The Endocannabinoid System and Its Therapeutic Potential

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The endocannabinoid system is a popular topic in regards to creating therapeutics for a multitude of nervous system disorders. The endocannabinoid system, a naturally occurring system in the human body, plays a key role in neuromodulation of the body's nervous system. It is believed that dysfunction of the endocannabinoid system may lead to the pathogenesis of nervous system disorders. Alternatively, exploitation of this system may pose therapeutic benefits for individuals who have certain disorders. CB_2 receptors have been implicated in both Parkinson's and Alzheimer's Disease. CB_2 receptors on microglia in the substantia nigra and striatum have been implicated in neuroprotection via anti-inflammatory processes, suggesting a role in Parkinson's Disease. $A\beta$ plaque deposition, a hallmark of Alzheimer's Disease, has also been shown to be related to cannabinoid CB_2 receptors in the central nervous system. However, CB_2 receptors were not shown to be involved in tau hyperphosphorylation along with $A\beta$ plaque deposition, suggesting that focusing on the endocannabinoid system may not be best for development of a therapeutic for Alzheimer's Disease. The widespread neuromodulatory role of the endocannabinoid system has shown promise in acute and chronic pain regulation, but the presence of cannabinoid receptors on both excitatory and inhibitory neurons demonstrates a need for further research that is more directed towards specific receptors. Lastly, the endocannabinoid system has shown promising anxiolytic effects even though the mechanism of anxiety is still not fully understood. Overall, recent literature has shown promise for endocannabinoid-related therapeutic targets in many disorders, but further investigations are necessary.

Abbreviations: CB_1 – cannabinoid receptor type 1; CB_2 – cannabinoid receptor type 2; Aß – Amyloid-ß; GPCR – G protein-coupled receptor; CNS – central nervous system; PNS – peripheral nervous system; FAAH – fatty acid amide hydrolase; MAGL – monoacylglycerol lipase; LPS – lipopolysaccharide; BCP – ß-caryophyllene; PD – Parkinson's Disease; AD – Alzheimer's Disease

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Introduction

The naturally-growing *Cannabis sativa* plant, more commonly known as marijuana, has historically been used in the treatment of chronic pain. However, scientific research investigating the interactions between the chemical compounds of this plant and the human body did not begin until the 1960's, when Western cultures began to use it recreationally (Marzo et al., 2004; Zuardi, 2006).

In 1970, the United States implemented the Comprehensive Drug Abuse Prevention and Control Act which re-categorized marijuana to be separate from other narcotics and repealed mandatory minimum sentences for possession of small amounts of marijuana. As the attitude towards marijuana became more lenient during this time in the United States, a survey in 1979 showed that 27.6% of adolescents and young adults aged 12 to 25 used marijuana within the previous 30 days (Yu et al., 2020).

In the 1980's and early 1990's, Presidents Ronald Reagan and George H. W.

Bush re-established harsh drug penalties with Reagan signing the Anti-Drug Abuse Act and Bush declaring a War on Drugs. Coinciding with these presidential actions, in 1992, only 8.1% of adolescents and young adults surveyed stated they used marijuana within the previous 30 days (Yu et al., 2020).

In 1996, California became the first state to legalize the use of marijuana for medical purposes. Since then the societal view of marijuana has become more accepting as 36 states have legalized medical marijuana and 18 states have legalized recreational use of marijuana. In 2016, the number of adolescents and young adults surveyed who stated they used marijuana within the previous 30 days rose back to 14.7% (Yu et al., 2020). Additionally, while the age at which adolescents first use alcohol and/or cigarettes is increasing, the age at which they first use marijuana is stable (Keyes et al., 2019).

The increased legalization of marijuana and discoveries of cannabinoid receptors and ligands sparked further research into the newly termed, "endocannabinoid system" to determine the system's potential therapeutic benefits. Research investigating potential therapeutics involving the endocannabinoid system requires a knowledge of both exogenous and endogenous cannabinoids. Exogenous cannabinoids are substances that originate from outside of the body, such as drugs, that produce a biological effect on cannabinoid receptors. The main psychoactive component of marijuana, Δ9-tetrahydrocannabinol (THC), is an exogenous cannabinoid and it was one of the first compounds to be isolated and characterized in its effects (Gaoni & Mechoulam, 1964). Following the examination of THC, two membrane-localized cannabinoid receptors (CB_1 and CB_2) were also identified within the human body (Pertwee, 1997). Substances that exist naturally within the body and produce a biological response on these cannabinoid receptors are called endogenous cannabinoids (endocannabinoids). The most commonly studied endocannabinoids, N-arachidonoylethanolamine (anandamide) and 2-arachidonoylglycerol (2-AG), are the current focus of research to further understand the endocannabinoid system (Devane et al., 1992; Sugiuara et al., 1995).

The purpose of this review is to examine current literature on the endocannabinoid system in order to understand how the system functions and how it is involved in the neurological conditions of Parkinson's Disease, Alzheimer's Disease, chronic pain, and anxiety. Understanding how endocannabinoids function (or are dysfunctional) in these conditions is vital to determining how exogenous cannabinoids, such as marijuana and pharmaceuticals, can utilize this naturally-existing system to produce therapeutic effects.
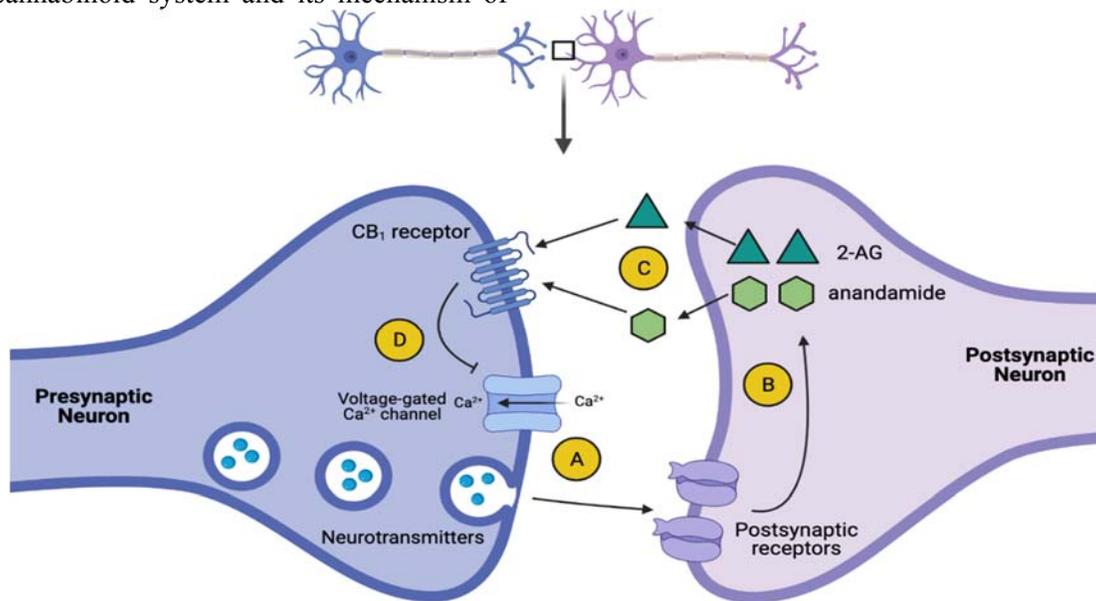
Understanding the Mechanism of the Endocannabinoid System

Before exploring the therapeutic potential of exploiting the endocannabinoid system, the mechanism by which it operates must be understood. The endocannabinoid receptor CB_1 is one of the most abundant types of G-protein-coupled receptors (GPCRs) in the central nervous system (CNS), but is also present in the peripheral nervous system (PNS) (Brady et al., 2012). CB_1 receptors are preferentially distributed on presynpatic neurons (Schlicker & Kathmann, 2001). The other major type of GPCR present in the endocannabinoid system, CB_2 receptors, are mainly found on blood cells and immune cells (Cabral & Griffin-Thomas, 2009). The endogenous substrates anandamide and 2-AG are synthesized by the postsynaptic neuron and exert mechanistic effects on CB_1 presynaptic receptors in a retrograde fashion (Survarna et al., 2016) (Figure 1B). The endocannabinoids are not stored in vesicles like other neurotransmitters, but are synthesized and released immediately into the synapse in response to physiological stimuli (Kano et al., 2009). Once in the synaptic cleft, hydrophobic endocannabinoids travel across the aqueous extracellular space through a mechanism that is not yet fully understood (Zou & Kumar, 2018). Then, the endocannabinoids interact with CB_1 receptors on the presynaptic neuron (Figure 1C). Through a GPCR signal, activated CB_1 receptors induce a cascading effect that inhibits voltage-gated Ca^{2+} channels (Wenzel & Cheer, 2018) (Figure 1D). Inhibition of voltage-gated Ca^{2+} channels stops the influx of calcium into the presynaptic neuron, which prevents

neurotransmitter release. Thus, the CB$_1$ receptors can elicit both short- and long-term reductions in presynaptic neurotransmitter release and postsynaptic firing frequency through the inhibition of presynaptic voltage-gated Ca^{2+} channels (Araque et al., 2017). Therefore, the endocannabinoid substrate exhibits an important neuromodulatory function. Once the neuromodulation by endocannabinoid substrates has occurred, the substrate is degraded. Fatty acid amide hydrolase (FAAH) is responsible for the degradation of anandamide (Alasmari et al., 2019). 2-AG is degraded by monoacylglycerol lipase (MAGL) (Grabner et al., 2017). Alternative enzymes participate in endocannabinoid metabolism, but FAAH and MAGL are the most common. Both anandamide and 2-AG are broken down into arachidonic acid and other compounds to fully inactivate the endocannabinoids in the synapse and allow the system to return to an unmodulated state.

Understanding the recently discovered endocannabinoid system and its mechanism of action is critical to understanding its potential association to nervous system disorders. Being widely distributed in the brain and throughout the periphery of the nervous system, the endocannabinoid system is involved in neuromodulation, as well as other functions such as homeostasis regulation, cytokine release from microglia, and synaptic plasticity (Cristino et al., 2019). Because the endocannabinoid system is involved in a multitude of functions, any dysfunction could lead to a wide variety of neurological disorders. The relatively recent identification of allosteric modulatory sites on cannabinoid receptors has opened the door for new therapeutic developments (Khurana et al. 2017). It is thought that enhancing or inhibiting endocannabinoid function, depending on the disorder, can provide therapeutic benefits to affected individuals.

Figure 1: A simplified mechanism of the neuromodulatory function carried out by the endocannabinoid system shows an action potential stimulating A) activation and influx of calcium through a voltage-gated calcium channel and subsequent neurotransmitter exocytosis across the synapse, B) synthesis of endogenous cannabinoids after postsynaptic receptor activation, C) diffusion of endocannabinoids into the synapse and retroactivation of CB$_1$ receptors, and D) inhibition of voltage-gated calcium channels and reduction in vesicular exocytosis of neurotransmitters.

The Endocannabinoid System and its Involvement in Neurological Conditions

Parkinson's Disease

One neurological disorder in which the endocannabinoid system is suggested to be involved with is Parkinson's Disease (PD). PD is characterized behaviorally by bradykinesia, tremors, muscle rigidity, and a shuffling gait (Dauer and Przedborski, 2003). Pathologically, PD is caused by the degeneration of dopaminergic neurons within the substantia nigra (Dauer and Przedborski, 2003). Cannabinoid receptors have been implicated in neuropathology and neuroprotection against the inflammation-induced degeneration observed with this disease (García-Arencibia et al., 2007). In regards to neuropathology, Navarrete et al. (2018) investigated the gene expression of three endocannabinoid system proteins: CB_1 receptors, CB_2 receptors, and MAGL. Levels of these three proteins within the substantia nigra and putamen of patients diagnosed with PD were compared to control samples (Navarrete et al). The authors used immunohistochemical and real-time PCR methodologies to determine localization and gene expression of these proteins (Navarrete et al., 2018). It was found that in the substantia nigra, CB_{2A} receptor gene expression was increased, MAGL gene expression was decreased, and CB_1 receptor gene expression was unchanged compared to controls (Navarrete et al., 2018). In the putamen, both CB_1 receptor and MAGL gene expressions were increased whereas CB_{2A} gene expression was decreased (Navarrete et al., 2018). The results by Navarrete et al. (2018) suggest that CB_1 receptors, CB_{2A} receptors, and MAGL are somehow involved in the neuropathology of PD, but their exact mechanisms are unknown.

In regards to the neuroprotective role performed by the endocannabinoid system, Gómez-Gálvez et al. (2016) examined how this system functions (or is dysfunctional) during neuroinflammation seen in PD. Neuroinflammation is observed pathologically in PD and is thought to be one of the causes of dopaminergic neuron death in the substantia nigra. In their study, Gómez-Gálvez et al. (2016) modeled PD in rodents via the injection of lipopolysaccharide (LPS) into the striatum, which causes inflammation and subsequent deterioration of dopaminergic neurons in the affected area. The striatal site lesions by LPS showed elevated levels of CB_2 receptors on the microglia that were recruited to the inflamed area (Gómez-Gálvez et al., 2016). In CB_2 receptor-deficient mice, the deterioration of dopaminergic neurons due to the LPS-induced inflammation is significantly greater (Gómez-Gálvez et al., 2016). Another study by Javed et al. (2016) further investigated the role of CB_2 receptors as they carry out neuromodulatory functions. Injection of rotenone into the striatum of a rat model mimicked PD in this study by inducing the loss of dopaminergic neurons (Javed et al., 2016). This model further investigated CB_2 receptor interaction in neuroinflammation by examining an endogenous CB_2 receptor agonist, ß-caryophyllene (BCP) (Javed et al., 2016). It was demonstrated that BCP reduced oxidative stress after rotenone injection by reducing the activation of proinflammatory cytokines, preventing the depletion of glutathione and increasing the number of antioxidant enzymes present (Javed et al., 2016).

All together, these studies provide evidence of CB_2 receptors on microglia being implicated in PD by demonstrating their neuroprotective function via their involvement in anti-inflammatory processes in both the striatum and substantia nigra. Directed targeting of CB_2 receptor-activation in these brain areas could prove to have therapeutic benefits by helping to reduce the inflammation and subsequent loss of dopaminergic neurons which are seen in PD pathology.

Alzheimer's Disease

Another neurological disorder in which the endocannabinoid system is suggested to play a role is Alzheimer's Disease (AD). AD is a progressive form of dementia that has major implications with memory, thinking, and behaviors. The common pathologies to confirm a diagnosis of AD include both amyloid plaques and hyperphosphorylated tau formation. Interestingly, when studying a rat model for AD to investigate a connection between AD and the endocannabinoid system, there was no trace of

CB_2 receptor expression in the "healthy" (without amyloid deposits) CNS (López et al., 2018). However, when neuroinflammation was induced by amyloid plaque formation, CB_2 receptors were observed to be upregulated in these areas (López et al., 2018). This effect is further shown in autopsies of post-mortem AD brains that reveal a significantly increased amount of CB_2 receptor expression in the microglia surrounding amyloid plaques (Aso & Ferrer, 2016). Another post-mortem AD brain study performed by Solas et al. (2013) carried out a correlational study between the endocannabinoid compounds and the AD pathology components. It was found that CB_1 receptors had significantly decreased expression in the AD brain (Solas et al., 2013). On the other hand, CB_2 receptor expression was found to be significantly increased (Solas et al., 2013). Interestingly, CB_2 receptor expression was correlated with the pertinent AD marker Aß42, but was not correlated with the cognitive status of the individual (Solas et al., 2013). This finding suggests that the pathogenesis of AD induces the expression of CB_2 receptors in microglia.

Research by Martín-Monero et al. (2011) provides a potential reason for this correlation between CB_2 receptor expression and aggregates of Aß42. In their study they found that the expression of selective CB_2 receptor agonist JWH-133 and mixed CB_1-CB_2 receptor agonist WIN55,212-2 resulted in microglial migration to the area of Aß aggregation (Martín-Monero et al., 2011). Additionally, JWH-133 and WIN55,212-2 expression resulted in the activated microglia phagocytosing the Aß plaques (Martín-Monero et al., 2011). These agonists have also been implicated in reducing proinflammatory cytokine levels, which could help alleviate inflammation experienced by Aß aggregates (Martín-Monero et al., 2011). In an AD mouse model, inducing a knockout of CB_2 receptors worsens the deposition of Aß plaques (Aso et al., 2016). In regards to the other major pathology observed in AD, knockout of CB_2 receptors has no effect on tau hyperphosphorylation (Aso et al., 2016).

Although CB_2 receptors are implicated in the deposition of Aß plaques, the fact that these receptors do not regulate both deposition of Aß plaques and tau hyperphosphorylation suggests that investigation into therapeutics for AD involving the endocannabinoid system may not be the best use of future research efforts.

Chronic Pain

One of the most commonly investigated research topics about the endocannabinoid system involves its role in pain production and suppression. Both acute and chronic inflammation are manifested as pain and it is thought that the endocannabinoid system, which is localized throughout nociceptive pathways, may be the solution to this undesirable sensation. Endocannabinoids are synthesized in response to increased activity levels, allowing them to exhibit antinociceptive effects through retrograde inhibition of presynaptic neurotransmitter release (Araque et al., 2017). Analgesic drug development of cannabinoid agonists has shown promise in the regulation of pain (Maldonado et al., 2016). Similar to the chemical compounds of endocannabinoid receptor agonists, such as anandamide and 2-AG, the drugs being studied are predicted to exhibit similar functions to endogenous cannabinoids at pain-inducing synapses (Maldonado et al., 2016). However, a problem lies in the fact that cannabinoid receptors are present in both excitatory and inhibitory neurons in the nociceptive pathways (Woodhams et al., 2017). Thus, cannabinoid agonists do not only have neuromodulatory effects on excitatory nociceptive pathways, but inhibitory ones as well. Therefore, it is not as simple as previously thought to synthesize a cannabinoid agonist or to fully inhibit degradative enzymes FAAH and MAGL. It has been suggested to inhibit multiple enzymes involved specifically in the degradation of endocannabinoids at lower dosages compared to full inhibition (Woodhams et al., 2017). Researchers continue to investigate regulation of nociceptive signal transmission as well as cytokine release onto local nerves in regards to the endocannabinoid system in hopes of finding a more effective analgesic for acute and/or chronic pain.

Anxiety

Another area of investigation when it comes to the endocannabinoid system and drug development is the system's involvement and regulation of emotional states such as anxiety. Anxiety disorders, though their mechanisms are

not yet fully understood, appear to occur due to improper neuronal activity in the limbic system. In recreational usage, marijuana is often used for the reduction of stress and anxiety, but the mechanism by which it performs this function is also not well understood. To investigate this mechanism, Bedse et al. (2017) altered levels of 2-AG and anandamide while monitoring behavior and electrophysiological data. The authors showed that depletion of anandamide in the limbic system resulted in anxiety-like behaviors in mice (Bedse et al., 2017). These behaviors were able to be restored to "normal" via the administration of 2-AG to this same area (Bedse et al., 2017). Another rodent study aimed at the inhibition of FAAH resulted in rapid onset and enduring anti-anxiety response via long-term depression of the amygdala (Duan et al., 2017). Moreira & Wotjak (2009) examined CB_1 receptors and how administration of the cannabinoids anandamide and 2-AG influenced anxiety. Surprisingly, they found that when low doses of cannabinoids were administered, anxiety-related behaviors were reduced, but when high doses of cannabinoids were administered, anxiety would increase (Moreira & Wotjak, 2009). Continuing, these authors also demonstrated that pharmacologically blocking or knocking out CB_1 receptors resulted in the anxiety-like states (Moreira & Wotjak, 2009). Overall, further research is required regarding the treatment of anxiety via the endocannabinoid system, as its mechanisms, dosages, and safety are still not fully understood.

Discussion

The endocannabinoid system has been shown to play numerous roles in the progression of neurological disorders. Specifically, CB_2 receptors on microglia demonstrate slowed progression of PD by reducing inflammation in the substantia nigra and striatum, suggesting the potential for therapeutic benefits in upregulating CB_2 receptors in these brain areas to reduce the rate at which dopaminergic neurons are lost. CB_2 receptors also appear to play a role in AD by contributing to the regulation of Aß plaque deposition and subsequent inflammation. However, targeting CB_2 receptors may be ineffective for therapeutic treatment of AD since CB_2 receptors do not play a role in tau hyperphosphorylation. The endocannabinoid system is also involved in pain reduction, but the therapeutic effects for chronic pain are complicated due to the presence of cannabinoid receptors on both excitatory and inhibitory neurons in the nociceptive pathway. Additionally, low doses of cannabinoids may reduce anxiety-related behaviors and CB_1 receptors may play a role in reducing anxiety-like states, but the treatment of anxiety via the endocannabinoid system is still not fully understood. Although the endocannabinoid system has been shown to carry out numerous roles such as neuromodulation, anti-inflammation, Aß plaque reduction, pain reduction, and anxiety reduction, the therapeutic potential of the endocannabinoid system appears most promising for PD, chronic pain, and anxiety.

The societal view regarding the use of marijuana was negative in the United States between the 1980's and early 1990's even though it was (and still is) one of the most commonly used psychoactive substances in the world (Antony et al., 2020). Marijuana and other cannabinoids are not normally thought of in a medicinal context. However, with the discovery of the endocannabinoid system and the increasing number of states legalizing medical marijuana, the social stigma is beginning to shift.

The therapeutic potential for this system is still being examined for a multitude of disorders due to its widespread distribution throughout the body. However, the widespread nature of the endocannabinoid system and psychoactive effects of exogenous cannabinoid compounds create challenges for drug development and clinical practice due to the many adverse side effects, such as anxiety, depression, and potential dependence (Alger, 2013). Furthering the current knowledge of endogenous cannabinoids and the endocannabinoid system will allow for the development of more specific and effective exogenous cannabinoid therapeutics that can be used to treat a variety of disorders.

Acknowledgements

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