich et al. 2004). It has been shown that ERK signaling in the BLA is required for the acquisition of extinction

(Herry et al. 2006), suggesting that impaired ERK signaling in CB_1 receptor KO mice could contribute to the impaired extinction observed in these mice. In addition, mice lacking forebrain calcineurin exhibit impaired extinction of conditioned fear behaviors (Havekes et al. 2008), supporting a role for this protein in the impaired extinction observed in CB_1 receptor KO mice. These data suggest that ECS could facilitate extinction of conditioned fear via activation of ERK and calcineurin signaling (Davis et al. 2003; Galve-Roperh et al. 2002).

4 Human Studies Suggesting a Role for ECS in Depression

4.1 Cannabis Use and Depression

The thousands of years of human use of the CB_1 receptor agonist, THC, in preparations of *Cannabis sativa* support the hypothesis that there is a relationship between cannabis use and depression. Elevation of mood is one of the commonly cited motivations for the use of cannabis. In a study of young, poly-substance users, 69% of the respondents reported that they used cannabis to "make themselves feel better when down or depressed" (Boys et al. 2001). While this is far less than the 97% who responded that they used cannabis to help relax, it argues that cannabis could exert anti-depressant effects in humans. Several clinical trials in the 1970s designed to determine the anti-depressant efficacy of THC found that it failed to improve symptoms of depression and produced unacceptable adverse effects (Ablon and Goodwin 1974; Kotin et al. 1973). Although it can be argued that these studies were small and did not take into consideration the heterogeneity in depressive illnesses, it is not likely that THC would be broadly useful as an anti-depressant in humans.

A similar hypothesis, that depressed individuals self-administer cannabis because it elevates mood, is not supported by available data (Kandel et al. 1986; Miller-Johnson et al. 1998; Patton et al. 2002). This hypothesis predicts that depressed people use cannabis to elevate mood more frequently than non-depressed users. This prediction was not upheld in a recent study (Arendt et al. 2007); in fact, depressed subjects experienced more depression, aggression and sadness when intoxicated with cannabis than when they were not intoxicated.

There are data to support an alternative hypothesis that cannabis use precipitates depression. For example, cannabis dependence and depression are co-morbid diagnoses more than would be expected by chance (Degenhardt et al. 2003). Furthermore, several prospective studies have found that cannabis use precedes the diagnosis of depression (Bovasso 2001; Patton et al. 2002; Rey and Tennant 2002). Cannabis use was identified in high-school students as a significant, independent predictor of suicidal behaviors after adjustment for depressive symptoms (Chabrol et al. 2008). However, a large (greater than 12,000 participants) longitudinal study did not find that past cannabis use was a significant predictor of depression in adults

when baseline differences between users and non-users were carefully controlled (Harder et al. 2006). The authors of this study concluded that the available evidence does not support a causal relationship between cannabis use and depression, but does suggest that a common factor or factors predisposes individuals to both depression and cannabis dependence. In this regard, the hypothesis of a shared genetic predisposition for both cannabis use and depression has received support in the literature. Both cannabis use and dependence (Fu et al. 2002a; Kendler et al. 2000; Lynskey et al. 2002) and depressive/suicidal behaviors (Fu et al. 2002a, b; Statham et al. 1998; Sullivan et al. 2000) are moderately heritable. More importantly, several recent studies have demonstrated that the genetic factors for cannabis dependence and depression/suicidality are moderately correlated (Fu et al. 2002a; Lynskey et al. 2004). Twin studies suggest that shared environmental factors also contribute significantly to the co-morbidity of cannabis dependence and depression (Lynskey et al. 2004).

4.2 Depression and the ECS

The data described above lead to the hypothesis that dysregulation of ECS results in depression. Support for this hypothesis comes from the adverse events profile in humans of the CB_1 receptor antagonist, rimonabant, which demonstrates a small, yet significant, increased likelihood for the development or exacerbation of depression (Van Gaal et al. 2008). The likelihood of depression or mood changes with depressive symptoms increases when patients with pre-existing depressive illness were not excluded from rimonabant treatment (Nissen et al. 2008). These data suggest that endogenous activation of CB_1 receptors serves as a buffer against depression and its elimination or reduction in susceptible individuals can result in depressive symptoms. In another study, the incidence of depression in patients with Parkinson's disease was found to be significantly correlated with polymorphisms in the CB_1 receptor gene (Barrero et al. 2005). There was a trend for the same observation in non-Parkinson patients, but the study was not sufficiently powerful to determine whether CB_1 receptor polymorphisms contribute to the likelihood of developing major depression in the general population.

There have also been some very interesting studies that have investigated the hypothesis that depression changes ECS. Patients with depression who died by suicide had significantly greater CB_1 receptor agonist binding site density and agonist signaling in the dorsolateral PFC than matched controls (Hungund et al. 2004; Vinod et al. 2005). Tissue contents of both AEA and 2AG in the dorsolateral PFC were also increased in alcoholic patients who were depressed compared to alcoholics without depression (Vinod et al. 2005). In a study using immunohistochemical approaches, neuronal CB_1 receptor density in the anterior cingulate cortex (ACC) was not found to be different between patients with major depression and controls (Koethe et al. 2007). However, CB_1 receptor density was significantly decreased in subjects with major depression taking selective serotonin re-uptake

inhibitors (SSRIs) compared to patients with major depression who were not being treated with SSRIs, suggesting that the drug therapy reduced CB_1 receptor expression (Koethe et al. 2007). CB_1 receptor density was also decreased in glial cells in the ACC of brains from patients who died with major depression compared to controls (Koethe et al. 2007). This finding is particularly interesting in light of other data suggesting that glial cell function and/or numbers are dysregulated in major depression (Cotter et al. 2001).

Our group has recently published a study in which circulating endocannabinoid concentrations were compared in non-medicated women with major depression and controls (Hill et al. 2008). 2AG contents in the serum were significantly lower in women with major depression than matched controls and were negatively correlated with the length of the current depressive episode. These data, while preliminary, support the possibility that some of the peripheral consequences of depression, such as cardiovascular and metabolic changes, could be related to ECS modulation.

To summarize, the available human data support the general hypothesis that CB_1 receptor activity is involved in the regulation of mood and that pharmacological dysregulation of ECS can alter mood in some individuals. Data suggest that depressed individuals have altered ECS; however, whether changes in ECS precede or follow the development of depression is unknown.

5 Animal Studies Suggesting a Role for ECS in Depression

5.1 Evidence That Alteration of CB_1 Receptor Signaling Results in Anti-Depressant-Like Effects

Immobility assays in rodents have been used extensively as preclinical models of anti-depressant efficacy of various pharmacologic agents. The Porsolt forced swim test is commonly employed; the time that rodents spend in an immobile, floating state is argued to represent a state of behavioral despair and is reduced by monoamine elevating anti-depressants (Porsolt et al. 1978). The highly efficacious CB_1 receptor agonists, HU210 (Hill and Gorzalka 2005b) and WIN55212-2 (Bambico et al. 2007) reduce immobility duration in the forced swim test in male rats at very low doses, consistent with anti-depressant efficacy. These agonist effects are blocked by co-treatment with CB_1 receptor antagonist. Indirect CB_1 receptor agonists, including AM404 (Hill and Gorzalka 2005b) and the FAAH inhibitor, URB597 (Gobbi et al. 2005; Hill et al. 2007b), also exhibit anti-depressant efficacy in the forced swim test. URB597 also has anti-depressant efficacy in a second immobility assay, the mouse tail suspension (Gobbi et al. 2005).

While the direct and indirect agonist data are fairly consistent and support a role for the ECS in the coping response of mice in the forced swim, antagonist data have been inconsistent. In both male and female C57Bl/6N mice, rimonabant had no

effect on the duration of immobility and increased struggling during the first exposure to the test (Steiner et al. 2008b). However, these investigators found that chronic treatment with high dose rimonabant significantly decreased immobility (Steiner et al. 2008a). Other studies using acute treatment with antagonists have also reported no effect (Bambico et al. 2007; Gobbi et al. 2005; Gobshtis et al. 2007; Hill and Gorzalka 2005b). On the other hand, several studies have demonstrated that acute treatment with antagonists, usually at high doses, reduces immobility (Shearman et al. 2003). The reasons for the discrepancies in these studies are not clear, but strain/species differences, differences in the parameters examined and differences in the environmental context of the assay (i.e., light vs. dark phase) are all plausible explanations.

Immobility tests comparing KO and wild-type mice have also been used to infer pro-depressant or anti-depressant roles for various proteins or signaling systems (Cryan and Holmes 2005). The duration of immobility of CB_1 receptor KO mice on a CD-1 background is not different from wild-type (Jardinaud et al. 2005). In one study, Steiner and colleagues reported that immobility (floating) was significantly increased in CB_1 receptor KO mice on a C57Bl/6N background compared to wild-type (Steiner et al. 2008b), while a second study from the same laboratory reported no difference in response when KO and wild-type mice were pretreated with a vehicle injection (Steiner et al. 2008a).

Taken together, these data suggest that activation of the CB_1 receptor exogenously can produce an anti-depressant behavioral phenotype in immobility assays; and they provide some support for ECS tone. On the other hand, they suggest that CB_1 receptor activation also contributes to behavioral despair since antagonist treatment can be anti-depressant as well. As for the effects of cannabinoid receptor ligands in anxiety discussed above, it is likely that there are "functional" pools of CB_1 receptors that subserve pro- and anti-depressant behavioral effects.

Most depressive disorders in humans include decreased incentive to seek positive reinforcers or anhedonia as a core symptom (Rush and Weissenburger 1994). This aspect of depression can be modeled using several rodent assays; the most common is the sucrose consumption test. Activation of CB_1 receptors results in a selective increase in the consumption of highly palatable foods, including increased sucrose drinking relative to the drinking of water (Sofia and Knobloch 1976). Inhibition of ECS by antagonists inhibits sucrose consumption in two bottle-choice paradigms (Arnone et al. 1997) and decreases responding reinforced by normal food and sucrose in operant procedures models (Freedland et al. 2001; Perio et al. 2001). CB_1 receptor KO mice also display reduced sucrose intake (Poncelet et al. 2003; Sanchis-Segura et al. 2004). Therefore, there are consistent data that inhibition or removal of the CB_1 receptor in otherwise normal rodents results in a decrease in their motivation to consume sucrose. These data lead to the hypothesis that reduced ECS could contribute to the anhedonia that occurs in depression. In support of this hypothesis, exposure of mice to stress results in a decrease in sucrose consumption that is reversed by direct and indirect CB_1 receptor agonists (Rademacher and Hillard 2007). Interestingly, in this study, rimonabant reduced sucrose consumption in the stressed mice at doses that did not affect sucrose

consumption in unstressed mice, consistent with a possible recruitment of ECS in the stressed condition (Rademacher et al. 2008).

5.2 *Evidence That Environmental Contexts That Produce Depression-Like Symptoms Alter ECS*

Repeated stress has been used to model depressive symptoms in rodents with a reasonable degree of biological and behavioral similarities to humans (Nestler et al. 2002). In particular, chronic exposure to an unpredictable and variable set of stressors (CUS) produces changes in rodents that parallel many aspects of human depression (Willner 2005). Several studies have demonstrated alterations in ECS in rodents exposed to CUS. Hippocampal CB_1 receptor density is reduced in rats exposed to CUS; and perseveration in the water maze induced by CUS is reversed by cannabinoid agonist treatment (Hill et al. 2005a). In another study, CUS was found to reduce body weight and sucrose intake in rats, both of which were reversed by treatment with a FAAH inhibitor (Bortolato et al. 2007). These studies suggest that down-regulation of ECS contributes to the detrimental effects of CUS. This conclusion is supported by the finding that CB_1 receptor KO mice exhibit increased sensitivity to the anhedonic effects of CUS (Martin et al. 2002).

Repeated exposure to the same stressor also recapitulates some of the behavioral effects of depression, including anhedonia. Repeated restraint results in changes in endocannabinoid content in several limbic regions, including a progressive increase in 2AG content within the PFC, amygdala and hypothalamus, as the number of restraint episodes increases (Rademacher et al. 2008). On the other hand, restraint decreases AEA contents in the PFC and amygdala regardless of the number of restraint episodes. These and other data support the hypothesis that repeated exposure to stress alters ECS and that these changes underlie the behavioral alterations induced by stress (Patel and Hillard 2008). Early life stress, which is known to promote the appearance of depression in adulthood, can be mimicked in mice using a 24-h maternal deprivation (Marco et al. 2009). Evidence from Macri and Laviola suggests that early life stress also down-regulates CB_1-receptor-mediated signaling (Macri and Laviola 2004).

In a recent study, Rubino and colleagues demonstrated that chronic THC exposure during adolescence resulted in significantly increased immobility in the forced swim test in females but not males, and significant anhedonia in both males and females (Rubino et al. 2008c). These studies are very interesting, particularly since they bear on the hypothesis that cannabis consumption predisposes humans to depression.

Therefore, an evolving body of evidence supports the hypothesis that altered ECS accompanies the development of depressive-like behaviors in rodents. The specifics of the alteration are not completely clear, but hypofunctional ECS in subcortical regions, particularly the hippocampus and hypothalamus, have been seen in several models.

5.3 *Evidence That Anti-Depressant Therapies Alter ECS*

While THC itself is not a good anti-depressant in humans, the role of ECS in mood regulation prompts the question of whether altered ECS contributes to the efficacy of other anti-depressant drugs or manipulations. Chronic exposure of rats to desipramine results in a significant increase in CB_1 receptor binding site density in the hippocampus and hypothalamus in non-stressed rats (Hill et al. 2006). Furthermore, the ability of chronic desipramine treatment to inhibit activation of Fos in the paraventricular nucleus (PVN) in response to stress was reversed by CB_1 receptor antagonist treatment. In addition, rimonabant inhibited the weight gain in response to desipramine, but did not affect the ability of desipramine to reduce immobility in the forced swim assay (Gobshtis et al. 2007). These data suggest that the ability of chronic desipramine to inhibit the activation of the hypothalamic–pituitary–adrenal (HPA) axis and increase weight in normal rats is mediated by an increase in ECS, perhaps in the hypothalamus. In contrast to these results, the effect of an acute injection of desipramine to induce immobility was absent in CB_1 receptor KO mice but the dampening effects of desipramine on HPA axis activation were intact (Steiner et al. 2008b). These results also suggest a difference in the mechanisms by which anti-depressants and ECS affect immobility and HPA axis activation, an observation that is discussed further below. The role of ECS in the effects of desipramine is not identical for other anti-depressants. For example, the SSRI citalopram significantly decreases CB_1 -receptor-mediated signaling in the PVN (Hesketh et al. 2008). Electroconvulsive shock treatment (ECT) is the most effective therapeutic option for depression in humans in that it benefits a higher proportion of patients than chemical anti-depressant therapy and requires substantially less time to see benefit (Silverstone and Silverstone 2004). ECT also produces alterations in ECS that can be summarized as an increase in subcortical ECS and a decrease in cortical ECS (Hill et al. 2007a).

Therefore, the treatments for human depression modulate ECS in a regionally specific manner. However, the changes are not consistent with respect to brain region or directionality and more studies are needed to determine which, if any, of these changes are relevant to ECS in depression.

6 Neural Mechanisms Underlying Endocannabinoid Modulation of Depression

The neurobiology of depression is complex; however, a large body of evidence supports the hypothesis that dysregulation of the HPA axis plays a critical role (Hill and Gorzalka 2005a). In particular, HPA axis hyperactivation and reduced feedback inhibition are seen in humans with depression and in animal models of depression. The ability of anti-depressants to suppress HPA axis hyperactivity is

coupled to their clinical efficacy (Appelhof et al. 2006). Recent studies strongly suggest that a primary role for ECS is to dampen HPA axis activation by stress and to allow for appropriate stress recovery (Barna et al. 2004; Di et al. 2003; Patel et al. 2004). These findings are consistent with the data obtained in rodents described above that inhibition of ECS is generally pro-depressive while its activation results in an anti-depressant phenotype, and lead to the hypothesis that dampening of the HPA axis is the mechanism by which ECS interacts with depression. However, HPA axis inhibition does not completely explain the effects of ECS to alter coping behaviors in the forced swim assay. For example, desipramine-induced behavioral effects are CB_1 receptor-dependent while its effects on HPA axis activation are not (Steiner et al. 2008a). Recent studies in our laboratory demonstrate that female CB_1 receptor KO mice exhibit normal HPA axis activation by stress but have increased immobility in the forced swim assay compared to wild-type (Roberts and Hillard, unpublished data).

The monoamine hypothesis of depression posits that dysregulation of serotonergic and noradrenergic signaling in the brain contributes to depressive symptoms (Belmaker and Agam 2008). ECS interactions with serotonergic signaling have been demonstrated in many studies. For example, serotonergic neurons have been shown to be involved in many cannabinoid effects, including hypothermia (Malone and Taylor 1998) and sleep (Mendelson and Basile 2001). The effect of WIN55212-2 to reduce immobility in the forced swim test is abolished by the serotonin (5-HT) depleting agent, para-chlorphenylalanine, indicating that this behavior is also 5-HT-mediated (Bambico et al. 2007). Low doses of WIN55212-2 enhance dorsal raphe serotonergic neuronal activity, an effect that is mimicked by the FAAH inhibitor URB597 (Gobbi et al. 2005). This effect appears to be due to ECS activation in the medial PFC since lesions there abolish the WIN55212-2 on raphe firing. Therefore, these studies suggest that activation of 5-HT-mediated signaling in the PFC is involved in the anti-depressant efficacy of activation of ECS. Recent studies have found that both CB_1 receptor blockade (Tzavara et al. 2003) and chronic administration of THC result in increased serotonin levels in the PFC (Sagredo et al. 2006). Chronic administration of another agonist, HU210, results in an enhancement of $5\text{-}HT_{2A}$ behavioral effects and a decrease in $5\text{-}HT_{1A}$ effects (Hill et al. 2005b). On the other hand, the FAAH inhibitor, URB597, increases firing of serotonergic neurons in the dorsal raphe and noradrenergic neurons in the locus coeruleus and ECS has been shown to subserve the regulation of glutamate-induced activation of serotonergic neurons in the raphe (Haj-Dahmane and Shen 2005).

CB_1 receptors are present throughout the limbic system (Herkenham et al. 1990) and can modulate both GABA and glutamate release (Freund et al. 2003). Therefore, it is not surprising that global activation or inhibition of ECS has confusing effects on behavior. A few studies have begun to dissect regional differences in the role of ECS in depression. HU210 injected into the rat hippocampus elicits reduced immobility in the forced swim test while URB597 is not active via this route (McLaughlin et al. 2007). These data, that activation of ECS in the hippocampus exerts anti-depressant effects, are consistent with findings that CUS, which produces

depressive-like symptoms, down-regulates hippocampal ECS (Hill et al. 2005a). WIN55212-2 is also an effective anti-depressant when injected into the ventromedial PFC; the effects of indirect agonists and antagonists were not determined (Bambico et al. 2007). The possible role of 5-HT signaling in the PFC effects is discussed above. Interestingly, CUS has been shown to increase CB_1 receptor mRNA expression in the PFC (Bortolato et al. 2007) and human suicides have increased CB_1 receptor density and signaling (Hungund et al. 2004). It will be very interesting to determine the neuronal site of these up-regulated receptors.

7 Clinical Implications for Endocannabinoid-Based Therapeutics for Anxiety and Depressive Disorders

The data reviewed above indicate that ECS has an anxiolytic function. Data from studies of unconditioned anxiety measures suggest that pharmacological augmentation of ECS could represent a novel approach to the treatment of generalized anxiety disorder, and anxiety symptoms associated with depressive disorders. Endocannabinoid augmentation could also be useful in the treatment of PTSD based on the role of ECS in stress response habituation (Patel and Hillard 2008) and enhancement of extinction of conditioned fear and anxiety.

Initial augmentation strategies have focused on inhibition of AEA catabolism by FAAH and endocannabinoid uptake inhibitors. Both of these approaches have been successful in preclinical models. Future drug discovery should be aimed at development of selective inhibitors of 2AG degradation, which could also have anxiolytic properties. It is likely that pharmacological augmentation of ECS will have several advantages over direct CB_1 receptor agonists including less likelihood of precipitating anxiety or panic reactions and less socio-political resistance to widespread clinical use. Lastly, these data suggest that the use of CB_1 receptor antagonists should be minimized in patients with anxiety disorders, due to an increased risk of exacerbating symptoms (Christensen et al. 2007).

The issue of treating depression with ECS-based therapies is far more murky. Human depression is a heterogeneous disease and only a fraction of those treated with conventional therapies have long-term disease remission. There are strong indications (discussed at length above) that ECS dysregulation could contribute to depression in some humans. The challenge to research at this stage is to further our understanding of both depression and ECS in order to elucidate which depressed patients will benefit from ECS-based therapy.

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