Chapter 10

Cannabinoids: Drug or Medication?

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Additional information is available at the end of the chapter

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Abstract

This chapter aims at exploring the use and misuse of cannabinoids as it has become a major societal issue. In the first section, we describe the historical use of cannabis as a natural cure in ancient civilizations. We then explore the current use of cannabinoids in medicine, which includes innovative strategies for treating various diseases such as multiple sclerosis or cancer-induced pain. In the second section, we consider how the discovery and characterization of the endocannabinoid system have increased knowledge of this system's mode of action. Consumption of cannabis for recreational use however is a significant public health issue today. Scientific advances are confronted with the adverse health effects that are demonstrated in preclinical and clinical studies based on the psychotic and addictive properties of this compound. In the third section, we therefore provide an overview of the recent findings on the endocannabinoid system using animal models with proposed molecular mechanisms and potential interactions with other neuromodulatory systems like the opioid system. Finally, through alternative strategies to current treatments with both phyto- and synthetic cannabinoids, we try to reconcile the beneficial aspects of the use of cannabinoids for medication and the aspects associated with addictive properties.

Keywords: animal models, cannabis, dependence, endocannabinoid, pharmacology, therapies

Abbreviations

2-AG, 2-arachidonoylglycerol; AEA, anandamide, *N*-arachidonoyl-ethanolamide; AIDS, acquired immune deficiency syndrome; ALS, amyotrophic lateral sclerosis; CBD, cannabidiol; CBN, cannabinol; CB1, type 1 cannabinoid receptor; CB2, type 2 cannabinoid receptor; CNS, central nervous system; CPA, conditioned place aversion; CPP, conditioned place preference; DA, dopamine; DAGL, diacylglycerol lipase; FAAH, fatty acid amide hydrolase;

G protein, guanine nucleotide binding protein; GABA, c-aminobutyric acid; GPCR, G-protein-coupled receptor; iv, intravenous; MGL, monoacylglycerol lipase; MS, multiple sclerosis; NAPE-PLD, *N*-acyl phosphatidylethanolamine phospholipase D; Δ^9-THC, delta-9-tetrahydrocannabinol

1. Cannabis as medication

1.1. Historical uses of cannabis

Cannabis is a botanical genus belonging to annual plants from the *Cannabaceae* family. According to botanical classification, this genus only contains one species *Cannabis sativa* L. subdivided into three subtypes: *sativa*, *indica*, and *ruderalis*. *Cannabis sativa* was one of the first plants cultivated by humans. In the 4th millenary BC in China, cannabis was mainly produced for its stalk fibers, which were used to manufacture strings, ropes, textiles, and paper. It was also consumed for its fruits [1]. Cannabis has been used as a natural medication for thousands of years. It can be traced back to 2700 BC, but at that time it was only transmitted by oral traditions [1]. The first written evidence of its therapeutic use was reported during the first century of the Christian era, with the world's oldest pharmacopoeia, the Chinese Pharmacopoeia *Shen Nong Ben Cao Jing* [2]. The main uses of cannabis were for treatment of rheumatic pain, constipation, disorders of the female reproductive system, and also malaria. In India, its use was mostly for analgesic, anticonvulsivant, tranquilizer, antispasmodic, digestive, appetite stimulant, or antitussive properties. Cannabis also spread throughout Asia for use in religious rituals as it was described as a magic and medicinal plant in the *Atharva Veda* (4th Veda) sacred text of Hindu and Vedic traditions [1].

As cannabis has been prohibited by law, it has been difficult to follow its use for its recreational properties. It has probably been used for recreational purpose since the beginning of the Christian era. Additionally, its consumption for its psychoactive effects became quite popular in the 1950s in the United States of America with the rise of jazz. It then spread among young people within Western countries with, for example, the explosion of its popularity in the 1960s–1970s with the hippie movement. Nowadays, cannabis and related compounds are the most abused illicit substances in Europe, the United States, and Australia [3–5]. Consumption of cannabis is still often underestimated in our modern societies, but the current prevalence has been estimated by the World Health Organization as about 3.9% among the global population (15–64 years old) [6].

1.2. Phytocannabinoids

Today in the common language, the terms “cannabis” or “marijuana” stand for mixtures of dried herbs (also named Bhang). In particular, the dried flowering tops, leaves, and stalks of the mature female plant (Ganja) are commonly used as cannabis herbs [2]. Resinous extracts of compressed flowering tops called “hashish” or “hash” are also consumed and are stron-

ger than marijuana [7]. Interestingly, the plant *Cannabis sativa* contains more than 400 different chemical compounds including over 60 cannabinoids which are specific to the plants of the genus *Cannabis.* These molecules are called phytocannabinoids. The most potent one is the (−)-Δ^9-tetrahydrocannabinol (Δ^9-THC) and has been purified and isolated in 1964 by Raphael Mechoulam's team [8]. This molecule is the main psychoactive component of *Cannabis sativa* and is therefore responsible for most of the psychoactive and physical effects of cannabis consumption [9]. It has been described as a partial agonist for both cannabinoid receptors, CB1 and CB2 (see below) [10]. The other main active constituents of cannabis are the (−)-Δ^8-tetrahydrocannabinol (Δ^8-THC), the cannabinol (CBN), and the cannabidiol (CBD) [11]. The Δ^8-THC is slightly less potent than Δ^9-THC, as CBN, and is present in very small quantities in the plant. CBD is devoid of psychoactive effects and of most of the other Δ^9-THC-like effects, but has other interesting features such as antiepileptic, antianxiety, antinausea, and antischizophrenic properties [12]. Phytocannabinoids other than Δ^9-THC can show additive, synergistic, or antagonist effects with Δ^9-THC and these interactions may modulate its actions when cannabis is consumed as an herbal mixture [13]. Noteworthy, Δ^9-THC content may strongly vary upon the origin of the plants and the type of cannabis preparations. Since Δ^9-THC is the main active compound of cannabis, refined farming methods and plant genetic crossbreeding technologies have been developed in order to increase Δ^9-THC content and therefore to increase the potency of cannabis [13]. Depending on their relative content of Δ^9-THC and CBD, cannabis plants are classified in three different types: "drug-type" plants (Δ^9-THC/CBD > 1), "intermediate-type" plants (Δ^9-THC/CBD = 1), and "fiber-type" plants (Δ^9-THC/CBD < 1) [14]. In addition to the relative content in various phytocannabinoids, consumption patterns also impact the effects of cannabis.

1.3. Modes of cannabis consumption

Although cannabis is often mixed with tobacco, then rolled and smoked as "joints," it can also be consumed in other forms [13]. It can be smoked from pipe or even from "buckets" by inhaling from a mass of plant or resin ignited in a plastic bottle. Moreover, it is ingested in the form of candies or baked into cakes (cookies, muffins). In the latter case, cannabis is mixed with butter to form "marijuana butter," also called "cannabutter" or "Marrakech butter," and then mixed with other baking ingredients ("space cake"). Finally, more rarely, macerated extracts of cannabis can be taken. Contrary to other drugs of abuse that can also be injected, cannabis is unsuitable for intravenous use due to its relative insolubility in water. When cannabis is taken as joints, the association of the phytocannabinoids with nicotine will produce the hedonic effects sought by the smokers. It is therefore more difficult to study these effects and the specific properties coming from cannabis alone. There are many factors influencing the global effect of cannabis in humans resulting from combination of the relative content in different phytocannabinoids (e.g., Δ^9-THC, CBD), the route of administration, the mode of consumption (concomitant intake of nicotine, polyconsumption), the previous experiences of the consumer with drugs, and the intake conditions [15]. Unlike other drugs of abuse such as heroin, cocaine, or benzodiazepines, the risk of overdose of cannabis is rather low and no sudden deaths directly linked to cannabis herbs have been reported so far [13].

1.4. Psychological and systemic effects of cannabis

People primarily use cannabis for its positive effect on mood. Indeed, it results in an euphoria or "high," characterized by a sensation of relaxation, decreased anxiety, awareness, depression, and increased sociability depending on the intake conditions [16]. Nevertheless, paradoxical reactions with dysphoria, anxiety, severe panic, and psychosis can be observed when high doses of Δ^9-THC are taken. In some cases, similar effects can also occur at the first consumption or for more psychologically vulnerable people [16]. Therefore, psychotropic effects of cannabis are dose-dependent and variable depending on the quantity of cannabis consumed and on its Δ^9-THC content.

Cannabis use induces some adverse effects. It impairs cognitive and psychomotor functioning [17]. Its consumption induces slowed reaction time, motor incoordination, short-term memory loss, as well as attention disorders, and so it thus disturbs driving capacities, explaining the observed link between cannabis consumption and road accidents [13]. Furthermore, chronic use of cannabis may lead to long-term effects on attention and memory and these disturbances may last weeks or even several months after stopping cannabis consumption [18]. Moreover, cannabis can increase the risk of psychotic symptoms. It has been shown to induce the emergence of chronic psychosis such as schizophrenia in young consumers [19–21]. Finally, high and frequent cannabis consumption can lead to dependence and therefore to development of tolerance and withdrawal syndromes (see below).

At the systemic level, cannabis has mainly cardiorespiratory effects [18]. Its impact on the cardiovascular system can be reflected by induction of dose-dependent tachycardia, vasodilatation, and reddening of the ocular conjunctiva, the latter being a typical sign of cannabis consumption. Postural hypotension and fainting can also be observed. Moreover, relationships between cannabis consumption and cardiac arrhythmias, cardiovascular ischemias, coronary insufficiency, and other cases of fatal cardiac accidents have been reported [2, 22, 23]. Abusive cannabis consumption can indeed induce atrial [23] or ventricular fibrillations [24] in predisposed patients and therefore represents a major risk for persons suffering from preexisting cardiac diseases. It is noteworthy that young cannabis consumers are also at risk in terms of cardiac consequences [25, 26].

Cannabis also has a strong impact on the respiratory system. The smoke of cannabis joints contains the same constituents as tobacco (nicotine, noxious mixtures of gases and particulates) and smoking cannabis chronically causes coughing, bronchitis, and emphysema. Even if the daily number of smoked cannabis joints is generally much lower than smoked conventional cigarettes, the differing manners in which cannabis is smoked may enhance the deposition of smoke particulates at the pulmonary level in cannabis smokers, and therefore explains specific respiratory consequences of cannabis [27]. It has been suggested that by increasing oxidative stress and inducing mitochondrial dysfunctions [27] Δ^9-THC may participate, in the long-term, to the development of chronic airway diseases, pulmonary infections, and even lung cancer in heavy smokers [13, 28, 29]. More attention should be given to these adverse respiratory effects as habitual smoking of cannabis has greatly increased in our modern society.

Among other adverse effects of cannabis use, immunosuppressive and endocrine properties have also been described as disturbing reproductive functions and fertility [18]. Depending on the individual, cannabis can also induce a reduction in salivary secretion (sensation of dry mouth) and an increase in appetite [30].

1.5. Current uses of medicinal cannabis

Medicinal or therapeutic cannabis refers to preparations using *Cannabis sativa* for a pure medicinal purpose. Intake of these phytocannabinoids can include smoking, inhaling, local application, or ingestion. Depending on the country, medicinal cannabis use is not allowed, permitted, or accepted. In France, any use of cannabis was removed from the French Pharmacopoeia in 1953 [31]. Its use, importation, sale, transportation, and production are strictly forbidden. On the other hand, medicinal cannabis use is authorized in several countries, including Canada, New-Zealand, Australia, Netherlands, Spain, United Kingdom, and more than 20 states in the United States of America. In Canada, for example, production and sale is allowed under the supervision of Health Canada [32]. In the United States of America, therapeutic cannabis is still classified as a "schedule I controlled substance" [33]. The American Medical Association recently proposed rescheduling it to become a "Schedule II controlled substance," meaning that it has potential abuse risk and is accepted for therapeutic use [34]. Some states have voted on an amendment to permit medicinal use in conditions under physician agreement for specific indications such as severe pain, cancer, cachexy, glaucoma, AIDS, multiple sclerosis (MS), severe nausea, or sleep disorders [33–37]. This creates a patchwork of state laws in the United States of America for legal use of medicinal cannabis, its indications and maximal amount tolerated.

International nonproprietary name	Brand name	Active ingredient(s)	Indications
Dronabinol	Marinol®	Δ^9-THC	Chemotherapy-induced nausea and vomiting AIDS-related loss of appetite and weight loss
Nabilone	Cesamet®	Synthetic analogue of Δ^9-THC	Chemotherapy-induced nausea and vomiting
Nabiximols	Sativex®	Δ^9-THC and CBD (ratio 1:1)	MS-related spasticity

Table 1. Marketed cannabinoid-based medicines.

Phytocannabinoids or derived compounds already exist on the market for some medicinal interventions (**Table 1**). Δ^9-THC is mostly used as a therapeutic compound for its antiemetic and orexigenic properties. It has been commercialized under Dronabinol (Marinol®) to suppress nausea produced by chemotherapy and anorexia symptoms associated with AIDS. A synthetic analogue of Δ^9-THC, Nabilone (Cesamet®), has also been produced to treat nausea and vomiting linked to chemotherapy. Nabiximols (Sativex®) contains an equal mixture of the

phytocannabinoids Δ^9-THC and CBD and is usually prescribed to relieve pain in adult patients with MS or advanced cancer. It is administrated as a buccal spray in several countries, including Canada, Spain, Denmark, Germany, and United Kingdom [38]. Interestingly, these compounds are reported to only be used for recreational purposes and are therefore abused extremely rarely [39]. Indeed, the mode of consumption (oral) and their pharmacokinetic features delays a potential high effect for several hours and is therefore much less euphoric than cannabis. Recently, Sativex has been suggested as a potential substitutive medication for cannabis dependence and may help decrease withdrawal syndromes [40]. **Table 2** summarizes the current pathologies or symptoms treated with the approved phytocannabinoids derivatives.

Pathologies	Symptoms of the pathologies targeted by the cannabinoids	References
Acquired immune deficiency syndrome (AIDS)	Anorexia (loss of appetite and weight loss related)	[38]
Cancers and chemotherapies	Nausea and vomiting	[38]
Neuropathic/chronic/inflammatory pain	Pain intensity, inflammation, spasticity	[35, 39, 42, 48]
Gilles de la Tourette syndrome	Motor and verbal tics	[43, 44]
Multiple sclerosis (MS)	Spasticity	[36, 45]
Amyotrophic lateral sclerosis (ALS)	Tremors, motor deficiencies	[234, 235]
Huntington's chorea	Motor and cognitive disorders	[232]
Psychosis and schizophrenia	Anxiety, hallucinations, paranoia	[219, 220]
Depression	Sadness, demotivation, loss of interest, sleep disorders	[255, 257]
Heroin addiction	Craving	[221]
Cannabis addiction	Withdrawal syndrome	[189, 268]
Cocaine addiction	Hyperlocomotion, rewarding effects of cocaine	[66, 127]
Osteoporosis	Osteoclastogenesis, bone resorption, and bone fragility	[236]

Table 2. Pathologies and associated symptoms targeted by cannabinoids.

For example, clinical studies have investigated the analgesic properties of medicinal cannabis [35]. A comparison of cannabis's analgesic effect was performed between patients smoking cannabis and a placebo. A significant improvement in various types of pain including chronic, postoperative or neuropathic pain, as well as pain in patients suffering of AIDS was obtained [35]. A randomized clinical assay was conducted on patients with neuropathic pain who smoked high or low concentrated cannabis (Δ^9-THC) or a placebo. This study showed that cannabis is effective at ameliorating neuropathic pain [41]. Similar results were obtained in another randomized study including adults with AIDS-associated sensory neuropathy where the effect of smoking low concentrated cannabis three times per day over 5 days was compared with a placebo [42]. Other studies have suggested a potential role of smoking

cannabis to alleviate some symptoms of Tourette's syndrome, a central nervous system (CNS) disorder characterized by motor or verbal tics [43, 44]. Also, orally administered cannabis (a mixture of Δ^9-THC and CBD) has been tested in cohorts of patients with MS and lower spasm frequency and increase mobility has been observed [45]. Symptoms in patients who have MS with muscle spasticity were treated with oral Δ^9-THC, cannabis extract, or placebo, and results have shown that the first compound is more efficient in alleviating some aspects of the disability [46]. These observations confirm the therapeutic interests of oral administration of drugs containing Δ^9-THC such as Marinol® or Cesamet®.

1.6. Adverse effects of medicinal cannabis

It has been observed that the more Δ^9-THC content in medicinal cannabis, the more efficient it is in alleviating pain [47]. But with high dose formulations, secondary effects such as headache, dizziness, sleepiness, dry mouth or eye sensation, sedative effects, hypotension, and impairments in memory and cognition can be observed by some patients [35, 47]. Also, the best equilibrium to have an analgesic effect with the minimum side effects is strongly dependent on the individual and the preparation of the drug (various content of phytocannabinoids), which renders the dosage regimen particularly difficult. Indeed, in certain cases it has also been shown that the benefit/risk ratio is not positive for chronic pain treatment as cannabis only moderately reduce pain [48]. Recently, dronabinol has been tested for treatment of opiate withdrawal in a clinical trial and some adverse effects, including increased heart rate, were observed at the examined dose [49]. These observations raise some concerns globally about the dosage of such compound for potential benefits. Besides these potential adverse effects, poor knowledge about the interaction of medicinal cannabis with other drugs and possible psychotic and cognitive effects on patients are high safety concerns. Altogether, these observations still limit current cannabis therapeutic use [35, 37].

It is noteworthy that current therapeutic use is directed to alleviate several symptoms of various pathologies rather than being a cure for the patient. Cannabis plants containing high level of CBD are associated with lower adverse effects [50] and therefore represent a major therapeutic line of research for further drug design together with some synthetic derivatives of cannabis (see below).

2. The endogenous cannabinoid system

In recent decades, the discovery and characterization of the endogenous cannabinoid system represents a great milestone for research in the field, with considerable efforts being given to create a better understanding of the mode of action of cannabinoids. The endocannabinoid system comprises two well-characterized receptors, cannabinoid receptors CB1 and CB2, and their lipid neuromodulators (endocannabinoids), enzymes for their synthesis and their degradation.

2.1. Cannabinoid receptors

Cannabinoid receptors are receptors that were discovered in the early 1990s with CB1 and CB2 cloned from rat brain in 1990 and rat spleen in 1993, respectively [51–53]. They both are membrane receptors coupled to G-protein (Gαi/o) (GPCR) and share common signaling properties (see below). They both bind Δ^9-THC, the main component of cannabis [54, 55].

CB1 and CB2 receptors share 48% peptidic sequence homology [10]. Radioligand binding studies revealed a rather different distribution of these two receptors [56]. CB1 receptors are mainly expressed in peripheral and central nervous systems as well as in the reproductive and neuroendocrine systems [55, 57]. This receptor is highly abundant in the brain in areas involved in memory and cognitive functions like the hippocampus, involved in reward processes such as the striatum, ventral tegmental area, and prefrontal cortex, as well as in motor coordination and psychomotor performances such as the cerebellum [58–62]. CB1 is also present in brain structures involved in the regulation of appetite and nociception. With this wide pattern of expression, CB1 receptors are therefore involved in various physiological functions and are responsible for most of the psychoactive and central effects of cannabis.

CB2 receptors are highly expressed in the immune system in spleen, tonsil, thymus, mastocytes, and blood cells. It has also been described in microglial cells [53, 55]. Several studies have reported significant mRNA expression of CB2 receptors in brain areas such as granular cells of the cerebellum [63, 64] in brain stem [65] as well as in dopaminergic neurons of the VTA [66] and in a subset of excitatory and inhibitory neurons in CA1, CA3, and dentate gyrus of the hippocampus [67]. Detection of CB2 proteins in brain structures is still controversial because of the low expression and/or low specificity of available antibodies. Very little data are available on the CB2 receptor's central function and a very recent study using a reporter mouse line may help better clarifying the potential role of this receptor in brain functions [68]. The authors generated BAC transgenic GFP reporter mice to trace CB2 expression and could detect, using fluorescence techniques, that the major sources for GFP-CB2 expression are in B-cells in the spleen, blood, and microglia in the brain.

Pharmacological studies have proposed that other non-CB1 and non-CB2 receptors interact with endocannabinoids such as channel vanilloid TRPV1 recognizing capsaicin [69] or the orphan GPCR GPR55 [70, 71]. These receptors are not yet classified among cannabinoid receptors, but interactions with cannabis could potentially explain some pharmacological effects of this drug that cannot be accounted for by CB1 and CB2 activation [72].

2.2. Endocannabinoid synthesis and degradation

Two main endogenous ligands were discovered in the early 1990s: *N*-arachidonoylethalonamine or anandamide (AEA) which was isolated from pig brain [51] and 2-arachidonoylglycerol (2-AG) which was isolated from rat brain and dog intestine [73, 74]. Other endocannabinoids have been identified, such as the *N*-arachidonoyl-dopamine (NADA), the *O*-arachidonoyl-ethanolamine (virhodamine), or the 2-arachidonoyl-glycerylether (2-AGE or noladin), but their specific physiologic role needs further clarification [72].The 2-AG is a full agonist at both CB1 and CB2 receptors, whereas AEA is a partial agonist [75]. Interestingly, it

has recently been shown that 2-AG can be self-administered by rats and can stimulate dopamine (DA) transmission [76]. AEA also acts at TRPV1 receptors [11]. Both ligands show no strong selectivity for CB1 and CB2, with a ratio generally below 10 [73, 77, 78]. Endogenous cannabinoids are lipid neuromodulators synthesized from distinct phospholipid precursors. Several pathways have been described for their biosynthesis, noticeably for AEA (for a recent review, see [79]). The *N*-arachidonoyl phosphatidylethanolamine (NAPE) is one precursor for AEA, which can be generated through a two-step process involving a calcium-dependent transacylase and phospholipase D (NAPE-PLD) [80]. 2-AG synthesis mainly involves phospholipase C and diacylglycerol lipases (DAGL) [74, 81, 82]. It has recently been demonstrated using genetically modified mice that the DAGLα enzyme is directly responsible for 2-AG synthesis in the CNS [83]. Interestingly, 2-AG participates in the synthesis of arachidonic acid used for prostaglandin synthesis.

Postsynaptic neurons release endocannabinoid lipids. They cross the membrane by passive diffusion or using still not well characterized transporter systems, and then mainly act on presynaptic cannabinoid receptors. They are therefore retrograde synaptic messengers which regulate synaptic transmission. The decrease of neurotransmitter release will occur both at excitatory (glutamate) and inhibitory (gamma-aminobutyric acid or GABA) synapses (recently reviewed in [79, 84]). Endocannabinoids' local action is fast, as they are rapidly degraded through a recapture step through transporters, followed by enzymatic degradation with the fatty acid amide hydrolase (FAAH) for AEA [85, 86] and the monoacylglycerol lipase (MAGL) for 2-AG [87].

2.3. Signaling pathways

Several signaling pathways are activated by cannabinoid receptors and have been extensively reviewed [54, 55, 88]. Agonist activation induces inhibition of adenylate cyclase and a reduction of intracellular AMPc levels and therefore a decrease of protein kinase A activity [89]. On the other hand, beta and gamma subunits inhibit calcium voltage-gated channels and activate the opening of potassium channels. This effect results in membrane hyperpolarization and a reduction in neurons excitability and therefore a decrease in neurotransmitter release. At the presynaptic level this effect decreases the release of glutamate and GABA as well as acetylcholine, noradrenaline, cholecystokinin, or corticotrophin [10]. Activation of cannabinoid receptors also activates intracellular effectors stimulating MAPKinase signaling. This regulation induces phosphorylation of transcription factors and leads to gene expression modulation. CB1 signaling is well characterized and additional pathways have been described, including activation by CB1 receptors of sphingolipid metabolism by either direct synthesis of ceramide by serine-palmitoyltransferases or hydrolysis of sphingomyelin [90]. In astrocytes and glial cells, CB1 receptors are coupled to sphingomyelinase enzymes by chaperone molecules to produce ceramide that participate to apoptotic signaling [91, 92]. On the other hand, CB2 activation modulates a similar range of signaling pathways as the CB1 receptor, but clearly adenylate cyclase and MAPK pathways have been the most studied in regard to selective CB2 agonists (recently reviewed in [93]).

2.4. Physiological roles of the endocannabinoids

Endocannabinoids, by regulating the release of many neurotransmitters, act on various biological processes in the nervous, digestive, reproductive, pulmonary, and immune systems. Therefore, this system plays a critical role in a wide range of physiological functions, including energy balance, nociception and modulation of pain responses, and cognition and emotion as well as in reward. The central and peripheral roles of the endocannabinoid system have recently been extensively reviewed (see [94–101]) and the physiology and pathophysiology of this system have been largely studied using classical pharmacology and genetically modified animals [102]. Here, we provide just a few important specific points to illustrate these many roles.

The endocannabinoid system participates in several perception processes. Among them, nociception and modulation of pain responses have been particularly well studied and stimulation of the endocannabinoid system globally decreases pain sensitivity [98, 103]. CB1 receptors are a central player in these responses, but CB2 receptors also play a crucial role in the modulation of the immune response of the nervous system during neuropathic or joint pain [104, 105]. The endocannabinoid system has been shown to participate in different types of pain including acute, inflammatory, and neuropathic pain. Endocannabinoids like AEA also have strong antinociceptive properties [106] and can decrease pain perception in a situation of chemical skin damage, for example [107]. In terms of perception, this system also plays a role in retina physiology [108]. CB1 receptors have been shown to be expressed in the inner and outer plexiform layers of the retina of several species. Its activation in retinal bipolar cells decreases the amplitude of voltage-gated L-type calcium channel currents and therefore modulates photoreceptor activity. Also, CB2 expression has been demonstrated at RNA [109] and protein [110] levels and a specific role for this receptor in shaping retinal responses to light has been proposed [111]. These observations illustrate a modulatory role for the endocannabinoid system in visual processing. Agonists for these receptors significantly decrease intraocular pressure indicating a potential therapeutic effect for glaucoma treatment [112].

Noticeably, energy balance is an essential basic function where the endocannabinoid system has been revealed to play a major role. Indeed, cannabis is well known for hunger activation and specifically stimulating an appetite for sweet. Orexigenic properties of Δ^9-THC have been confirmed on rat studies [113] and food intake is increased by CB1 receptor activation in hypothalamic structures of the brain [114]. The endocannabinoid system is therefore clearly involved, mainly through central CB1 receptor activation, in food intake behaviors and energy balance [114–116]. It also plays a role in these processes by directly acting on peripheral organs like the gastrointestinal tract where both CB1 and CB2 receptors are expressed and participate in the regulation of motility and barrier function [117].

In the periphery, cannabinoid receptors are also expressed in cardiovascular tissues and their endogenous ligands have distinct effects. For example, it has been shown that AEA and 2-AG induce vasodilation which can trigger hypotension [118]. They can also induce depressor effects and bradycardia [119]. The endocannabinoid system clearly plays a role in cardiovascular-related diseases and opposing effects of activation of CB1 and CB2 have been suggested, highlighting potential therapeutic intervention [117].

In the brain, CB1 receptors are highly expressed throughout many structures including the hippocampus, cerebellum, and prefrontal cortex, suggesting a crucial role in cognitive functions such as learning and memory, motor coordination, and emotions like anxiety or depression and reward. Cannabis has been initially consumed to modify one's mood state and CB1 is proposed as a strong contributor for this effect. A rodent model for depressive-like behaviors has recently been proposed with genetically modified animals where this receptor is absent [120]. Dysfunction of many other processes is also produced by cannabis consumption and these events are thought to be mainly mediated through CB1 receptors. Interestingly, a possible implication in psychiatric disorders has been more recently proposed for CB2 receptors. A link between CB2 activation and schizophrenia has been considered [121, 122]. In addition, recent evidence suggests a neuromodulator role of CB2 receptors in addictive processes, with an implication in cocaine, nicotine, or ethanol effects [122–127]. Whether these central effects are due to CB2 expression in neurons, microglia, or inflammatory cells is still under investigation (see in [79]).

The endocannabinoid system also plays a neuroprotective role in some pathological conditions as it has been shown that CB1 receptors expressed specifically in glutamatergic hippocampal neurons are both necessary and sufficient to provide substantial endogenous protection against kainic-acid-induced seizures [128]. This system also modulates adult neurogenesis in the hippocampus with a pivotal role in some steps of this process, probably through activation of both CB1 and CB2 receptors (reviewed in [129].

Altogether pharmacological studies and preclinical models have allowed substantial progress in understanding the cellular and molecular mechanisms of the prolonged use of cannabinoids [130, 131]. In particular, genetically modified animal models, using knockout and conditional knockout methodologies together with viral approaches (inactivation or re-expression of components of the endocannabinoid system), have greatly improved our knowledge on the physiological and pathophysiological relevance of endocannabinoid signaling (see [102]). The use of animal models is also greatly evolving with rapid genome engineering technologies being developed using CRISPR-Cas9 and will surely improve our knowledge of the cannabinoid system [132, 133].

In conclusion, endocannabinoids are neuromodulators of important homeostatic mechanisms, including nociception and control of pain, vision, digestive and reproductive systems, energy balance, mood regulation, cognitive functions, and immune system and reward processing. Dysfunction of the system may induce pathologies and we particularly explore the role of this system in the development of addiction.

3. Cannabis misuse and dependence

3.1. Cannabis misuse

Besides its medicinal use, cannabis has been used for its psychotropic effects and the addictive potential of its components, including Δ^9-THC, has been well described since the discov-

ery of the endogenous system. Indeed, cannabis and its derivatives represent the most consumed illicit drug in modern countries worldwide. More popular than cocaine, amphetamines, ecstasy, or opiate in Europe, about 21.7% of citizens (15–64 years old) have taken cannabis at least once in their life. This represents an estimated 14 million Europeans aged 15–34 using the drug in the last year and about 3 millions using it daily. Among intensive cannabis users, close to 7% of individuals have become dependent [134] and are now seeking treatment for cannabis-induced disorders. Even if this represents a minority of consumers, it globally represents a large number of people that may develop a cannabis-related health problem and is therefore a growing recognized public health threat [4]. Prevalence among young people is greater and corresponds to recreational use. Moreover, it has been reported that more men use cannabis, which may correspond to increased risk-taking behavior, but one cannot exclude a further increase of this behavior toward cannabis in women, as it has been observed for other drugs of abuse such as tobacco or alcohol in recent years. Also, global intake of cannabis is rather stable in Europe (Norway, Germany, France, and United Kingdom). Nevertheless, some European countries where prevalence used to be low now show a noticeable increase in cannabis use (Italy and Bulgaria). Internationally, cannabis use is still controversial, as a strong disparity for its use and legal state is observed [4]. In the United States of America, about 3 million adults and adolescents tried cannabis for the first time and about 10% of the population (32 million citizens) have used this drug in 2012 [135]. It is noteworthy that cannabis consumption is easier in the United States of America as it is more and more accessible due to its legalization in some state for either therapeutic intervention (20 states in March 2014: Alaska, Arizona, California, Colorado, Connecticut, Delaware, Hawaï, Maine, Massachusetts, Michigan, Montana, Nevada, New Jersey, New-Mexico, Oregon, Rhode Island, Vermont, Virginia, Washington DC, and Washington state) [33, 34, 136] or even more recently for recreational use (Alaska, Colorado, and Washington state, [137, 138]).

In conclusion, misuse or repeated use of cannabis may lead to societal and health issues. Cannabis abuse is correlated with poor academic performance, legal problems, risky behaviors, unemployment, road accidents, and a higher risk of developing psychological disorders, respiratory diseases, and cardiovascular problems [6, 19, 139–141]. In addition, its consumption is often associated with other psychostimulants to potentiate their effects, which makes this polyconsumption more dangerous for the individual. For example, alcohol taken together with cannabis may increase the risk of death car accident of about 15 times [142].

3.2. Cannabis and adolescents

The popularity of cannabis has grown substantially in recent years among young people. In Europe, a survey performed in 2011 (European School Project on Alcohol and other Drugs) showed that about 24% of the young population has taken cannabis at least once, 20% in the year 2011, and 12% within the last month [4, 142]. Similar to patterns of adult use, more boys are consumers of cannabis (1.5 times more than girls), probably because risk-taking behavior is more pronounced in boys. Consumption is recreational for most young users, but about 2% of them become intensive users. Even though cannabis is still illegal in most places, it is largely perceived as harmless. Thus, there is a normalization of usage among the young population

which has spread among many countries and is therefore a growing health concern. Indeed, the most striking observations related to consumption during adolescence are a predisposition to develop psychiatric and cardiovascular pathologies [6, 134]. In the latter case, a recent study was performed on data collected by the French Addictovigilance Network from 2006 to 2010, a nationwide network of regional addictovigilance centers focused on achieving reliable surveillance of abuse and pharmacodependence cases. They analyzed spontaneous reports of cardiovascular complications related to cannabis use and showed some death cases from coronary syndromes, juvenile arteriopathies, and acute cerebral angiopathy [143]. Association with an increased risk of myocardial infarction has also been reported, with aggravation of coronary ischemia and even triggering of myocardial infarction [144]. Also, myocardial infarction, sudden cardiac death, cardiomyopathy, stroke, transient ischemic attack, and arteritis have been described [136]. Complications can occur in young users without preexisting cardiovascular problems [136] and at a greater frequency [143]. Therefore, potential for marijuana-associated adverse cardiovascular effects is of extreme seriousness as it may even have been underestimated if the analysis were based on spontaneous reports [143].

Recreational consumption by adolescents may lead to subsequent drug abuse. Cannabis has been proposed as a gateway drug [145, 146]. This hypothesis is still debated and neurobiological mechanisms are still not fully understood. Preclinical studies suggest nevertheless that cannabis may facilitate the sampling of other drugs of abuse such as alcohol, cocaine, and heroin. Indeed, rat exposure to Δ^9-THC during adolescence increases voluntary heroin intake in adulthood [147]. Furthermore, data analysis of transcriptomic and DNA methylation modifications has revealed a generational transmission of adaptations both at the neurobiological and behavioral levels in animals with parental exposure to Δ^9-THC [148, 149]. These observations suggest long-term adaptations and germinal transmission which may involve epigenetic events with gene expression modulations such as DNA methylation or histone post-translational modification [150, 151].

Academic difficulties have also been observed among occasional cannabis users during adolescence [139]. Regular consumption is predictive of a person having a higher risk of problems with other drugs in adulthood, along with psychological and societal issues [139]. It has been shown that consumption during adolescence may increase chronic psychosis and this phenomenon is reinforced when there is a genetic predisposition to the problem [19].

3.3. Dependence on cannabis

As opposed to cocaine or heroin, cannabis has often been considered a less harmful drug with low dependence properties and minimal negative effects, but its addictive potential has long been questioned [152]. Recent research has made strong progress in the knowledge of the mechanisms of action of cannabis and no doubt has subsisted that cannabis is an addictive drug. Moreover, an increasing number of cannabis consumers are seeking efficient treatments indicating that a growing fraction of the population is being dependent on cannabis.

Chronic consumption of a drug may lead to addiction and this brain disease can be characterized by specific behavioral consequences: compulsive drug seeking, uncontrolled-drug intake, craving for the drug, and strong potential for relapse. This addictive behavior evolves

despite its adverse consequences on everyday life. This phenomenon has been well documented by several reviews which propose a model for this spiral of addiction [62, 153–156]. It develops in a small proportion of casual users and relies on psychological, genetic and environmental factors participating to the individual vulnerability [157–160]. To precisely evaluate this pathological process, the American Psychiatric Association has proposed the DSM-V (Diagnostic and Statistical Manual of Mental Disorders) as a reference for diagnosing addiction as a mental disorder [161]. Several criterions are documented for this evaluation and allow classifying the severity of the individual addiction depending on the numbers of criterions identified.

When cannabis plant is taken, Δ^9-THC enters the body through the lungs and circulating blood, which causes it to quickly reach the brain. When cannabis is eaten (space-cake for example), absorption is slower [35]. Δ^9-THC targets the cannabinoid receptors and therefore heavily activates the endocannabinoid system, which triggers the psychological and physical effects of the drug. Among the criterion listed for cannabis, the development of tolerance [162] and withdrawal syndromes caused by the interruption of consumption [152] are well recognized. Tolerance is characterized by a decrease of the effects following repeated drug consumption or by a need to increase the amount taken to reach similar effects [163]. Nevertheless, development of tolerance can vary between individuals in terms of physiological responses or behavior [164]. Withdrawal syndromes appear when the individual is under abstinence following chronic intake of the drug or when the individual seeks for the same drug or another one to alleviate these symptoms [15, 163]. Spontaneous withdrawal is observed in humans to cause increased agitation and excitability, insomnia, anxiety, aggressiveness, depression state, anorexia, and tremors [13, 152]. Interestingly, the criterion of craving cannabis has been added for the first time to the DSM-V.

Specific animal models have been developed to study drug rewarding effects mediated by specific brain structures in preclinical research. In order to characterize cannabis effects observed in humans, rodent models using repeated administration of cannabinoid agonists have been elaborated to evaluate consequences of such a chronic exposure as well as the addictive power of these cannabinoids [165]. This allowed a better understanding of motivational and reinforcing properties of these drugs [15]. The self-administration paradigm (SA) is recognized as the most powerful model for measuring both the rewarding and motivational effects of a drug [166]. It is an operant system based on a voluntary procedure to obtain the drug, coupled with the association of a signal [167]. Intravenous (iv) SA has been rather difficult to establish for Δ^9-THC (probably due to its partial agonistic nature) and several adaptations were necessary to obtain a reliable model. Indeed drug priming, low doses, food restriction, and animal restraint were useful to measure cannabinoid agonists properties in this task [96, 165, 168]. Both Δ^9-THC and the synthetic cannabinoid WIN55,212-2 have been successfully described to promote self-administration in rats and mice, and extended to the study of mice deficient for cannabinoid receptors [169–172]. Other synthetic compounds were also successful, such as CP 55,940 and HU-210 [173]. Moreover, the selective antagonist of CB1 receptor (rimonabant or SR141716A) was able to reverse cannabinoid-induced iv SA, illustrating the major role of this receptor in reinforcing properties of the drugs [171]. Interestingly, stable iv

SA of Δ^9-THC was obtained in squirrel monkeys, with much lower doses than for rodent studies [174, 175], illustrating potential differences in the pharmacodynamic and kinetic properties of this drug across species.

A task evaluating the conditioned place preference (CPP) has been developed to study reinforcing properties of drugs associated with an environmental cue like the context in which the drug is administered [176]. Interestingly, conflicting evidence exist about either positive (CPP) or negative (CPA) properties of cannabinoids, depending on experimental conditions used [96]. Agonists such as Δ^9-THC and CP 55,940 used at high doses produce aversion, therefore animals will avoid the compartment where the drug was injected [177–180]. Also, the antagonist SR141716A induced CPP at low and high doses in rats, revealing reinforcing properties [180]. Nevertheless, reinforcing properties can be observed with low doses of Δ^9-THC, longer conditioning periods or priming injections of the drug in the home cage prior to conditioning [181]. This nonoperant paradigm with specific experimental conditions has been used to study cannabinoid effects in genetically modified mice (for review see [182]). Finally, withdrawal signs following repeated exposure to Δ^9-THC can be measured in rodents. They may be either spontaneous or precipitated by a selective CB1 antagonist, and somatic signs can be scored for providing an index of dependence [152, 183]. For example, mice exhibited several signs including tremor, ataxia, piloerection, ptosis, and decreased motor activity [177, 184].

3.4. Medications for cannabis dependence

Besides preclinical evidence of cannabis-induced dependence in rodent studies, demand for treatment of cannabis use disorders is increasing. This illustrates the fact that an increasing number of people are dependent on cannabis. In the United States of America, more people are dependent on cannabis than cocaine (4.5 and 1 million in 2010, respectively) and therefore more people are seeking treatment for the first, rather than second, drug [185]. In 2013, about 845,000 people aged more than 12 years old received treatment because of their cannabis consumption [5]. In general, adults seeking treatment have been regular users for more than 10 years and have already tried to reduce or stop their behavior [134, 152].

There are currently no approved medications for the treatment of cannabis dependence and cannabinoid antagonists could be a potential pharmacotherapy [186]. The use of selective CB1 antagonists for the treatment of drug dependence has been investigated in preclinical studies as CB1 receptors are highly expressed in brain structures related to reward (see Section 2.1) [187, 188]. In a study using squirrel monkeys, rimonabant, a selective CB1 antagonist with some inverse agonistic properties, blocked cue-induced drug seeking, Δ^9-THC-induced drug seeking, and the direct reinforcing effects of Δ^9-THC suggesting that this compound may help to maintain abstinent behavior [189]. Such medications might be effective treatments for cannabinoid dependence, but they have not been tested on humans' cannabis reinforcing effects.

Cognitive behavioral therapy associated with contingency management is quite efficient for treating cannabis dependence [4, 134]. Moreover, family-based interventions may help adolescents dealing with cannabis withdrawal and craving and social help for employment is

also crucial for limiting relapse [4]. Proposed therapeutic approaches are based on existing ones known to be effective in the treatment of other drug use disorders like baclofen for alcoholic withdrawal. Some clinical studies suggest that existing medications for other indications may be promising target for cannabis use disorder. Among them, buspirone, bupropion (Zyban®), sodium divalproate (Depakote®), and lithium may have therapeutic benefits [190]. Bupropion (Zyban®) is an inhibitor of noradrenalin and dopamine recapture, and an antagonist of nicotinic receptors. It is used to treat nicotinic withdrawal, and is an antidepressant. Sodium divalproate (Depakote®) is an antiepileptic drug used to treat bipolar symptoms. Buspirone is used as an anxiolytic and lithium as a thymoregulator. Finally, novel approaches have focused on cannabinoid replacement therapy (CRT) and showed that Sativex®(Nabiximols), a buccal spray containing Δ^9-THC and CBD used to treat spasticity associated with MS, may be a useful substitutive medication for cannabis dependence [40]. Nevertheless, controlled clinical trials are needed to confirm potential therapeutic efficacy of these molecules in cannabis-dependent treatment [190].

3.5. Synthetic cannabinoids

A more recent problem that has strongly increased the risks of drug use is the proliferation worldwide of new derivatives of synthetic cannabinoids. These drugs, also known as synthetic marijuana, "Spice," or "K2," are often less controlled and are being sold on the Internet which greatly facilitates their access [2, 191, 192]. Spices usually look like a mixture of dry herb plants where a multitude of compounds have been sprayed. More recently, these molecules have been sold for liquid preparation used in e-cigarettes [193]. These synthetic compounds are analogs of cannabinoids, but the exact content of the mixtures is not fully known and many chemical types of compounds are now being produced. They induce similar euphoric and relaxing effects as classical cannabis derivatives but they present a much higher potency. It is not even clear that they all contain Δ^9-THC [194]. Also, they do not contain CBD, which is suggested to balance the antipsychotic effects of Δ^9-THC in cannabis [195]. There may be specific risks associated with these new drugs compared to those known for cannabis. Indeed, they produce increased or even additional adverse effects, such as tachycardia, hypertension, chest pain, cardiac palpitation, intense sweating, convulsions, drowsiness, and agitation [192, 193, 196]. In adolescents, hallucinations, paranoia, and myocardial infarction have been reported [197]. More toxicology studies are needed to better characterize the adverse effects of these substances [198]. Altogether, synthetic cannabinoids represent a significant public health issue with an evolving legal market place that specifically target young populations and is difficult to control.

3.6. Cannabinoid-opioid interactions

Mechanisms of action are yet more complex as the endocannabinoid system interacts with other neuromodulatory systems such as the hypocretin, dopaminergic, adenosinergic, and opioid systems. The latter is of particular interest as the endocannabinoid and the opioid systems share neuroanatomical, neurochemical, and pharmacological characteristics [199–202]. The opioid system consists of three GPCR named mu, delta, and kappa receptors which

interact with endogenous ligands (enkephalins, dynorphins, and endorphins) as well as exogenous ligands, including morphine or heroin. A hypothesis of cross-modulation includes the release of opioid peptides induced by cannabinoids (or reversely of endocannabinoids by opioids), and possible direct interaction at the level of receptors or signaling pathways. Evidence for specific interactions in the modulation of nociception has been provided both with *in vitro* and *in vivo* approaches [203, 204]. In the context of responses associated with reward and relapse, specific mechanisms have been highlighted, in particular with the use of knockout approaches [182]. Noticeably the mu opioid receptor has been proposed as a convergent molecular target mediating rewarding properties of opioid compounds but also of other drugs of abuse, including cannabinoids [205, 206]. The participation of the enkephalinergic system, with a joint action of mu and delta receptors, in behavioral responses associated with cannabinoid dependence has been clearly demonstrated. Moreover, chronic exposure to cannabinoid agonists induced a modification in both the density of mu opioid receptors and their activity in structures related to reward, which may contribute to the development of cannabinoid dependence [207]. Interestingly, studies using cannabinoid iv SA experiments have shown that opioid antagonists can block cannabinoid intake in mice and rats [169] and in squirrel monkeys [175]. Moreover, iv SA of morphine is abolished in animals deficient for the CB1 receptor [208], confirming a role for CB1 receptor in the modulation of opioid reward [182, 209]. Additionally, the antagonist rimonabant can precipitate withdrawal signs in morphine dependent animals and reciprocally, the opioid antagonist naloxone can precipitate these effects in cannabinoid dependent rats [210]. Another cross-interaction occurs at the cellular levels with colocalization of CB1 and opioid receptors observed in several brain structures including limbic areas, mid-brain, brain stem, or spinal cord [182, 211, 212]. Also, heterodimerization processes between cannabinoid and opioid receptors have been reported in both *in vitro* and more recently *in vivo,* in neuronal populations [213, 214]. This physical proximity is suggested mainly for CB1 receptors and delta or mu receptors and may impact on signaling properties of these receptors in specific brain structures, therefore possibly influencing analgesic or addictive responses involving these receptors. More research remains to be done to decipher the physiological role of such heteromers [214, 215].

4. Therapeutic perspectives

Even though cannabinoids are considered a drug of abuse and can induce dependence, they are used to treat several pathologies, including drug dependence. As developed earlier in this chapter, there are several risks associated with cannabis use, including altered short-term memory and decision making, increased anxiety and psychosis, and an increased risk of cardiovascular and lung diseases. On the other hand, the beneficial effects of cannabinoids in specific pathologies are worth trying to develop for medical use and therefore represent a main therapeutic challenge for health science. Several therapeutic strategies are currently being developed with some limitations. **Figure 1** and **Table 3** summarize some of these approaches.

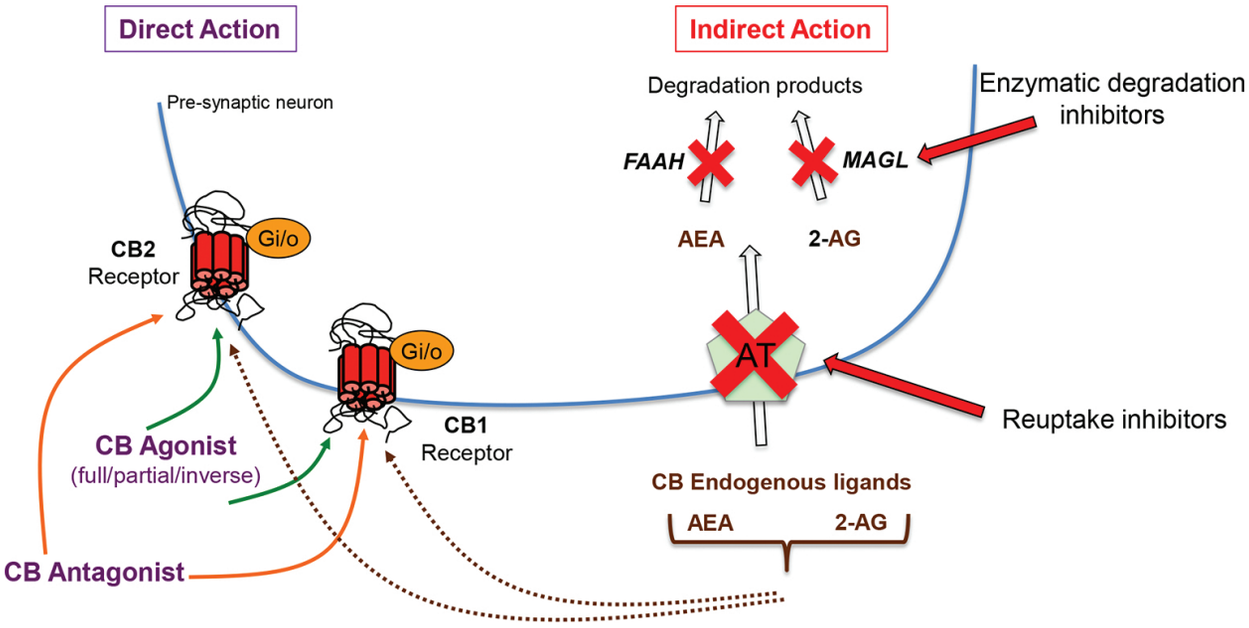

Figure 1. Therapeutic interventions targeting the endocannabinoid system. Commercialized compounds or molecules under study for targeting the endocannabinoid system are acting on different processes. Two main strategies are being developed to target the endocannabinoid system. A **direct** process will focus on compounds that bind to the cannabinoid receptors CB1 and/or CB2 receptors, with full, partial, or inverse agonists or antagonists (see text for details). An **indirect** process will aim at increasing endocannabinoid levels *in situ* and, therefore, target either the reuptake of anandamide and 2-AG or their degradation by their specific enzymes, FAAH and MAGL, respectively. Only the presynaptic neuron is represented here. Thin arrows represent the action of endogenous (hatched) or exogenous (plain) ligands on cannabinoid receptors. For detailed references, see the text. Abbreviations: AEA, anandamide; 2-AG, 2-arachidonoylglycerol; AT, anandamide transporter; CB, cannabinoid; FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase.

4.1. Cannabidiol as a promising therapeutic medication

Cannabidiol (CBD) is a phytocannabinoid that has a low affinity for both receptors CB1 and CB2 with inverse agonistic properties [96, 216] and antagonistic effects, with CB2 receptors in particular [216] (see Section 1). This action explains the opposite effects of CBD toward Δ^9-THC and illustrates the interest to associate both compounds for developing a new medication. As mentioned earlier, CBD is commercialized in association with Δ^9-THC as Sativex®. Other properties such as anxiolytic, antidepressor, and antipsychotic effects have been observed with CBD [217]. Recent studies have revealed that CBD can decrease the cognitive and memory impairments induced by Δ^9-THC in male rhesus monkeys [218]. Evidence from several research domains suggests that CBD can be used for antipsychotic treatment (for reviews, see [219, 220]). A clinical study shows that anxiety and psychotic effects produced by a high concentration of Δ^9-THC can be reversed by CBD administration. Animal studies investigated the pharmacological profile of this phytocannabinoid and revealed a similar pattern to atypical antipsychotic drugs (clozapine or risperidone). Also, clinical studies on schizophrenic patients using CBD demonstrated a potential for this drug to be used as an alternative treatment for schizophrenia [220]. More investigations are still needed to better demonstrate the potential of this phytocannabinoid as a medication.

Strategy	Therapeutic potential	Limitation
Medicinal cannabis (phytocannabinoid including: Δ^9-THC, CBD, Δ^8-THC, CBN, etc.)	Severe pain (neuropathic, AIDS-associated, postsurgery, chronic) Muscle spasticity; cancer; cachexia, glaucoma, nausea	Adverse effects (psychosis) Safety issues (drug interaction unknown) Therapeutic benefit not fully proved Various pharmacodynamic and pharmacologic profiles Difficult dosage regimen
Cannabidiol	Anxiety; depression; psychosis(schizophrenia, bipolar disorders) Opiate dependence Pain Neurodegenerative diseases (AD, PD) Inflammatory diseases (rheumatoid arthritis) Brain ischemia Diabetes; nausea ; cancer	Clinical trials needed
CB2 receptor agonist	Chronic inflammatory and neuropathic pain Neurodegenerative and neuroinflammatory diseases (AD, MS, HD, ALS, brain ischemia) Osteoporosis Peripheric inflammatory disorders (atherosclerosis, nephropathy, liver disease) Cocaine dependence	Poor results in clinical trials compare with preclinical data
CB1 receptor antagonist	Drugs of abuse dependence(cannabinoids, nicotine, alcohol, opiates) Obesity; metabolic disorders	Adverse effects (anxiety, depression, suicides) Clinical trials needed
Peripheric CB1 receptor antagonist	Weight gain Atherosclerosis Gastrointestinal, liver, pancreatic or coronary artery diseases Arthritis	Preclinical studies to complete Clinical trials needed
Inhibitors of endocannabinoids degradation or inhibitors of recapture transporter	Anxiety; depression Opiate dependence	Clinical trials needed

This table lists examples of strategies targeting directly or indirectly the endocannabinoid system being developed or in progress for treating several pathologies. Most of trials were perform in preclinical studies. For most of candidate medications clinical trials must be perform or completed to confirm the efficiency in human and the safety of the various compounds. For detailed references, see the text.

Table 3. Examples of therapeutic strategies targeting the endocannabinoid system.

Interestingly, a rodent study using iv SA of heroin has revealed a potential for CBD as a medication for heroin dependence [221]. Indeed, the authors show in their model that CBD does not alter the intake of heroin, but specifically impairs the seeking behavior reinstated by a conditioned stimulus cue. This CBD effect is associated with the normalization of neurobiological changes observed in this model, noticeably in the CB1 receptor expression in structures related to reward like Nucleus Accumbens. In humans, opiate dependence is mostly treated with substitutive therapy like methadone but this molecule does not affect heroin craving. Therefore, CBD represents a potential alternative to treat heroin craving and relapse [221].

CBD has been shown to have analgesic properties. Paclitaxel is an anticancer drug that induces neuropathic pain. In a rodent model with chemotherapy-induced neuropathic pain and this medication, CBD was able to decrease mechanical sensitivity [222]. It had no conditioned rewarding effects and did not affect conditioned learning and memory. The precise mechanism of action is still not clear and may partly involve the serotonin system [222]. An interaction of CBD with CB2 receptor has also been suggested in heterologous system [216]. CBD may be an efficient treatment to reduce the neuropathic pain induced by chemotherapy without any of the potential side effects of cannabinoids.

Finally, other pathological situations, such as neurodegenerative diseases (Parkinson and Alzheimer), cerebral ischemia, diabetes, nausea, rheumatoid arthritis, or other inflammatory problems could be treated with CBD [223, 224]. Additionally, the potential for CBD as a medical intervention in psychotic disorder has been reviewed recently [219]. CBD represents a therapeutic approach for several disorders, but more clinical studies are needed. Also, plants with higher content in CBD or a low ratio Δ^9-THC/CBD are being produced for medical application and may not be of interest for euphoric purposes.

4.2. CB2 receptors as targets for medication

Pharmacology has provided many synthetic cannabinoid ligands that specifically interact with cannabinoid receptors and therefore represent great tools for research and clinical applications. The limitation for the use of these compounds as therapeutic drugs so far is that most of them target CB1 receptors and therefore may lead to adverse psychotic effects [225]. Indeed, CB1 receptor activation induces most of the central and psychotic effects of cannabis (see Section 2.1). Even though CB2 receptors are expressed in the CNS (see Section 2.1), they are mostly involved in inflammatory processes and are therefore less implicated in adverse central effects. Noteworthy activation of CB2 is not associated with tolerance or withdrawal syndrome in animal models of neuropathic pain [226]. In addition, no CPP or CPA could be measured using the CB2 agonist JWH-133 at the doses tested in mice, neither SA with this same agonist in mice able to self-administer cocaine, revealing no direct role of the CB2 receptor associated with reinforcing properties of cannabinoids [127]. Targeting these receptors for therapeutic purposes is therefore of strong interest [93].

As CB2 is highly expressed in immune cells, a potential role in treating several diseases including inflammation, cancer, osteoporosis, and liver diseases has been proposed (for a recent review, see [227]). Thus, CB2 agonists represent promising medication strategies in

several therapeutic applications with modulation of inflammatory processes without triggering psychotic effect [228, 229]. For example, selective activation of CB2 receptors by the synthetic cannabinoid JWH-015 suppresses CD40 expression in a model of cultured microglial cells activated by IFN-gamma, suggesting a beneficial role of CB2 activation in pathological activation of microglial cells [230]. This effect may be of interest in the context of neurodegenerative and neuroinflammatory diseases such as Alzheimer disease (AD), MS, or Huntington's disease [230–233]. Interestingly, another selective agonist of CB2, AM1241, was effective at slowing signs of disease progression in an amyotrophic lateral sclerosis (ALS) mouse model (G93A-SOD1 transgenic mice) when administered at the onset of tremor signs [234]. Daily injections of this CB2 agonist also increased the survival interval after disease onset by 56%, with reduction of motor neuron degeneration and preservation of motor function. Interestingly, a strong increase of CB2 mRNA expression is observed in the spinal cord of this mouse model [235]. These observations highlight the therapeutic potential of CB2 agonists for the treatment of these chronic pathologies.

A study using genetically modified mice deficient for CB2 receptors has revealed a low bone mass phenotype, suggesting that endocannabinoids play an essential role in the maintenance of bone mass by signaling through CB2. The authors showed that CB2 receptors are expressed in cells of both the osteoblast and osteoclast lineages and that exposure of these cells to a CB2-specific agonist (HU-308) results in direct stimulation of osteoblasts and inhibition of osteoclasts, suggesting that CB2 signaling contributes to the maintenance of normal bone mass [236]. Thus CB2 selective agonists could play a protector role in osteoporosis and represent a treatment strategy. In addition, selective agonists for CB2 receptors have been proposed for the treatment of inflammatory disorders in periphery, including atherosclerosis [237], nephropathy [238] or chronic liver disease [239].

Finally, CB2 receptor expression has been detected in neurons and a modulator role of this receptor has been proposed in drug addiction (see Section 2.4). Chronic administration of JWH-133 CB2 agonist inhibits iv SA of cocaine, cocaine-induced hyperlocomotion, and cocaine-induced levels of dopamine in the Nucleus Accumbens, in wild-type and mice deficient for CB1 receptors, but not in knockout mice for CB2 [127]. A similar effect is observed in the ventral tegmental area with inhibition of dopaminergic activity both *in vivo* and *in vitro* [66]. Therefore, the development of CB2 agonists for the treatment of cocaine dependence may be a future strategy.

In conclusion, preclinical studies are encouraging for CB2 agonist use in therapeutic approaches, but clinical results are rather poor and more studies are still needed. These limited results may be due to low *in vivo* selectivity of the tested compounds (which may also interact with CB1 receptors), individual (gender, age) or interspecies differences in CB2 receptors and associated signaling pathways [93].

4.3. Cannabinoid antagonists to treat several disorders

Other therapeutic strategies have explored the use of specific antagonists to block cannabinoid effects [188]. Rimonabant was the first CB1 antagonist introduced into clinical practice [240]. In 2006 in Europe, it was initially developed as a medication (Acomplia®) to treat obesity

disorders and associated risks such as dyslipidemia, diabetes, and metabolic syndromes [241–243]. The anorexigenic properties of rimonabant were also encouraging to evaluate the potential of this compound to avoid weight gain when stopping nicotine consumption. A random double-blind clinical study has revealed that the addition of a nicotinic patch with rimonabant was more efficient than a placebo patch associated with rimonabant to prolonged abstinence following 6–9 weeks of treatment, but weight gain was similar in both groups [244]. Besides promising effects of this compound, it was withdrawn from the market in 2008 as strong psychiatric adverse effects have been observed during clinical trials (increased anxiety, deep depression, and suicide) [38, 240, 245] and was therefore not authorized by the Food and Drug Administration in the United States of America.

Knowing the adverse effects of rimonabant in human, development of any new selective cannabinoid antagonists with different pharmacodynamics properties (more neutral antagonist) that would possess the same activity both in animals and humans is greatly needed. In addition, such compound would have to yield a positive benefit/risk ratio to be considered for a therapeutic use and be tested in clinical research under strictly controlled circumstances that maximize safety [240]. Alternative perspectives for specific medical conditions are oriented toward cannabinoid antagonists that will only act on the periphery without CNS related adverse effects. Such compounds are being developed to treat pain (see [246]) and may also be useful for obesity and metabolic disorder, as preclinical studies have demonstrated decreased food intake using LH-21, an antagonist with a poor penetration rate into the central nervous system [247]. Likewise, peripheral antagonism may be beneficial for other pathologies with noticeable peripheral pathophysiologic mechanisms including gastrointestinal, liver, pancreatic, or coronary artery diseases ([240] and references therein).

Cannabinoid antagonists have also been evaluated for their potential in opiate-dependence therapy. Indeed, bidirectional interactions between cannabinoid and opioid systems on reward processes revealed by both pharmacological and genetic approaches (see Section 3.6) suggest a possible therapeutic intervention with cannabinoid antagonist for opiate dependence. For example, rimonabant administration suppresses morphine-induced CPP and morphine SA in mice, and heroin SA in rats [173], with the latter effect appearing only in opiate-dependent rats but not in nondependent animals [248]. Another therapeutic use for cannabinoid antagonists would be for treatment of nicotine abuse. Preclinical studies have revealed that CB1 selective antagonists, including rimonabant and AM251, reduced nicotinic SA, as well as nicotinic-induced CPP behaviors (see [249]). Besides, clinical studies have shown that rimonabant was efficient for tobacco smoking cessation, but the therapeutic effects were not better than other substitutive medications and results for abstinence were not fully convincing [250].

Moreover, CB1 antagonists have been evaluated for their use in alcohol dependence (recently reviewed in [251]). In preclinical studies, evidence accumulates for the good efficiency of cannabinoid antagonists to significantly reduce alcohol consumption and attenuate alcohol withdrawal symptoms. For example, a preclinical study demonstrated that rimonabant may be effective in reduction of alcohol consumption, most probably by indirect modulation of dopaminergic transmission [252]. On the other hand, results obtained in animals do not

necessary translate to human studies. Indeed, a double blind clinical trial with placebo has been conducted to examine the effect of a 12-week rimonabant treatment on alcohol-dependent patients under detoxification and only a mild effect was observed for efficiency against relapse [253]. Globally, results on cannabinoid efficiency for alcohol dependence are highly inconsistent and more clinical studies are needed to confirm an effect in human for this major health concern worldwide.

4.4. Inhibition of endocannabinoid degradation as a therapeutic strategy

An indirect strategy that is currently developed to target the endocannabinoid system is to limit the endocannabinoid degradation in order to increase their natural concentration *in situ* and amplify their effects. The most targeted enzyme is the FAAH enzyme (see Section 2.2) which hydrolyses AEA and therefore, by developing potent and selective inhibitors AEA actions may be prolonged. For example, the URB597 is a selective inhibitor of this enzyme [254, 255]. In both rats and mice it elicits antidepressive and antianxiolytic like effects, likely via CB1 receptor mediated modulation of serotonin and norepinephrine neurotransmission [255–258]. These observations highlight FAAH as an interesting pharmacological target to directly modulate endocannabinoid levels in the brain and therefore offer a potential treatment for depression and anxiety phenotype.

In addition, several preclinical studies have shown that the inhibitors of catabolic enzymes (FAAH and MAGL) may be useful against opiate abuse. Indeed, URB597 protects against tolerance and memory deficits in chronic morphine treatment and does not interfere with drug-induced reinstatement of either conditioned floor preference or avoidance [259]. Moreover, these inhibitors reduce somatic morphine withdrawal signs but not aversive aspects (CPA paradigm) [260]. The MAGL inhibitor JZL184 attenuates spontaneous withdrawal signs in morphine dependent mice. Morphine-dependent mice challenged with the opiate antagonist, naloxone, display a profound withdrawal syndrome. In these conditions, both PF-3845 (FAAH inhibitor) and JZL184 reduce these withdrawal signs, a process that is reversed by a CB1 antagonist SR141716A [261]. Interestingly, the FAAH inhibitors do not show any adverse effects such as hypothermia, hypomotility, or catalepsia [81, 262]. In addition, they do not show reinforcing properties and therefore are a promising therapeutic strategy to treat opiate dependence with the minimal risk of abuse that is classically observed with cannabinoid agonists [257, 260]. Interestingly, other authors have evaluated the effects of structurally different FAAH inhibitors in an animal model of working memory known to be sensitive to impairment by Δ^9-THC and showed that one FAAH inhibitor (AM3506) decreased accuracy in the memory task via a CB1-dependent mechanism, whereas the others had no effect [263].

Another target for increasing endogenous levels of the cannabinoid-receptor agonist would be to block the AEA transporter. The endocannabinoid uptake inhibitor AM404 can have antidepressant effects in the forced swim test in rat (decreased immobility), suggesting a potential therapeutic effect as for the FAAH inhibitors [264]. On the other hand, a very recent study demonstrated that AM404 was able to effectively reinforce SA behavior and induce reinstatement of drug-seeking behavior in abstinent squirrel monkeys, indicating that such a compound that promotes increased endocannabinoids may have a potential for abuse [265].

All these indirect strategies are of particular interest as they amplify cannabinoid receptor activation specifically where the endocannabinoids are produced, therefore increase signaling in defined brain structures [255]. Nevertheless, clinical studies are needed to confirm the therapeutic potential of these molecules in human and it will be crucial to evaluate the effects of such inhibitors with respect to their potential for memory impairment, abuse liability, and probably other cannabis-like effects in clinical trials before any specific therapeutic application.

5. Conclusion

The growing consumption of cannabis and its derivatives in the population and particularly in the adolescent population represents a real public health challenge. A growing interest has been developed in cannabis and related compounds in research. Furthermore, considerable debates involving its legalization are still being conducted and this may have political consequences. Risk should not be neglected and it seems crucial to widely disseminate more scientific knowledge about this family of compounds before legalization becomes a normalization. Cannabis exposure produces a range of behavioral and neurobiological adaptations and the general public should be more aware of the clinical implications of the long-term impact of this drug. Among adaptations, long-term exposure to drugs of abuse or specific exposure during a critical period of development may elicit gene expression changes through epigenetic mechanisms. Recent research using genomic technology has investigated plasticity mechanisms taking place in brain structures involved in reward circuitry and highlighted epigenetic control of gene transcription (see Section 3.2). An expected increase of scientific data in this field will help clarify the molecular mechanisms of drug abuse vulnerability. This research will also be a new avenue for proposing novel therapeutic interventions for long-term cannabis exposure or spreading abuse of synthetic cannabinoids.

Cannabinoid derivatives have positive effects on several other pathologies besides drug dependence. These applications need further rigorous clinical trials to ensure efficiency and safety in human and additional cannabinoid-related compounds need to be developed. In conjunction, a combination of strategies may be foreseen, as this is the case in other pharmacological fields, with specific care to the dose and duration of treatments. Inventive therapeutic approaches for treating pain or dependence may also consider targeting heterodimers of cannabinoid and opioid receptors using antibodies or bivalent ligands or indirectly acting on both systems using dual enkephalinase and cannabinoid catabolic enzyme inhibitors [266, 267]. Intensive research is now oriented toward such perspectives.

To conclude, and as for the opiate compounds that are used as medication, in particular, to treat pain (e.g., morphine) or abused for euphoric effects (e.g., heroin), cannabinoids by targeting a complex endogenous system also constitute a powerful pharmacological tool with both drug and medication properties. Therefore, future investigations are necessary in order to propose optimal therapeutic approaches for managing complex diseases and promising strategies for reducing dependence. The ultimate goal is to propose innovative strategies to current treatments with increased safety usage.

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