From Cannabis to the Endocannabinoid System: Refocussing Attention on Potential Clinical Benefits

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ABSTRACT

Cannabis sativa is one of the oldest herbal remedies known to man. Over the past four thousand years, it has been used for the treatment of numerous diseases but due to its psychoactive properties, its current medicinal usage is highly restricted. In this review, we seek to highlight advances made over the last forty years in the understanding of the mechanisms responsible for the effects of cannabis on the human body and how these can potentially be utilized in clinical practice. During this time, the primary active ingredients in cannabis have been isolated, specific cannabinoid receptors have been discovered and at least five endogenous cannabinoid neurotransmitters (endocannabinoids) have been identified. Together, these form the framework of a complex endocannabinoid signalling system that has widespread distribution in the body and plays a role in regulating numerous physiological processes within the body. Cannabinoid ligands are therefore thought to display considerable therapeutic potential and the drive to develop compounds that can be targeted to specific neuronal systems at low enough doses so as to eliminate cognitive side effects remains the 'holy grail' of endocannabinoid research.

Del Cannabis al Sistema Endocannabinoide: Re-enfocando la Atención hacia los Potenciales Beneficios Clínicos

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RESUMEN

La cannabis sativa *es una especie herbácea usada en uno de los remedios herbarios más viejos conocidos al ser humano. Durante los últimos cuatro mil años, se ha usado para el tratamiento de numerosas enfermedades, pero debido a sus propiedades psicoactivas, su uso medicinal actual se halla muy restringido. En este estudio, se busca resaltar los adelantos hechos durante los últimos cuarenta años en cuanto a entender los mecanismos responsables de los efectos del cannabis sobre el cuerpo humano, y cómo éstos pueden utilizarse potencialmente en la práctica clínica. Durante este tiempo, se han aislado los ingredientes activos primarios en el cannabis, se han descubierto receptores cannabinoides específicos, y se han identificado por lo menos cinco neurotransmisores endógenos (endocannabinoides). Juntos, éstos forman la estructura de un complejo sistema de señalización endocannabinoide, el cual tiene una amplia distribución en el cuerpo y desempeña un papel en la regulación de numerosos procesos fisiológicos dentro del organismo. Por tanto, se piensa que los ligandos cannabinoides despliegan un considerable potencial terapéutico. Así, el dinamismo para desarrollar compuestos que puedan ser dirigidos a sistemas neuronales en dosis suficientemente bajas como para eliminar los efectos cognitivos secundarios, sigue siendo el "santo grial" de la investigación de los endocannabinoides.*

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INTRODUCTION

In January 2009, in Britain, there was a reversal of a decision taken five years earlier to de-classify cannabis from a Class B to a Class C drug. This apparent U-turn in policy exemplifies the long and controversial history associated with cannabis use, a history that has spanned some 4000 years and affected practically every major civilization. In the

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Caribbean, cannabis is often referred to as ganja but the more widespread name used in North America is marijuana. The numerous stereotypes associated with cannabis, in addition to the well known adverse health consequences associated with its chronic smoking, does little to help sell the concept of cannabis as a substance with enormous therapeutic potential. Recent advances in the understanding of the actions of cannabis and related ligands on the body suggest that now is an opportune time to change public opinion in this exciting field.

Cannabis is derived from three plant species, the most common being *Cannabis sativa,* which was originally cultivated for its fibres and is commonly known as hemp. Traditionally, hemp was used extensively to manufacture rope, cloth and even paper. The discovery of the active ingredient in *Cannabis sativa,* delta-9-tetrahydrocannabinol (THC) in the 1960s triggered a renewed focus on the potential medical benefits of the plant, with two Caribbean scientists, Manley West and Albert Lockhart at the forefront of these endeavours during the 1970s and 1980s. Two observations started West and Lockhart on their journey: (i) they noted a reduction in glaucoma among Rastafarians who traditionally used cannabis and (ii) persons from rural communities who used eyewash purportedly derived from cannabis claimed improved eyesight. This triggered ten years of pioneering research that culminated with the development and patent of a drug, Canasol, for the treatment of glaucoma. Canasol made use of the non-psychoactive components of the cannabis plant but still maintained its medicinal usefulness as demonstrated in clinical trials (1).

Thirty years on, understanding of the active ingredients in cannabis, the receptors to which they bind and their functioning within the body has advanced and cannabinoid physiology is now a major focus of research across Europe and North America. A 2005 review noted that since the discovery of the endogenous cannabinoid system in 1992, over 3500 reports have been published examining this system and its role in physiology (2); today, the number is probably well over 4000. Despite this explosion in cannabinoid research across the developed world, the Caribbean has not kept pace. This review seeks to highlight the major features of the cannabinoid system, enumerate its role in physiological processes and explore how the potential benefits of such understanding may impact on clinical practice. It is hoped that this effort will rekindle and refocus research into cannabinoids within the Caribbean region.

The Endocannabinoid System

A new era in cannabinoid physiology and pharmacology began in the 1960s with the discovery of THC amongst over 60 active ingredients in the cannabis plant. This propelled a wave of cannabinoid research but progress was slow and often limited to somewhat 'crude' clinical applications as the molecular basis for the effects of cannabis continued to elude scientists. The matter was resolved almost thirty years later when the first cannabinoid receptor was discovered (3). To date, two cannabinoid receptors have been definitively identified and characterized: namely CB1 and CB2. However, there is considerable interest in the possibility that cannabinoids may affect additional molecular targets, with the hunt for a putative 'CB3' receptor very much in vogue.

The CB1 receptor was first cloned from rat DNA in 1990 (3) with a human variant identified in the same year (4). The CB1 receptor belongs to the G-protein coupled receptor superfamily with a characteristic serpentine structure and is found almost exclusively within the central nervous system (CNS). The CB1 receptors are widely distributed within the CNS, being found in the cerebellum, hippocampus, olfactory bulb, striatum, forebrain and spinal cord. In fact, CB1 receptors have been shown to be the most abundant G-protein coupled receptor within the CNS, being present at many central synapses. The details of CB1 receptor distribution are beyond the scope of this review but have been well documented elsewhere (5, 6). This widespread distribution of CB1 receptors in the brain is now known to be responsible for the numerous physiological and psychotropic effects of cannabis as the blockade of CB1 receptors eliminates the 'high' associated with cannabis inhalation (7). In addition, animals in which CB1 receptors have been 'knocked out', *ie* the gene for the CB1 receptor has been deleted, do not demonstrate the typical behavioural effects *eg* reduced memory and analgesia, associated with CB1 receptor activation (8, 9). The CB2 receptor was identified three years after CB1 in 1993 (10) and was originally believed to be confined to peripheral tissues, in particular the immune system. Recent studies though have also identified CB2 on microglia, the immunocompetent cells of the CNS (11, 12) which may play a critical role in mediating neuroinflammation. CB2 receptors are also G-protein coupled receptors but have only 44% homology with the CB1 receptor (10).

While the term 'exogenous cannabinoids' refers to active derivatives of the cannabis plant or synthetic compounds derived in the laboratory, the discovery of specific cannabinoid receptors gave credence to the idea that endogenous cannabinoid neurotransmitters are present within the body. This was confirmed with the discovery of the first endogenous cannabinoid, N-arachidonoylethanolamide in 1992 (13). It was subsequently named anandamide (AEA), being derived from the Sanskrit word *'ananda'* meaning inner bliss and tranquillity and was followed shortly by the identification of a second endocannabinoid, 2-arachidonoylglycerol [2-AG] (14). Even though anandamide and 2-AG appear to be the most abundant endocannabinoids, at least three others have been identified including virhodamine (OEA) and N-arachidonoyldopamine [NADA] (15).

Two other unique features of the endocannabinoid system have contributed to the understanding of its functioning. Gamma-amino-buytric acid (GABA) is the most abundant inhibitory neurotransmitter in the CNS and glutamate, likewise, is the most abundant excitatory neuro-

transmitter in the CNS. This gives rise to the first unique feature regarding CB1 receptors, their localization, the vast majority being found co-localized on GABAergic and glutamatergic synapses. The second feature is, unlike most other neurotransmitter receptors that are predominantly found on the post-synaptic membrane and participate in an anterograde flow of information, the majority of CB1 receptors are found pre-synaptically (16, 17).

Taken together, these features reveal a novel signalling mechanism utilized by the endocannabinoid system. It is now known that endocannabinoids are stored within the post-synaptic membrane in their precursor form and released on demand following post-synaptic depolarization (15, 18). Once released into the synapse, they diffuse in a retrograde manner towards the pre-synaptic neuron where they bind to the CB1 receptors. Binding to the CB1 receptor leads to the inhibition of neurotransmitter release *via* modulation of pre-synaptic calcium and potassium channels (19, 20). This on-demand negative feedback system (Figure) is considered cri-

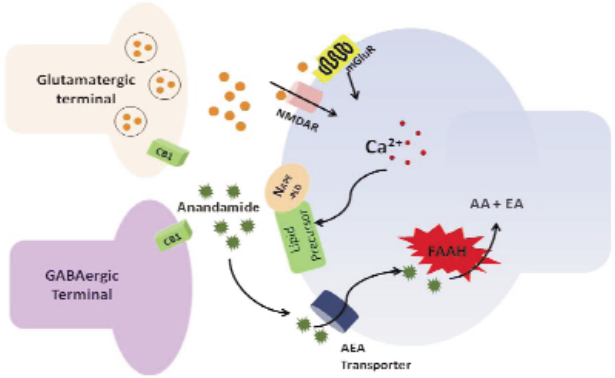

Figure: Schematic diagram depicting a typical central synapse with endocannabinoid signalling. Endocannabinoids are synthesized on demand in the post-synaptic neuron from fatty acid precursors. They then travel in a retrograde manner and bind to receptors found on the pre-synaptic terminal, thus modulating neurotransmitter release. Anandamide (the first endocannabinoid discovered) is broken down by neuronal re-uptake and the enzyme fatty acid hydrolase. Calcium ions (Ca^{2+}); NMDA Receptor (NMDAR); Metabotropic receptor (mGluR); Cannabinoid Receptor 1 (CB1); Anandamide (AEA); Arachidonic Acid (AA); Ethanolamine (EA); N-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD); fatty acid amide hydrolase (FAAH).

tical to maintaining overall efficacy and efficiency of the synapse and disruption is hypothesized to be a key player in neurological disease processes (18, 21).

The classic example of this negative feedback effect described above is termed depolarization-induced suppression of inhibition (DSI). The mechanism of DSI was originally unknown but it was soon determined to have a pre-synaptic locus and eventually shown to be mediated *via* endocannabinoids (22). A similar effect has also been observed at excitatory synapses, depolarization-induced suppression of excitation (DSE) and this too was shown to be mediated by endocannabinoids. Demuth and Molleman (23) and Kano *et al* (24) provide a full review of cannabinoid signalling mechanisms.

Endocannabinoid Physiology

Given the widespread distribution of cannabinoid receptors in the CNS, it is not surprising that endocannabinoids have been implicated in a number of distinct physiological processes. Mechanistic knowledge of these processes and how they can be manipulated is important as they offer novel opportunities for intervention in numerous clinical scenarios. It should be noted that the vast majority of researchers do not advocate the imbibing of cannabis, *via* smoking or otherwise, as an effective clinical intervention. What is being considered, though, is the possible manipulation of the endocannabinoid system by targeted drug delivery systems that minimize unwanted side effects. This association with the use of cannabis as an illicit drug has made endocannabinoid research fraught with controversy but, given the increasing amount of empirical evidence, further research into the role and functioning of the cannabinoid system and its potential benefits must continue.

Modulation of Pain

Cannabis, controversially, has long been used to treat chronic, intractable pain and it is thought that this results from modulation of the endocannabinoid system (25, 26). In fact, cannabinoids have been suggested to be up to ten times more potent than morphine in animal models of pain (27); cannabinoids also attenuate different types of pain (neuropathic and inflammatory) and the endocannabinoid system itself can be modulated by various chronic pain states (28).

This modulation has been demonstrated in the periphery, in the spinal cord and at a supra-spinal level. Within the periphery, cannabinoid agonists have demonstrated analgesic properties (29). In experiments in which peripheral nociceptors had their CB1 receptors removed *via* gene deletion, the anti-nociceptive effects of cannabinoid agonists was attenuated by up to 50% (30). Interestingly, it has long been known that the anti-nociceptive properties of non-steroidal anti-inflammatory drugs (NSAIDs) cannot be fully attributed to the blockade of prostaglandin synthesis and there is now evidence to suggest that cannabinoid receptors may be involved in NSAID mediated analgesia (31−33). In the spinal cord, activation of CB1 receptors reduces the transmission of pain *via* dorsal horn neurons (29, 34), essentially 'closing the gate' and preventing ascending pain signals reaching higher structures within the CNS. Cannabinoids are also active at supra-spinal structures including the peri-aqueductal grey matter (26, 35) and the amygdala (36).

Given the wealth of pre-clinical data available and the numerous reports of individuals successfully utilizing cannabis for chronic, intractable pain states, a number of clinical trials have been implemented seeking to evaluate the efficacy of cannabinoid agonists. Emerging evidence from these suggests that there is much potential in the use of these compounds although not all studies show a clear benefit (37, 38). Indeed, in 2005, the cannabis-derived drug Sativex® was approved in Canada as a prescription medicine for the treatment of pain associated with cancer and neuropathic conditions including multiple sclerosis. Sativex contains both the active cannabinoid THC and its inactive counterpart cannabidiol and is currently available in over twenty countries worldwide.

Effective therapeutic doses in humans still result in too many side effects, mandating that more targeted application of cannabinoids be achieved. One such possibility involves the modulation of endogenous cannabinoid levels by endocannabinoid uptake or inhibitors of enzymes that breakdown endocannabinoids; this would lead to an increase in the functionality of endogenous cannabinoids at the site thereby potentiating the endogenous analgesic response (39). Another avenue may involve the development of novel cannabinoid receptor targets that activate G-protein coupled receptors associated with pain pathways.

Learning and Memory

The adverse effects of cannabis use on memory have been repeatedly seen in chronic users (40) but the advent of our understanding of the endocannabinoid system has now allowed a much more thorough investigation of these effects. It is now generally agreed that cannabinoids can modulate short term memory but have little impact on long term memory. This has been demonstrated in a variety of situations including the Morris Water Maze (41) and fear conditioning (42). In addition, extensive work has been done examining the role of cannabinoids on molecular engrams of memory, in particular long term potentiation (LTP) and long term depression [LTD] (43). Most studies have demonstrated that cannabinoids inhibit LTP and potentiate LTD (44). Studies have not demonstrated that cannabinoid antagonists can improve memory.

Given the work cited above, impairment of memory represents one of the side effects that must be avoided when utilizing cannabinoids. However, there may be a potential role for cannabinoids in Alzheimer's disease (45). Post-mortem studies have noted an increase in CB receptor expression among microglia found in senile plaques within Alzhemier's brains (46). Despite this finding, perhaps most hope lies in the previously noted role of CB2 receptors found upon microglial cells in the brain. Microglia represent the immune-competent cells of the brain and thus help regulate many of the neuro-inflammatory processes within the brain, including Alzheimer's disease (47). Despite much interest, to date little empirical evidence has been collected to support these theories (48).

Appetite

One of the more remarkable effects of endocannabinoids is their ability to influence appetite. Administration of anandamide into the ventromedial hypothalamus stimulates feeding (49) while CB1 receptor antagonists inhibit food intake, an effect that was not demonstrated in CB1 knockout animals (50).

Again, this has led to a number of clinical trials in which the cannabinoid antagonist rimonabant was tested as an anti-obesity medication and initially found to be effective in reducing body weight (51), eventually being licensed within the European Union. However, due to the number of side effects, including increased suicidal tendencies, the medication was never approved in the United States of America and eventually removed from use among the European Union countries. This has resulted in a re-examination of the use of endocannabinoids in obesity management (52).

Reward Pathways and Addiction

Most of the drugs of addiction including alcohol, nicotine, cocaine and morphine all interfere with the natural reward pathways in the brain. These pathways include the ventral tegmental area (VTA) and its connections to the amygdala, nucleus accumbens, prefrontal cortex and the limbic system. Within the past decade, evidence has accumulated that these effects may be modulated by endocannabinoids. One of the first such reports demonstrated that cannabinoid agonists could reinstate drug-seeking behaviour (relapse) in rats after withdrawal from cocaine (53); similar results were not observed in CB1 knockout mice (8) implicating the CB1 receptor.

It is now generally believed that cannabinoids facilitate dopamine release in the VTA and thus may play a key role in the rewarding properties of addictive compounds (24, 54). This work has led to several clinical trials, also making use of the cannabinoid antagonist rimonabant, as a treatment for addiction, in particular nicotine addiction. To date, results appear promising, demonstrating a reduction in relapse rates; however, they may not be any better than nicotine replacement in preventing initial cessation. As was the case in other clinical trials, there was an increased risk of side effects that needs to be managed if these compounds are to become front-line strategies in the management of addiction (55).

Immunological Function and Multiple Sclerosis

One of the very earliest accounts of the activity of cannabis from ancient China highlights its ability to attenuate rheumatism and thus its anti-inflammatory properties (56). In more modern times, cannabinoids have been shown to lower the resistance of a variety of animal species to infection and

these effects have been confirmed in a number of *in-vitro* studies (57). Also, endocannabinoids are elevated in tissues where ongoing inflammation is present (58). In the latter case, it has been suggested that the accumulation of endocannabinoids is an adaptive attempt by the body to alleviate inflammation. While it was originally thought that immune function was only mediated *via* CB2 receptors, it has now been demonstrated that CB1 receptors can also modulate inflammation and the interplay between these receptors may be particularly important in modulating neuroinflammation (59).

These effects on the immune system have perhaps been best harnessed in the treatment of multiple sclerosis (MS), a chronic autoimmune disease in which there is inflammation within the CNS. In particular, there is an attack upon the myelin sheath of neurons leading to progressive motor and sensory deficits often accompanied by pain of varying severity. In animal models, both CB1 and CB2 agonists have been shown to reduce the neuroinflammation and axonal damage associated with MS leading to improve functioning (60, 61). Given these results, numerous clinical trials have already been conducted to evaluate the effect of cannabinoids in multiple sclerosis (62). These trials have focussed on alleviating many of the symptoms associated with MS including spasticity, bladder control, motor control and pain. To date, results have been mixed but encouraging enough to suggest that cannabinoids do have a role to play in the management of symptoms. Beyond managing symptomology, efforts are underway to determine whether or not cannabinoids can actually slow disease progression. The Cannabinoid Use in Progressive Inflammatory brain Disease (CUPID) study recently begun at Peninsular Medical School in the United Kingdom is seeking to address these questions and results are expected in early 2012.

Endocannabinoids and Neuroprotection: Ischaemia

Given the ubiquitous nature of cannabinoid receptors in the brain and their ability to modulate synaptic transmission, the endocannabinoid system is widely viewed as a fine-tuner of neural function. Connected to this has been the suggestion that the endocannabinoid system offers a means of neuroprotection to a variety of different insults and pathological processes. In this last section, the potential role of endocannabinoids in modulating hypoxia/ischaemic neuronal injury in the brain is briefly reviewed.

In animal models, endocannabinoids have been shown to increase after ischaemia (63) and there has also been an increase in expression of cannabinoid receptors following transient ischaemia (64). Numerous studies have demonstrated the neuroprotective effects of cannabinoids in a variety of different models including permanent middle cerebral artery occlusion [MCO] (65), transient ischaemia (65) and closed head injury (66). Further evidence was gleaned from CB1 knockout mice where it was demonstrated that infarct volume was increased following transient ischaemia (67).

However, it should be pointed out that this effect is not so clear cut, as other studies have shown that cannabinoid antagonists can also be neuroprotective (63, 68). We also showed similar results in an *in-vitro* model when it was demonstrated that the cannabinoid antagonist AM-251 improved recovery of synaptic transmission following oxygen-glucose deprivation [OGD] (69).

Given these contradictory findings, it is also important to appreciate that cannabinoids also have a number of non-receptor mediated effects including antioxidant properties (70, 71), their ability to interact with other neurotransmitter systems *eg* adenosine (72) and also the ability to lower body temperature (73). Taken together, the results suggest that cannabinoids can indeed modulate cell death following ischaemic injury but the mechanisms and conditions under which this takes place remain unclear, despite the vast amount of research that has taken place over the past twenty years.

CONCLUSION

Understanding of cannabinoids and the endocannabinoid system has dramatically increased within the past 20 years. This understanding has shed new light on the numerous physiological processes in which endocannabinoids are involved and offered new vistas for modulation of these same processes. Despite the tremendous advances that have been made, successful pharmacological interventions have yet to be fully elucidated. Key to this process still remains the ability to disentangle the potential benefits of cannabinoid receptor activations from its pitfalls, including the psychotropic side effects. Until this is done, the full potential of harnessing this system remains locked away, though results to date provide ample incentive for those currently working in the field.

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