A Review of the Relationship between the Endocannabinoid System and the Reduction of Depression and Anxiety

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The growing acceptance of cannabis use for medical and recreational purposes across the world has sparked interest in evaluation of the therapeutic potential of the drug. While the beneficial effects of cannabis use to treat physical pain are well-known, its efficacy as a treatment for mental health disorders has not been as extensively investigated. As the most prominent and widespread of these disorders, depression and anxiety have been diagnosed in individuals across the world. Despite the wide range of severity for these disorders, tricyclic antidepressants and selective serotonin reuptake inhibitors are predominately prescribed to treat any case. However, these compounds are not always effective treatments, which leaves a need to investigate alternative treatment options for depression and anxiety. This review article aims to identify prominent research studies focused on evaluating the potential of the human endocannabinoid system, which consists of two main cannabinoid receptor subtypes (CB1 and CB2). Prior studies have focused on the use of exogenous cannabinoids such as oleamide or phytocannabinoids such as delta-9-THC, but this review gives more consideration to endocannabinoids that are produced by the human body. In addition to experiments testing the independent capacity of endocannabinoid receptor ligands as antidepressants, the additive and synergistic potentials of these ligands have been examined in conjunction with cholinergic receptor ligands through the use of mice FST. Recent studies have also indicated that certain genetic variants within the endocannabinoid system such as the CB1 rs1049353 G allele have been linked to increased prevalence of mental health disorders and provide a rationale for gender discrepancies in disorder incidence. Although current research into the prospective use of endocannabinoids as antidepressants is limited, this review details the field's most salient advancements toward potential clinical applications.

Abbreviations: CB1 – cannabinoid receptor type 1; CB2 – cannabinoid receptor type 2; delta-9-THC – delta-9-tetrahydrocannibinol; CBD – cannabidiol; FST – forced swim test

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Introduction

Across the world, depression affects individuals from different ages, ethnicities, and socioeconomic classes. This condition can range in severity from increasing an individual's reclusiveness to causing severe disruptions in an individual's daily life, sometimes resulting in fatal suicide attempts. The disease is difficult to diagnose because symptoms are difficult to measure and there are many distinct disorder subtypes (Brigitta, 2002). Females are generally twice as likely to develop depression when compared to males and approximately twenty percent of the world's population has the condition for some period during their lifetime. Additionally, the recurrence of depression after recovery exacerbates the risk of cardiac disease and other psychiatric disorders (Brigitta, 2002). Anxiety, like depression, has emerged in a sizable portion of the United States' population (over 13 percent) and also has its own set of debilitating

symptoms. Specifically, anxiety has been linked to increases in unproductivity, drug abuse, and even mortality rates, which often persist due to lack of individualized management beyond a primary care provider (Bystritsky, 2013). The ubiquitous nature of depression and anxiety as well as their correlation to potentially fatal health outcomes has sparked research efforts to identify risk factors and understand their biological basis. This review manuscript aims to provide an in-depth overview of research on the endocannabinoid system as a treatment option to improve the health outcomes of individuals with mental disorders.

Research has outlined that depression emerges from a dynamic combination of stressful life events and genetic predispositions that is specific to each patient. Stressful life events have been repeatedly found to increase depressive symptoms in adults and children, which has led to the development of the stress sensitization hypothesis that "subsequent episodes [of depression] require less stress to elicit a depressive recurrence" (Shapero et al., 2014, 2). The stress sensitization hypothesis has been supported in cases where childhood emotional abuse is the stressful life event, as these individuals exhibited more symptoms of depression during adulthood than the general population (Shapero et al., 2014). Thus, it is known that life events have a role in causing depression, but it is difficult to quantify the correlation between the stress of an event and the severity of the condition.

Depression has a projected heritability of sixty percent, revealing that genetics are likely to have an influence on the conditions' emergence (Wang et al., 2015). In addition, genetic regions have emerged that appear to deviate solely in patients with depression conditions, such as the T-182C polymorphism in the "5' promoter and coding region of the norepinephrine transporter (*NET)* gene" (Wang et al., 2015, 4). Experiments that have attempted to identify genetic loci responsible for the onset of depression never reach a consensus, which elucidates the complexity of depression as a preventable condition. Given the difficulty of managing the risk factors for depression, most research has instead focused on understanding the biological

pathways that are affected in patients for possible treatment.

The predominant biological theory for depression and other affective disorders is the monoamine hypothesis. Monoamines, including serotonin and dopamine, stimulate G protein-linked receptors on postsynaptic neurons to respond to neurotransmitters responsible for affecting brain responsiveness (Kalia, 2005). Pharmacologically, the monoamine hypothesis has been supported, as reserpine-like drugs that deplete monoamines have been found to promote depression. Also, the brains of suicidal individuals had levels of serotonin (5-HT) content that were comparable to controls (Kalia, 2005). The identification of low serotonin levels being correlated to depression has influenced the types of drugs that have been manufactured to treat the condition.

Tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors (MAOIs) were the primary option for the treatment of depression, until the more recent development of selective serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs). While these compounds have the same basic biological mechanism involving the "inhibition of reuptake of monoamine neurotransmitters from the synaptic cleft," TCAs and MAOIs have distinct "degrees of direct receptor antagonist activity" that cause them to have more detrimental side effects (Bodkin et al., 2007). SSRIs have been developed to have less hazardous side-effect profiles and higher serotonin specificity than the traditional TCAs; this has led to them being prescribed more often by physicians (Sangkuhl et al., 2009). SSRIs, however, are more expensive to produce and administer than TCAs. Furthermore, meta-analyses revealed there was negligible difference in efficacy and side effects between the two classes (Anderson, 2000). In the case of anxiety treatment, SSRIs and SNRIs are also the most commonly administered classes of medications. However, the effectiveness of SNRIs largely varies based on the patient case and SNRI treatment can even result in a worsening of physiological symptoms (Bystritsky, 2013). These findings reveal that SSRIs and SNRIs may not have been as revolutionary a development in

the treatment of depression or anxiety as previously hoped.

Another issue complicating the treatment of depression and anxiety is the existence of patient cases where the condition is unresponsive to treatment with conventional TCAs or SSRIs. In these cases, physicians have switched the type of TCA or SSRI being used, but the response to this switch is limited. The maximum increase in patients' improved response was twenty-seven percent when switching between TCA compounds and seventy-five percent when switching between SSRI compounds (Tundo et al., 2015). While these two classes may treat most cases of depression, there exists a need for the discovery and production of a therapeutic alternative.

The endocannabinoid system has demonstrated some potential to be a novel target for the development of this alternative. In order to further understand the novelty of using endocannabinoids as antidepressants, it is important to differentiate between exogenous cannabinoids, phytocannabinoids, and endogenous cannabinoids (endocannabinoids). Exogenous cannabinoids refer to any cannabinoid compound that originates outside of the human body and includes those that are pharmaceutically manufactured or stem from plants (phytocannbinoids). Contrarily, endocannabinoids refer to cannabinoid compounds that originate from the human body.

There are currently two known subtypes of cannabinoid receptors in the human body: CB1 and CB2 receptors (Kruk-Slomka et al., 2015). CB1 receptors are the more abundant subtype and are concentrated in the central nervous system (CNS), especially the limbic system and brain areas associated with emotion (Kruk-Slomka et al., 2015). Contrarily, CB2 receptors are mostly found in peripheral tissues of the immune system, but have also been discovered to exist in the cerebellum and the hippocampus (Kruk-Slomka et al., 2015). Both receptor subtypes belong to the same class of G-protein coupled receptor that regulates the release of neurotransmitter when stimulated (Huang et al., 2016). The location of these receptor subtypes and their mechanism of action involving neurotransmitter inhibition have created speculation that they could be involved in the treatment of depression. There has also been more direct biological evidence that these receptor subtypes might be involved in the development of depression as well. Studies have indicated that CB1 and CB2 receptors genetic polymorphisms have been associated with depression and even treatment resistance in some depression patients (Huang et al., 2016). Although cannabis has been mostly restricted to a recreational context because of existing stigma, behavioral mice studies revealed that their coping mechanisms for fear and stress involved upregulation of their endocannabinoid system (Alger, 2013). This biological evidence of the endocannabinoid system being altered in patients with depression and behavioral evidence of the endocannabinoid system being involved in the extinction of stress validates investigation into whether endocannabinoids and/or exogenous cannabinoid ligands can act as effective antidepressants.

There are two primary endocannabinoids identified in the human body, anandamide and 2-arachidonyl glycerol, whose role as ligands in the endocannabinoid system have been studied extensively. Anandamide has unique properties in that its concentration determines the number of receptors that are activated and that it can both make and destroy short-term neuronal connections related to memory (Sallaberry and Astern, 2018). Although anandamide is considered to have analgesic potential because of its high affinity for CB1 receptors, this affinity and its role in memory have led to its consideration for the treatment of psychological disorders like post-traumatic stress disorder (PTSD) (Sallaberry and Astern, 2018). These properties of anandamide further lend support to the mice study mentioned earlier in which the endocannabinoid system has been identified as part of the coping mechanism to deal with anxiety or depression. 2-arachidonyl glycerol (2-AG) is the most prevalent endocannabinoid present in the human body, but unlike anandamide it is considered both a CB1 and CB2 receptor agonist with a more prominent role in anti-inflammation as part of the human immune response (Sallaberry and Astern, 2018). Although both of these endocannabinoids have the ability to stimulate cannabinoid receptors, their relevance in preventing the uptake of serotonin and acting as antidepressants is still being explored.

While the endocannabinoid system has shown potential to be involved in the treatment of depression, there have been studies depicting cannabis use as a cause of depression rather than a treatment. A longitudinal study conducted in New Zealand revealed that increased cannabis usage between the ages of fourteen and twenty-one was associated with statistically significant reductions in reported satisfaction with life, which is a common symptom of depression (Fergusson and Boden, 2008). This illustrates a lasting effect of cannabis on brain chemistry beyond the period of frequent ingestion as depression was still observed at a later stage in life. Although this study could be criticized for its qualitative nature, other experiments have found that acute administration of delta-9-tetrahydrocannabinol (THC), the main psychoactive component of cannabis and a CB1 receptor agonist, increased "subjective ratings of anxiety" and led to a "greater response to fearful than to neutral faces" (Bhattacharyya et al., 2017, 6-12). These studies pose a threat to the viability of endocannabinoids being used as substitutes for antidepressants because they reveal CB1 receptor agonists that have caused symptoms regularly associated with depression. However, the administration and dosing of endocannabinoids that would be designed as antidepressants varies significantly from the recreational conditions in the longitudinal study and the acute conditions of the delta-9-THC study. Given the possibility that endocannabinoid system may provide an alternative treatment options for depression and anxiety, the conflicting results from existing studies warrant extensive research to unearth whether there is therapeutic potential.

State of the Field: Investigating the Endocannabinoid System

As the stigma surrounding marijuana use for medicinal purposes has decreased over the last couple decades, research has focused on examining the use of both endocannabinoids and exogenous, synthetic cannabinoids for the treatment of diseases ranging from Alzheimer's disease to cancer. Since the CB1 and CB2 receptor subtypes of the endocannabinoid system have been found to have a role in CNS areas associated with emotion, many of these research studies have focused on understanding the role of these receptors in the reduction or treatment of depression and anxiety. In this section, some of these studies will be evaluated to provide a more thorough understanding of how relevant the endocannabinoid system is to the manufacturing of cannabinoid receptor-based antidepressants.

CB1 Receptor Agonists and Antagonists' Effects on Anxiety

In 2006, a study at the Medical College of Wisconsin evaluated the effects of direct and indirect CB1 receptor agonists and antagonists on anxiety-related behaviors in mice using the elevated-plus maze paradigm. This study was particularly noteworthy because it deviated from solely testing the exogenous cannabinoids that had been the focus of previous studies. Instead, the study used multiple drugs belonging to each class of agonist and antagonist with a range of doses to evaluate the role of endocannabinoid signaling in mediating anxiety. The mice were housed in treatment groups of up to ten mice, each mouse was tested once in the elevated-plus maze. The design of the maze consisted of a central platform with two open and two enclosed arms extending off the platform (Patel and Hillard, 2006). The percentage of time spent in open arm exploration was the primary indicator of reduced anxiety, but percentage and number of open arm entries (four paws placed on the platform) were also measured as indicators of reduced anxiety. Contrarily, closed arm exploration along with percentage and number of closed arm entries were measured to be markers of anxiety-like behaviors (Patel and Hillard, 2006).

The two CB1 receptor agonists selected for the study significantly increased the percentage of time spent in open arm exploration. CP 55,940, an effective CB1 receptor agonist, increased all three components of open-arm exploration that were deemed to be associated with reduced anxiety. Furthermore, this reduction in anxiety was visible at almost all concentrations across the dosing range (Patel and Hillard, 2006). These findings were replicated by another high-efficacy agonist, WIN 55212-2, at dosages of 1 and 3 mg/kg, but were not supported by treatment of the mice with THC across all

dosages (Patel and Hillard, 2006). Expectedly, both of the CB1 receptor antagonists AM251 and SR141716, had an adverse effect on anxiety-like behaviors. At 3 and 10 mg/kg of both drugs, the time spent in open arm exploration was significantly decreased, which indicates the promotion of anxiety-like behaviors (Patel and Hillard, 2006). Finally, the study tested the effects of pharmacological augmentation on endocannabinoid signaling through the use of two modulators: URB597 and AM404. At 0.1 and 0.3 mg/kg of URB597 along with 1 and 3 mg/kg of AM404, there was a significant increase in time spent in open arm exploration that was similar to the effects of the high-efficacy CB1 receptor agonists (Patel and Hillard, 2006). This study shows a statistically significant dichotomy in anxiety-like behaviors between CB1 receptor agonists and antagonists. However, there does seem to be some importance regarding the structure and type of receptor ligand being used, as THC failed to decrease anxiety-like behavior. Furthermore, this study's finding that the augmentation of endocannabinoid signaling is sufficient for significant reduction of anxiety-like behavior proposes another mechanism of treatment that may be effective in humans.

Phytocannabinoids Act on Endocannabinoid System as Antidepressants

Since most research studies focus on the evaluation of synthetic CB1 ligands for anti-depressant action, researchers at the University of Mississippi focused their 2010 study on elucidating the role of phytocannabinoids like delta-9-THC, delta-8-THC, cannabidiol (CBD), cannabigerol (CBG), cannabinol (CBN), and cannabichromene (CBC) that are more commonly found in the types of cannabis ingested by recreational users. In this study, these phytocannabinoids were obtained from potent *Cannabis sativa*, and tested alongside desipramine and fluoxetine hydrochloride for comparison to TCAs and SSRIs (El-Alfy et al., 2010). Eight-week-old mice were injected intraperitoneally with either a vehicle control or the assigned dosage of a test compound prior to participation in either a mouse tetrad assay, forced swim test (FST), or tail suspension test (TST). The mouse tetrad assay measuring rectal temperature and locomotor activity was a vital control because it determined the doses of treatments that caused hypothermia or catalepsy, respectively. These doses were subsequently removed prior to testing via the FST or TST (El-Alfy et al., 2010). The FST consisted of the mice being placed in a cylinder filled with water, and then measuring subsequent locomotor activity and immobility. The TST involved hanging mice by the tail after treatment and then measuring the time each mouse spent motionless (El-Alfy et al., 2010). In the FST and TST, locomotor activity was classified to be a marker of anti-depressant activity and immobility was a marker of a depressed state.

As expected, both the TCA desipramine and the SSRI fluoxetine caused significant decreases in FST immobility times. While delta-9-THC and delta-8-THC revealed non-significant reductions in immobility, CBD and CBC caused a significant reduction in mice immobility, which suggests that these compounds have antidepressant potential (El-Alfy et al., 2010). Both CBG and CBN failed to cause significant change in mice locomotor activity in the FST when compared to the control, which weakens their potential to act effectively as antidepressants. In the TST, delta-9-THC and CBC caused a significant decrease in immobility time, but none of the compounds caused a significant change in locomotor activity (El-Alfy et al., 2010). The highly predictive nature of the FST and TST procedures underscores that certain phytocannabinoids have the potential to cause antidepressant action. Specifically, delta-9-THC and CBC were successful in reducing immobility in both procedures, which entails further investigation into the antidepressant potential of these compounds. Even though delta-8-THC behaved similarly to delta-9-THC in the mouse tetrad assay, delta-8-THC did not demonstrate anti-depressant effects at any dose. A significant difference between delta-8-THC and delta-9-THC is their binding affinity for the CB1 receptor; in-vitro assays have revealed that delta-8-THC's binding affinity is three-fold lower than delta-9-THC (El-Alfy et al., 2010). Thus, an indirect but important finding of this study is the role of cannabinoid receptor ligand's binding affinity for the CB1 receptor in developing a cannabinoid receptor-based antidepressant.

Another salient takeaway from this study is that CBD, the non-psychoactive component of cannabis, exhibited antidepressant activity in the FST even though it is a "potent antagonist of CB1 and CB2 receptors agonists" (El-Alfy et al., 2010, 440). This shows that there is a need for further investigation into the role of CB2 receptors, as the antidepressant activity was observed through the administration of the CB2 agonist.

Endocannabinoid and Cholinergic Receptor Ligands Acting in Conjunction

Researchers at the Medical University of Lublin in Poland evaluated the antidepressant efficacy of CB1 and CB2 receptor ligands alone and in combination with cholinergic receptor ligands (nicotine and scopolamine), as the cholinergic system has previously been thought to be involved in the antidepressant activity of endocannabinoids. Similar to the previous study, the FST was used to evaluate depression-related responses and experimental groups were designed with eight to ten mice. The cannabinoid receptor ligands chosen were oleamide (CB1 receptor agonist), AM 251 (CB1 receptor antagonist), JWH 133 (CB2 receptor agonist) and AM 630 (CB2 receptor antagonist). Each ligand was administered via intraperitoneal injection at one of four selected concentrations. The cholinergic receptor ligands chosen were nicotine hydrogen tartrate (a nicotinic acetylcholine receptor – nAChR agonist) and scopolamine hydrochloride (a muscarinic acetylcholine receptor – mAChR agonist) (Kruk-Slomka et al., 2015).

In the first phase of the experiment, CB1 and CB2 receptor agonists and antagonists were tested independently in the FST. Oleamide and JWH 133, the cannabinoid receptor agonists, both caused a statistically significant decrease in mice immobility times during the FST, which reveals that they both exhibited an antidepressant effect. However, while the CB1 receptor antagonist, AM 251, did not reduce immobility times, the CB2 receptor antagonist, AM 630 (at a concentration of 0.5 mg/kg), caused a statistically significant decrease in immobility times when compared to the control (Kruk-Slomka et al., 2015). These initial findings are intriguing because they suggest that the antidepressant

activity of the CB2 receptor may be bidirectional as both its agonists and antagonists displayed antidepressant potential.

In the second phase of the experiment, co-administration of non-effective doses of antagonists with effective doses of agonists was performed for each receptor subtype. For both oleamide and JWH 133, immobility times in the FST were still significantly reduced, but AM 251 and AM 630 attenuated the strength of this reduction for each agonist, respectively (Kruk-Slomka et al., 2015). While these results may be expected for the CB1 receptor antagonist, the attenuation of antidepressant activity by the CB2 receptor antagonist when administered with both the CB1 and CB2 receptor agonists is contradictory to its previously observed independent antidepressant effect. This directly reflects the complexity and subtlety of the endocannabinoid system's involvement in the reduction of depression and supports the notion that there is a biological interaction between the receptor subtypes when simultaneously activated.

The third part of the experiment evaluated the antidepressant effects of the cholinergic receptor ligands both individually, and in combination with both effective and non-effective doses of the cannabinoid receptor ligands. Both the nAChR ligand nicotine and the mAChR ligand scopolamine significantly reduced immobility times in the FST, which demonstrates their antidepressant potential (Kruk-Slomka et al., 2015). Pretreatment with cannabinoid receptor ligands had no significant effect on the action of nicotine. However, the administration of some CB compounds at non-effective concentrations before a non-effective dose of scopolamine did have an antidepressant effect (Kruk-Slomka et al., 2015).

This finding is of particular significance in distinguishing the biological mechanisms involved in the antidepressant action of endocannabinoids. While endocannabinoids did not improve the antidepressant effect of nicotine (an agonist of nAChRs), it did improve the antidepressant effect of scopolamine (an antagonist of mAChRs). This may reveal that endocannabinoids actually engage the activation of nAChRs, which aligns with the earlier finding that they exhibit antidepressant activity when in

combination with a mAChR antagonist. From this study, the key takeaways are the complexity surrounding the antidepressant potential of the CB2 receptor and the potential for synergistic combinations of endocannabinoids with cholinergic ligands or other classical antidepressants.

The Potential of Endocannabinoids to Reduce Anxiety

While the previous studies have mainly focused on the therapeutic potential of exogenous CB1 and CB2 receptor ligands, some research has focused more on the potential of endogenous cannabinoids to reduce anxiety. A group of researchers at Johannes Gutenberg-University Mainz in Germany have attempted to explore the efficacy of endogenous cannabinoids by inhibiting the action of fatty acid amid hydrolase (FAAH). FAAH is an enzyme responsible for the degradation of endocannabinoids like anandamide and 2-AG, and inhibitors of FAAH have been developed to increase brain levels of anandamide (Moreira et al., 2008). As an endocannabinoid, anandamide is distinct from many synthetic cannabinoid receptor ligands that operate solely through CB1 receptor activation because it is a neuromodulator (Moreira et al., 2008).

This study aimed to test whether FAAH knockout mice would present reduced anxiety-like behavior when compared to wild-type mice in the elevated-plus maze and a light dark test (LDT). The elevated-plus maze was designed similar to the previously discussed experiment, and the LDT was conducted in a box divided into a dark and lit compartment (with or without a roof, respectively) (Moreira et al., 2008). The percentage of entries and time spent in the open arms of the elevated-plus maze and the percentage of time spent in the lit compartment of the LDT were measured and classified as reduced anxiety-like behavior (Moreira et al., 2008). The experiment was conducted in two main phases, one using genetic inactivation and the other using pharmacological inhibition of FAAH.

In the phase of the experiment involving genetic inactivation, the FAAH knockout mice were found to have reduced anxiety-like behavior in the elevated-plus maze when compared with wild-type mice (Moreira et al., 2008). The study also tested diazepam, a compound commonly used to treat anxiety, to set a benchmark for reduced anxiety-like behavior of the mice in the elevated-plus maze. Although FAAH knockout mice statistically reduced anxiety-like behavior when compared to the wild-type mice, they still had many more entries into the enclosed arms (16.10 average entries) than mice treated with diazepam (9.38 average entries).

The researchers then tested the effect of the addition of rimonabant, a CB1 receptor antagonist, on the behavior of the FAAH knockout mice in the elevated-plus maze. Despite rimonabant treatment, the FAAH knockout mice still resided in the open arms for significantly longer than the other groups (Moreira et al., 2008). Theoretically, if CB1 receptor activation was the primary mechanism through which endocannabinoids were reducing anxiety-like behavior, they would no longer exhibit reduced anxiety-like behavior upon the introduction of a CB1 receptor antagonist like rimonabant. The results of the FAAH knockout mice in the elevated-plus maze displaying reduced anxiety-like behavior were replicated in the LDT as the FAAH knockout mice were drawn to the illuminated section of the apparatus more than the wild-type mice (Moreira et al., 2008). Although this held true upon the addition of rimonabant, there was a significant effect of the drug on the behavior of the FAAH knockout mice as there was reduced exploration of the lit compartment (Moreira et al., 2008). This indicates that even if activation of CB1 receptors is not the only mechanism through which endocannabinoids reduce anxiety-like behavior, it is responsible for a significant portion of endocannabinoid's therapeutic potential in treating anxiety and depression.

In the phase of the experiment involving pharmacological inhibition, an FAAH inhibitor URB597 was administered to a treatment group at a concentration of 1 mg/kg prior to testing in the elevated-plus maze or LDT. Through this experiment, it was discovered that the CB1 receptor facilitates the anxiolytic qualities of FAAH inhibition (Moreira et al., 2008). Furthermore, the inhibition of FAAH by URB597 did not result in FAAH-inhibited mice spending more time in the lit compartment during the LDT,

which reveals a failure to reduce anxiety-like behavior when compared to wild-type mice (Moreira et al., 2008). When comparing the two phases of this study, it is apparent that the genetic inactivation of FAAH has more therapeutic promise than the pharmacological inhibition of FAAH. Although the genetic inactivation of FAAH did not reduce anxiety-like behavior as effectively as the conventionally-used diazepam, its endogenous therapeutic mechanism validates further exploration because of its reduced side-effect profile and natural occurrence. This study has underscored the importance of exploration into endocannabinoids as neuromodulators that may have therapeutic mechanisms in the treatment of anxiety beyond CB1 receptor activation.

The Potential of Endocannabinoids to Reduce Depression

Beyond the consideration of endogenous and exogenous cannabinoid receptor ligands for their potential as antidepressants, other studies have centered on studying the genetic variations in the endocannabinoid systems of patients with depression. One such study was conducted as a collaborative effort between researchers in Germany and Australia, who collected a sample of 256 depression patients at the University of Muenster. The Hamilton Depression Rating Scale (HAM-D 21) was used to track the weekly self-reported depression level of each patient throughout their individualized treatment regimen, which consisted of numerous classical antidepressants including citalopram, venlafaxine, and other TCAs or MAOIs (Domschke et al., 2008). From the 256 patients, exclusion criteria were followed to create a subset of 33 patients that would undergo a fMRI imaging study aimed at examining particular CB1 gene variants (Domschke et al., 2008). Despite the lack of previous literature on the subject, the researchers selected CB1 rs1049353 variant because of its relation to neuronal striatal activity in response to depression stimuli and CB1 rs12720071 variant because of its relation to substance abuse (Domschke et al., 2008). As part of the fMRI study, the subjects "viewed alternating 30 second blocks of masked happy, sad, angry, and neutral facial stimuli interleaved with no-face stimulus baseline blocks"

(Domschke et al., 2008, 753). Specific brain regions including the amygdala and thalamus were regions of interest.

From the HAM-D 21 score tracking, patients were found to respond worse to antidepressant treatment if they possessed the CB1 rs1049353 G allele rather than the homozygous rs1049353 AA genotype (Domschke et al., 2008). This outcome is unsurprising given the prior finding that this CB1 variant had been linked to earlier depression stimuli. However, when the results were stratified by gender, this trend was only visible in the subsample of female rather than male patients (Domschke et al., 2008). The gender-based discrepancy in the therapeutic effectiveness of antidepressants because of the presence of the CB1 rs1049353 G allele reflects the repeated finding that females are almost twice as likely as males to become depressed during their lifetime. Specifically, this genetic polymorphism may be a direct mechanism of heritability that is responsible for the higher incidence of depression in females. These results were replicated in the fMRI study as individuals with the homozygous rs1049353 AA genotype had stronger brain area activity to masked happy faces than those with the variant G allele (Domschke et al., 2008). One consideration affecting these results is that the detrimental effect of the CB1 G allele variant on antidepressant treatment was evidenced only in patients with high anxiety (Domschke et al., 2008). The interplay between the effects of genetic polymorphisms on the coding of CB1 receptors and the agonists of non-variant CB1 receptors on the development of anxiety must be further evaluated. Unlike the CB1 rs1049353 variant, the rs12720071 genotype had no significant effect on response to antidepressant treatment in both the analysis of HAM-D 21 scores and fMRI imaging (Domschke et al., 2008). Overall, this study has unearthed another manner in which the endocannabinoid system may be involved with depression and its treatment beyond just its receptor ligands. Research should focus on developing a more complete understanding of the network of endocannabinoid receptors' genetic polymorphisms that are related to the treatment of depression.

Discussion and Conclusion

The current body of research investigating the antidepressant and anxiolytic properties of endocannabinoids and synthetic cannabinoid receptor ligands has revealed that there exists therapeutic potential for these compounds in the treatment of disease. While anxiety and depression are distinct conditions, their comorbidity has been replicated in numerous research studies. While uncertainty remains regarding the exact biological pathways through which cannabinoid receptors act to reduce anxiety and/or depression, there have been significant developments in the medical research community's understanding of the endocannabinoid system. CB1 receptor agonists and antagonists have consistently been linked to beneficial and adverse effects, respectively, on the treatment of depression and anxiety in mouse behavioral studies. The role of the CB2 receptor, however, seems much more complex as both agonists and antagonists have shown beneficial effects when administered independently, but vary when administered in conjunction with each other or with CB1 receptor ligands.

While most research has prioritized the development of synthetic cannabinoid receptor ligands for their therapeutic potential, endocannabinoids (specifically anandamide) offer a novel treatment option as neuromodulators beyond their role as CB1 receptor agonists. Furthermore, endogenous cannabinoids exist within the human body and thus avoid the significant and sometimes severe side-effect profiles customary of most conventional antidepressants like SSRIs or TCAs. Phytocannabinoids, including delta-9-THC, cannabidiol, and cannabichromene that are often found in strains of recreational cannabis, have also demonstrated potential antidepressant and anxiolytic activity in mouse studies (El-Alfy et al., 2010). Much like endocannabinoids, phytocannabinoids pose less of a detriment to health than synthetic cannabinoid ligands because they are present in naturally growing strains of cannabis. Research has also revealed the potential for cannabinoid receptor ligands to exhibit synergistic antidepressant effects with other compounds, specifically muscarinic acetylcholine receptor antagonists like scopolamine (Kruk-Slomka et al., 2015).

Although the majority of research surrounding the endocannabinoid system and the treatment of depression/anxiety is focused on the types of receptor ligands best suited to create an antidepressant/anxiolytic effect, some research has emerged studying the relation of cannabinoid receptor genetics to antidepressant treatment response. Research has discovered that certain genetic polymorphisms like the CB1 rs1049353 G allele directly hamper the efficacy of antidepressants (Domschke et al., 2008). Additionally, this particular genetic variant appears to be a uniquely female issue, which may elucidate the increased incidence of depression in women and provides a theoretical mechanism of heritability for the disease. Despite these significant advancements in the field of cannabinoid receptor ligands and the treatment of depression/anxiety, there are still intriguing gaps in our understanding that may translate into viable human treatments.

More research should be performed to understand the therapeutic advantages to endocannabinoids versus synthetic cannabinoid ligands. While synthetic cannabinoid ligands can be varied in structure and chemical makeup to enhance various therapeutic components like binding affinity, there are other advantages to endocannabinoids that should be investigated. Endocannabinoids are naturally occurring in the human body and thus inherently pose less of a risk to the overall health of a patient. Furthermore, endocannabinoids are neuromodulators that can bind with a variety of other brain areas, so they are not entirely reliant on CB1 receptor activation for their therapeutic potential. Additionally, the genetic inactivation of FAAH may attribute greater therapeutic ability to endocannabinoids since CB1 receptor antagonists were found to have a minimal adverse effect on endocannabinoids' antidepressant activity (Moreira et al., 2008). The development of a synthetic cannabinoid receptor ligand that is able to overcome an effective dose of a CB1 receptor antagonist is a significant pharmacological challenge that endocannabinoids naturally overcome. Additionally, the other therapeutic mechanisms through which endocannabinoids have an

anxiolytic effect should be investigated as an alternative for patients who are unresponsive to treatment with CB1 receptor agonists. Finally, studies should evaluate which types of existing compounds have the most potential for synergistic effects with cannabinoid receptor ligands.

Further research is needed into the field of genetic polymorphisms and variants in the coding of the two cannabinoid receptor subtypes. Given the finding that a single genetic polymorphism in the CB1 allele correlated to a gender-specific incidence of failed antidepressant treatment, this type of research has an immense potential to evaluate mode of inheritance for cannabinoid receptor genetic variants. A glaring complication of the discovery of cannabinoid receptor polymorphisms in patients is how the treatment of the condition can be altered to improve outcomes. Moreover, studies should be catered to understand how the presence of genetic polymorphisms in the makeup of cannabinoid receptors affects the curative prospect of endocannabinoids and synthetic cannabinoid receptor ligands for conditions like anxiety and depression. The role of the CB2 receptor subtype needs to be thoroughly evaluated. Currently, the agonists of this receptor seem to have therapeutic potential, but the antagonists vary based on the other elements of the treatment group (Domschke et al., 2008). While CB1 receptor agonists seem to be more clearly linked to antidepressant effect, the potential of the CB2 receptor subtype should not be ignored especially given the possibility of treatment-resistant depression. The breadth of discussed studies highlighting the possibly therapeutic relationship between the endocannabinoid system and anxiety and depression validates the need for thorough research in the field to continue. Patients with severe mental disorders who do not respond to classical antidepressants would benefit tremendously from this research into the prospective applications of the endocannabinoid system.

Acknowledgements

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