What do we know about Endocannabinoid System

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(Ann Coll Med Mosul 2023; 45 (2):247-252).
Received: 23th May 2023; Accepted: 5th July 2023.

ABSTRACT

Cannabis has been known for many centuries for its recreational and medical uses. The use of medical cannabis (MC) is on the rise with growing body of evidence about the benefits and safety of MC treatment. The use of MC represents a very interesting area for both patients and researchers for its potential therapeutic uses which include wide array of benign and malignant diseases across the board including both sexes and different age groups. Currently more than 40 countries have approved the use of Cannabis for medical purposes.

This article will review the history, terminology, mechanism of action, metabolism, pharmacological forms, legality and adverse effects profile. It represents an essential source of knowledge for clinician and researchers interested in pursuing the therapeutic potentials of MC. Proper understanding of such introduction is pivotal for safe and effective use of this multipurpose medicine.

Keywords: cannabis, medical cannabis, phytocannabinoids, endocannabinoid.

ماذا نعرف عن جهاز ال Endocannabinoid

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الخلاصة

عُرف القنب لقرون عديدة لاستخداماته الترفيهية والطبية. يتزايد استخدام القنب الطبي (MC) مع تزايد مجموعة الأدلة حول فوائد وسلامة علاج .MC يمثل استخدام MC مجالًا مثيرًا للاهتمام لكل من المرضى والباحثين لاستخداماته العلاجية المحتملة والتي تشمل مجموعة واسعة من الأمراض الحميدة والخبيثة في جميع المجالات بما في ذلك كلا الجنسين والفئات العمرية المختلفة. في الوقت الحالي ، وافق أكثر من ٤٠ دولة على استخدام القنب للأغراض الطبية.

ستراجع هذه المقالة التاريخ والمصطلحات وآلية العمل والتمثيل الغذائي والأشكال الدوائية والشرعية وملف الآثار الضارة. إنها تمثل مصدرا أساسيا للمعرفة للأطباء والباحثين المهتمين بمتابعة الإمكانات العلاجية لـ MC. يعد الفهم الصحيح لمثل هذه المقدمة أمرًا محوريًا للاستخدام الآمن والفعال لهذا الدواء متعدد الأغراض.

الكلمات المفتاحية: القنب , القنب الطبي , phytocannabinoids , endocannabinoid .

HISTORICAL ASPECT

The plant *Cannabis*- in its two varieties of *sativa* and *indica,* has been exploited for centuries to obtain relief from pain and improvement in mood status. Every part of the plant (flower, leaves, seeds and hemp fibre) has been used in different forms by many nations over many years. The first person reported to recommend cannabis as an herbal remedy was the Chinese Emperor Shennong Bencao Jing, 5000 years ago [1]. In addition to that, there is evidence that ancient India, Egypt, Greece and the Roman Empire have also used it as a medicinal therapy [2]. O'Shaughnessy and Moreau reported beneficial impact of this plant on muscle spasms, vomiting, convulsions, joint pain, tetanus and rabies [3]. Also, it has been used by Queen Victoria for painful menses, and by Empress Elisabeth (Sissi) of Austria for cough, and as an appetite stimulator [2]. Other non-medical uses include the hemp which has been used for the manufacturing of rope, canvas, paper and cloth [4].

The original isolation of cannabinol from cannabis resin was done by Wood *et al*. 1896 [5]. Afterward, there were trials to extract and study other cannabis compounds till 1964 when Raphael Mechoulam (the God father of modern studies on Cannabis) identify Δ^9 -tetra-hydro-cannabinol (THC) as the principle psychoactive ingredient of Cannabis and the prototype among the 60-100 bioactive metabolites named cannabinoids (highly lipophilic bi- or tricyclic ringed structures) extracted from this plant [1]. The revolution in the field of cannabis research flourished at the beginning of 1990s, when the first cannabinoid receptors was discovered and only three years later, the second was also unveiled [6,7].

Terminology and Definitions

The term "weed" is a slang for marijuana and it is made from dry leaves and flowers of female cannabis herb. Other names include Mary Jane, pot, herb, grass, and ganja. On the other hand, hash (or hashish) is a secreted gum (or resin) that is extracted from the trichomes which located on the surface of marijuana leaves and flower buds. It is the part of the plant that offers the greatest intoxication level because of its high THC content of over 40%. Both marijuana and/or hashish is ideally mixed with tobacco and rolled into a cigarette named "joint" that was widely spread in Europe and north America in the 1970s [4]. Other minor routes of delivery involve eating, drinking, dermal delivery and most recently vaporiser. Each of which has its own onset and duration of action [4]. After consumption, the symptoms are quite variables but typically there is an early phase of the "high" (euphoria) followed by the "drop" (drowsiness). In addition, there is an altered time perception, difficulty in concentration/thinking, hearing and/or visual distortion, impairment of the short memory and others [1].

Cannabinoids include endocannabinoids (ECB, also called endogenous cannabinoid), phytocannabinoids, and synthetic or semi-synthetic cannabinoids [8]. Phytocannabinoids are natural compounds derived from the plant with an affinity for and activity at cannabinoid receptors. There are two main cannabinoid receptors: CB1 and CB2; however, cannabinoids can activate other receptors, see details below. Thus, human cannabinoid system is made up from cannabinoids, receptors and their regulatory enzymes (controlling synthesis, transport and degradation) [9].

Endocannabinoid System

It is a lipid signalling system. Its components are distributed all over the body. It regulates huge varieties of physiological functions composing of metabolism, mood, appetite, motor skills, cell biology, stress, immune and inflammatory response, cardiovascular, reproductive, neuroendocrine and digestive tracts control and most importantly in pain and analgesia [4, 8, 10, 11]. Detailed pharmacokinetics of cannabinoids was best reviewed here [8].

Endogenous Ligands of Cannabinoids: ECB

All the endocannabinoids that have been identified till now are of lipid origin and allocated in two major categories: N-acylethanolamines (NAEs) and 2-monoacylglycerols (MAGs) which show similar pharmacological profiles, but little structural similarity, to THC [8]. The prototypes for the above for NAEs families are N-arachidonoylethanolamine (anandamide, AEA) and N-arachidonoyl dopamine (NADA) while 2-arachidonoylglycerol (2-AG), and 2-oleolyglycerol (2-OG) represent those for MAGs [4]. The discovery of the two principle most abundant, well-established, polyunsaturated fatty acid derivatives, AEA and 2-AG dated back to the mid-nineties and both of them have been shown to display nanomolar affinity to CB1 and CB2 receptors (like THC) [6, 7] in both man and other mammals. In fact, AEA is a CB1 partial agonist (despite of the high affinity) and a relatively weak ligand at CB2 receptors [10, 11], while 2-AG is a full agonist at both receptors and is much more abundant than anandamide in the mammalian brain (1000-fold) tissue [10].

All ECB have precursors existing in the lipid membranes and on demand (by depolarization or by stimulation of certain G protein-coupled receptors, GPCR), endocannabinoids are synthesized and released into the extracellular space. This is an important feature of ECB and by this they contrast the classical neurotransmitters which are synthesized in advanced and stored in synaptic vesicles [9]. It is not proven till now if there is a transporting system to facilitate the movement of these endocannabinoids within cells and across cell membranes [8]. The best characterized endocannabinoids are AEA and 2-AG. There are numerous endocannabinoid ligands with various activities and affinities outside the scope of this review.

Synthesis and Transformation

AEA and 2-AG are endocannabinoids that have distinct synthetic pathways. Regarding AEA, it has been proposed to be synthesized by multiple pathways [8, 11]. In immune cells and some brain region [9], AEA is synthesized by hydrolysis of N-arachidonoylphosphatidylethanolamine (lipid membrane precursor) by a distinct phospholipase D activity, called NAPE-PLD, to generate AEA and phosphatidic acid [12], see Figure -1-. Endocannabinoids which are not bound to the receptors are degraded. Intracellularly, AEA is metabolized in the CNS by microsomal enzyme fatty acid amide hydrolase (FAAH) [13], amidase subfamily of serine hydrolase, found abundantly in the liver and brain [14], to give arachidonic acid and ethanolamine. There is a second isoform of FAAH known as FAAH2, which has been recognised in humans, rabbits and elephants but not in rats, mice and dogs [15]. Both FAAH2 and N-acylethanolamine acid amidase (NAAA) may hydrolyse AEA in particular cell types [13], see Figure -1-.

On the other hand, the hydrolysis of 1,2-diacylglycerol (diacylglycerol lipases, DAGL) generates 2-AG and free fatty acids. As of today, there are two variants of DAGL: DAGLα and β [16]. DAGLα is the primary isoform for most 2-AG production which plays an important role in synaptic plasticity in an adult CNS based on knockout mice data [9]. Furthermore, monoacylglycerol lipase (MAGL) -from the lipase subfamily of serine hydrolase- is the primary metabolizing enzyme for 2-AG to generate arachidonic acid and glycerol [8]. Two other serine hydrolases enzymes, α / β- hydrolase domain 6 (ABHD6) and ABHD12 are also considered to be responsible for 2-AG degradation [4, 8, 9] albeit to a minor degree in a membranous preparation of mouse brain.

Both AEA and 2-AG are arachidonic acid derivatives and because of that they are metabolised by lipoxygenases (LOX) [17], cytochrome P450 isoforms (epoxygenase) [18] and cyclooxygenase-2 (COX-2) [19] to generate bioactive products [16] that apparently exert their effects through non cannabinoid receptors, see Figure -1-.

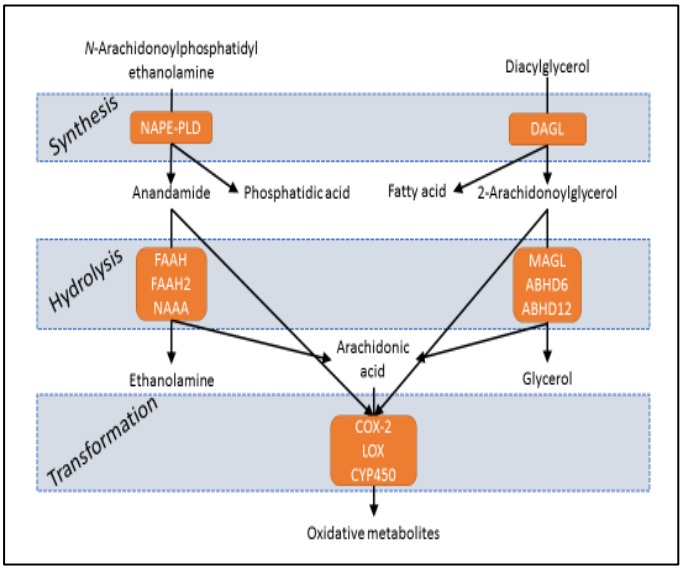

Figure -1 -Representation of the major pathways of anandamide and 2-arachidonyl glycerol synthesis, degradation and transformation.
Adapted from (Fowler *et al.*, 2017)[21].

Receptors: CB1 and CB2

As of now, there are two kinds of classical cannabinoid receptors; distinguished by their tissue distribution, amino acid sequences and signalling mechanisms. Both CB1 and CB2 are GPCRs, and are coupled via Gi/o proteins inhibiting adenylyl cyclase and stimulate mitogen-activated protein kinases. In mankind, CB1 receptor shares only 48 % homology of protein sequence with CB2 [20].

CB1 receptors are heavily distributed in the central nervous system (CNS), predominantly in cortex, cerebellum, hippocampus, basal ganglia, hypothalamus and brain stem; areas responsible for cognitive control, motor and emotional function, perception of pain and thermoregulation, therefore, the stimulation of CB1 receptors cause the typical behavioural and psychotropic actions of cannabinoids [9]. Interestingly, CB1 receptors are mostly absent in the respiratory area of the brainstem and that is why there are no respiratory symptoms upon overdose of cannabinoids [4]. In neurones, the CB1 receptor is positioned pre-synaptically [8, 9, 21] to control neurotransmitter release (for example: acetylcholine, 5-hydroxytryptamine, noradrenaline, dopamine, glutamate and GABA) [22]. Peripherally, CB1 receptors have also been detected in various body organs and tissues to regulate different physiological processes such as energy control and reproduction [23]. CB2, on the contrary, is primarily expressed in the periphery; mainly expressed in immune cells. Accordingly, it was

proposed to take part in immunological signalling. In response to injury, inflammation or pathophysiological states, CB2 can be induced in the CNS [8, 9]. Thus, overall activation of CB1 or CB2 receptors employs numerous implications on cellular physiology, including synaptic function, gene transcription, cell motility, etc [23].

Other Receptors

Endocannabinoids not only work through CB1 and CB2 receptors but there are additional receptors that can be activated by cannabinoids. These include:

-Orphan GPCR: GPR55, which has been involved in neuropathic pain [24]. GPR119, which has been implicated in obesity [25] and regulates glucose and insulin level [26]. OEA (an analogue of AEA) and 2-OG (an analogue of 2-AG) are natural agonists for GPR119 [27]. GPR18, where by three cannabinoids were reported as full agonists of GPR18: AEA, N-arachidonoyl glycine (NAGly) and THC [28].

-Ion channels: in which the involvement of Ca2+ and K+ channels are more than Na+ channels. It is well reviewed in [29].

- The Transient Receptor Potential Vanilloid subfamily member 1 (TRPV1, capsaicin receptor), its ligands are anandamide and its analogues (reviewed in Basso *et al*., 2017 [30]).

-Peroxisome Proliferator-Activated Receptors (PPARs): Generally, there are three mechanisms by which varieties of cannabinoids are reported to activate PPARs: direct binding, conversion to multiple compounds that immediately bind to PPARs or via intracellular signalling processes [31].

Clinical Applications and Restrictions

Currently, the plant cannabis has been dubbed as a "storehouse" because of numerous pharmacologically related compounds. Previously, cannabis was considered as of no medical use and was classified as a Schedule I agent in the USA in 1940 [32]. In 1941 and as a consequence of several legal limitations, cannabis was taken out of the American Pharmacopoeia [3]. The dilemma probably was with the symptoms that frequently upsurge upon its consumption, examples include: euphoria, dizziness, loss of memory, exhaustion, and paranoia that are not always well received by patients and which we know nowadays that they are developed upon the activation of the central CB1 receptors. However, the wide array of its therapeutic practices over the centuries was the impetus for continuing and directing research on cannabinoids toward discovering either new analogues or new pharmacological uses.

Cannabis-based medicines (CBM) are approved only for some indications. In 2005, Canada approved a multinational pharmaceutical laboratory which was seeking authorization in the United Kingdom and the European Union, to sponsor a medication containing CBD and D^9-THC (in a ratio of 1:1) for patients with multiple sclerosis [3]. Sativex® (Nabiximols)- as an oral spray- was the first medication licenced in UK for treating multiple sclerosis spasticity and peripheral neuropathic pain as an add-on drug [33]. This medication's examples of off-label use are chronic pain and symptomatic treatment of selected neuropsychological conditions (such as anxiety and sleeping disturbances)[33].

In addition, Cesamet® (Nabilone) (synthetic Δ^9-THC analogue) and Marinol® (Dronabinol) (synthetic Δ^9-THC) have therapeutic applications to treat nausea and vomiting (induced by chemotherapy and in acquired immune deficiency syndrome), to increase appetite and to provide neuropathic pain relief for those with multiple sclerosis or advanced cancer) [8,33]. They are available as capsules or oil/alcohol based drops and were FDA approved on 1985 [34]. Presently, however, the likely usage of both of these agents would be in those patients who are refractory to other medications due to the availability of 5HT3 receptor antagonists, such as ondansetron [4]. Lastly, Epidiolex® (an orally pure CBD), is-in specific circumstances-used for the treatment of childhood epilepsy, FDA approved it in 2018 [8].

In 2013, the American Herbal Pharmacopoeia recalled cannabis species back again, and several positive legislative changes had made by the US, European Union and Canada reflecting the mounting interest in the therapeutic capability of cannabinoids and the progress in scientific research [4].

On the other hand, Acomplia® (rimonabant) (a CB1 receptor antagonist) [35] was developed by Sanofi and got its approval in Europe (not in America) in 2006, as a medication for metabolic syndrome or obesity for 2 years before being abandoned because of the high incidence of depression and the increased suicidal thoughts [4, 8].

The rapid inactivation of endocannabinoids in vivo and/or their short life within the synapse may stand behind the difficulty of exploiting the therapeutic characterisation of AEA and 2-AG [14]. In addition, the wide spread of cannabinoid receptors all over the body and the presence of unknown/not discovered yet off targets all hinder the exploration and increase the complexity of this system.

The failure of drugs targeting cannabinoid receptors directly has led researchers to investigate the therapeutic potential of compounds targeting specific enzymes within the cannabinoid system. Selective FAAH and MAGL inhibitors have been identified and there is evidence suggesting promising therapeutic actions [21, 33]. Therefore, a

versatile activity assay is required to allow profiling of a large library of possible inhibitors/substrates for those regulatory enzymes. Another challenge now is developing approaches that will make an improvement to the effectiveness and/or the benefit-to-risk ratio of the ligands and to identify additional possible clinical applications [22]. An interesting research path would be the technological development of the current pharmaceutical formulations which may suggest that different preparations (ethanol vs aqueous extract for example) might have different efficacy in different disorders based on the concentration and/or formulation type [36]. Likewise, synthetic cannabinoids may potentially reveal more potency and selectivity than their natural counterparts which consequently might pave the way towards a new therapeutic strategy.

There are many known disorders in which cannabinoid ligands have been shown to have a clinical impact and a promising future outcome (well-reviewed in [21]), such as: schizophrenia, multiple sclerosis, neuro-degenerative disorders (as a neuroprotector), glaucoma, psychosis, feeding disorder, nausea and vomiting, pain, anxiety, stress and insomnia, epilepsy and cancer.

CONCLUSION

Although the mankind's knowledge of cannabis has been developing over many eras, we still have a long way to discover the intricacy of the network of endocannabinoid system. To this day, there is a false misconception about CBM, such as the myth that it can lead to psychological addiction. However, the reality is that only THC is responsible for such obsession. Only time can tell if we can exploit the endocannabinoid system further to the benefit of humankind.

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