Therapeutic Potential of the Endocannabinoid System in the Brain

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Abstract: Cannabinoids have been predominantly considered as the substances responsible of the psychoactive properties of marijuana and other derivatives of *Cannabis sativa*. However, these compounds are now being also considered for their therapeutic potential, since the term "cannabinoid" includes much more compounds than those present in *Cannabis sativa* derivatives. Among them, there are numerous synthetic cannabinoids obtained by modifications from plant-derived cannabinoids, but also from the compounds that behave as endogenous ligands for the different cannabinoid receptor subtypes. Within the family of "cannabinoid-related compounds", one should also include some prototypes of selective antagonists for these receptors, and also the recently developed inhibitors of the mechanism of finalization of the biological action of endocannabinoids (transporter + FAAH). All this boom of the cannabinoid pharmacology has, therefore, an explanation in the recent discovery and characterization of the endocannabinoid signaling system, which plays a modulatory role mainly in the brain but also in the periphery. The objective of the present article will be to review, from pharmacological and biochemical points of view, the more recent advances in the study of the endocannabinoid system and their functions in the brain, as well as their alterations in a variety of pathologies and the proposed therapeutic benefits of novel cannabinoid-related compounds that improve the pharmacokinetic and pharmacodynamic properties of classic cannabinoids.

Keywords: Endogenous, plant-derived and synthetic cannabinoids, CB_1 receptors, anandamide, 2-arachidonoylglycerol, endocannabinoid transporter, FAAH, therapeutic potential.

1. A GENERAL APPROACH ON THE ENDOCANNABINOID SYSTEM

Mimicking the process followed in 70th decade when several studies demonstrated that the active principles of the plant *Papaver somniferum*, which serves to obtain the *opium*, act on the brain by activating various membrane receptors which serve for the binding of several endogenous compounds, so-called opioid peptides (enkephalins, β-endorphins and dynorphins) [1], during the 90th decade, we have known a similar development with the active principles of another plant, *Cannabis sativa*, also used by humans since ancient times with a dual purpose, medicinal or recreational [2]. These studies have also resulted, as happened with the "endogenous opioid system", in the description of the so-called "endogenous cannabinoid system", whose physiological, biochemical and pharmacological characteristics, as well therapeutic potential, are presently being to be elucidated. The endocannabinoid system represents a novel mechanism of cellular communication, and is formed basically by three types of elements: (i) endogenous ligands, (ii) membrane receptors, and (iii) mechanism for the inactivation of the endocannabinoid signal.

1.1. Endocannabinoid Ligands

The endocannabinoid ligands are mainly derivatives of the arachidonic acid, such as the arachidonoylethanolamide ("anandamide") and the 2-arachidonoylglycerol [3]. In recent years, it has been demonstrated how these endocannabinoids are synthesized, released, reuptaken and/or metabolized in the nervous cells, which support a neuromodulatory function for these compounds [4]. The synthesis of anandamide is Ca^{++}-dependent and is produced locally, upon demand, by the phospholipase D-mediated cleavage of the membrane precursor called *N*-arachidonoyl-phosphatidylethanolamine. However, instead accumulating in synaptic vesicles, anandamide is immediately released to the synaptic cleft where it is able to bind to several membrane receptors (see Fig. **1**), frequently located presynaptically, which enable anandamide-cannabinoid receptor signaling to control presynaptic events mainly the release of several neurotransmitters such as GABA, acetylcholine, glutamate and others [5]. Also concordant with this presynaptic location of cannabinoid receptors is the recent proposal that endocannabinoids may act as retrograde signal molecules at synapses (for review, see [6]). 2-Arachidonoylglycerol works in the same direction, although the biosynthesis of this endocannabinoid differs from that of anandamide and involves at least two different hydrolysis pathways both from inositol-phospholipids (for review, see [7]). In the last two years, additional active endocannabinoid ligands have been identified, among them, arachidonoyl-glyceril-ether (noladin-ether) [8], N-arachidonoyl-dopamine [9] or virodhamine [10], although their biochemical and pharmacological characteristics remain to be described.

1.2. Cannabinoid Receptors

To date, at least two different cannabinoid receptor subtypes have been cloned and characterized. They belong to

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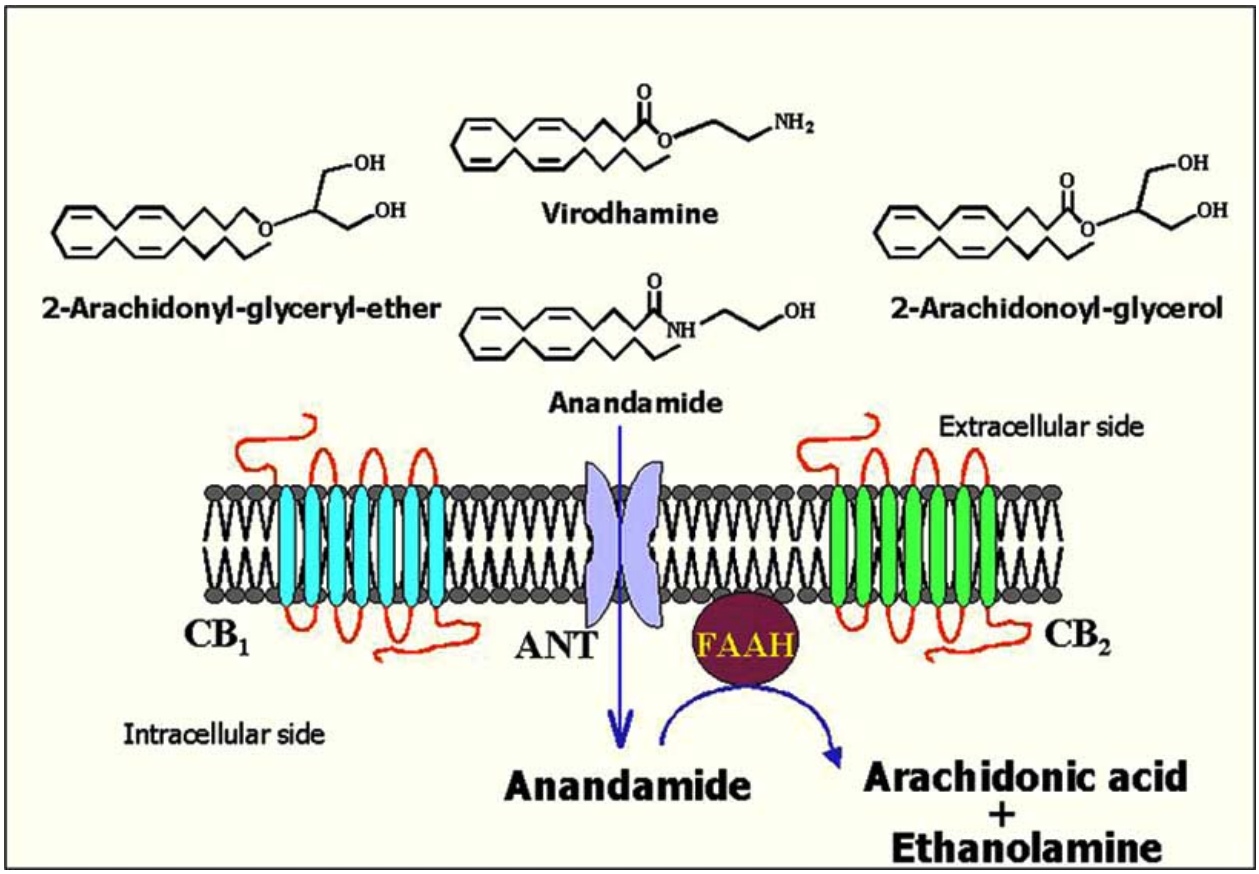

Fig. (1). Major constituents of the endocannabinoid signaling system (diagram kindly provided by Dr. María Luz López Rodríguez, Complutense University).

the seven transmembrane domain and GTP-binding protein coupled receptor superfamily (see Fig. **1**) (for review, see [11]), and have been called: (i) CB_1 (present preferentially in the brain, although also present in the periphery), and (ii) CB_2 (located almost exclusively in the immune system). However, the potential existence of another cannabinoid receptor subtypes (not CB_1, not CB_2) [12], or of molecular variants of these major subtypes [13], have been suggested although the evidence is still poor. A special mention deserves the case of the vanilloid VR1 receptors which were initially described as molecular integrators of nociceptive stimuli, abundant in sensory neurons (for review, see [14]), but that have been recently located in many brain structures [15]. Anandamide, as well as other related compounds, such as AM404, an inhibitor of the anandamide transporter, may also bind VR1 receptors, thus representing an alternative target for this endocannabinoid in the control of specific brain functions [16]. On the other hand, although some studies have suggested that endocannabinoid ligands might have a certain selectivity for the different cannabinoid receptor subtypes, in general, all ligands are able to bind and activate both receptor subtypes. The last years have served to progress significantly in the knowledge on the anatomical distribution and cellular localization of these receptors, mainly in the brain (for review, see [17]), in the description of their pharmacological properties [11], and in the elucidation of the intracellular signaling mechanisms to which they are coupled (see chapter by Díaz-Laviada and coworkers in this special issue).

Cannabinoid CB_1 or CB_2 receptors represent currently the major molecular targets to design and synthesize compounds with a selective action at the different key proteins of the endocannabinoid system. The importance of these synthetic compounds is that they serve, not only for the study of the physiological processes in which these receptors are involved, but also because they are susceptible to be used in some pathologies in which the occurrence of hypo- or hyperfunctionality of the endocannabinoid system has been recently reported [18]. The synthetic work has been focused to develop novel agonists with pharmacokinetic or pharmacodynamic advantages over the classic cannabinoids, for instance, compounds: (i) that possess better metabolic stability than anandamide, such as R-(+)-methanandamide [19], (ii) that exhibit selective affinity for the two major receptor subtypes, such as the anandamide analogs arachidonoyl-ciclopropylamide (ACPA) and arachidonoyl-2-chloroethylamide (ACEA) [20], that mainly bind to the CB_1 receptor, or the compounds HU-308 [21] or JWH-133 [22], that are selective agonists for the CB_2 receptor and then are devoid of psychotrophic side-effects of those compounds that also bind to CB_1 receptor, and (iii) that improve the water solubility of classic cannabinoids, such as O-1057 [23], which will facilitate the routes for administration of these compounds when used as medicines. Another important line of synthetic work includes the development of selective antagonists for CB_1 and CB_2 receptors [24-26], which are able to block the *in vivo* and *in vitro* actions of cannabinoids, and that would be useful in those disorders

where a hyperfunctionality of the endocannabinoid system has been postulated (see chapters #1 and #2 in this special issue for an overview on the pharmacological properties of selective ligands for both receptor subtypes).

1.3. Inactivation of the Endocannabinoid Signaling

To terminate the activation of cannabinoid receptors by their ligands, these endogenous compounds must be uptaken by a specific carrier-mediated system that is present in both neurons and glial cells [27], although the protein responsible of this function has not been isolated and/or cloned yet, which has generated certain debate about the existence of this transporter [28]. Once within the cell, the anandamide is hydrolyzed to its two components by the action of an amide hydrolase selective for fatty acid amides, called "fatty acid amide hydrolase" (FAAH) [29], whereas 2-arachidonoyl-glycerol is degraded by the action of a monacylglycerol-lipase [30]. FAAH is widely distributed in the CNS [31], in particular in those regions where CB_1 receptors are abundant, despite the role of FAAH is not confined to inactivate endocannabinoids (for review, see [6]). FAAH knockout mice have been recently developed and they displayed elevated concentrations of anandamide in the brain and high sensitivity to the biological actions of this endocannabinoid [32].

The proteins involved in the inactivation of the endocannabinoid signaling, in particular, the transporter and the FAAH, are also serving as templates to develop new compounds able to inhibit their functioning and, then, to enhance endocannabinoid tone, thus mimicking frequent pharmacological approaches used for other neurotransmitters. For instance, there are some interesting compounds that behave as transporter inhibitors, such as AM404 [33], VDM11 [34] or UCM707 [35]. These compounds, called "indirect agonists", act by potentiating the endocannabinoid action in those processes in which the finalization mechanism involves an uptake system. However, some of these compounds, such as AM404, may also behave as direct agonists for the VR1 receptors [16]. There are also inhibitors of the FAAH activity, also able to prolong the endocannabinoid action, such as AM374 [36] or the compounds URB532 and URB597 [37], that have been proposed as potential anxiolytic substances. All these "indirect agonists" might be useful in those pathologies in which a reduction of the endocannabinoid activity has been postulated. By acting through prolonging the presence of endogenous ligands at the synapse, these compounds are able to minimize the psychotrophic effects frequently observed when using direct CB_1 receptor agonists.

2. FUNTIONS AND THERAPEUTIC POTENTIAL OF THE ENDOGENOUS CANNABINOID SYSTEM IN THE BRAIN

The endocannabinoid system plays a modulatory role in several physiological processes, mainly in the brain [4,6,38] although also in peripheral processes such as the immune regulation [39], the cardiovascular system [40], the reproductive endocrine processes [41], and the control of energetic metabolism [42]. In the brain, endocannabinoids participate in processes such as the control of movement [43,44], learning and memory [45] and nociception [46], as well as they play an important role in various events of brain development [38]. This can be concluded from numerous data that showed: (i) the distribution of CB_1 receptors in the brain [17], (ii) the neurobiological effects of synthetic, plant-derived or endogenous cannabinoids [4,6,11], and/or (iii) the changes found in mice lacking CB_1 receptor gene expression [47,48]. Table **1** summarizes the most relevant functions attributed to the endocannabinoid system in the brain, including a description of the effects observed after the activation of CB_1 receptors by their endogenous ligands in nerve cells. This table also includes the potential therapeutic relevance of each of these functions, which explains the increasing development of the cannabinoid pharmacology in recent years.

2.1. Pain

The administration of different types of cannabinoid agonists, such as levonantradol, Δ^9-tetrahydrocannabinod (Δ^9-THC), 11-hydroxy-Δ^9-THC or CP55,940, produced analgesia in various species and using different methods to analyze pain sensitivity (for review, see [46,49]). This effect seems to be produced through the activation of CB_1 receptors located in central areas that control nociception [46,49] and that include both spinal (neurons of the dorsal horn at lumbar level) and supraespinal (certain thalamic nuclei, periaqueductal gray matter, rostral ventromedial medulla and other brainstem areas) structures, and even located in peripheral sensory nerve terminals. In concordance with these anatomical observations, CB_1 receptor knock-out mice exhibit significant alterations in pain sensitivity compared to wild type animals [47,48], although cannabinoid-induced analgesia is not completely absent in these animals, thus suggesting a role for CB_2 receptors and, in particular, for VR1 receptors, to which anandamide is a full agonist (see details in [6]). Another evidence in favour of the antinociceptive potential of cannabinoids is the fact that endocannabinoid levels increased in the brainstem in response to a painful stimulus [46].

Two important findings derive from the above observations. First, it has been postulated that there would be specific endocannabinoid pathways, and pharmacologically distinct to those of opioids, involved in the nociceptive control (for review, see [46,49]). However, it is also assumed that opioid elements might be also implicated in mediating certain events of endocannabinoid-induced antinociception, since substances that block opioid transmission, such as antagonists for the different opioid receptor subtypes or antibodies against the opioid ligands, were able to reduce cannabinoid-induced analgesia in laboratory animals (for review, see [50]). In addition, Δ^9-THC potentiated the antinociceptive effect of morphine and viceversa [50]. Second, it has been suggested that cannabinoid-based compounds might be used as analgesic medicines, in particular for chronic or neuropathic pain. In addition, considering the synergism between analgesic effects of cannabinoids and opioids, it has been claimed that cannabinoids might be used to reduce morphine dose in treatments of chronic pain, without reducing the analgesic capability but with a significant reduction in the addictive potential of the opioid [50]. However, the clinical progress in recent years has been still poor and major challenges remain for the future.

Table 1. Brain Processes in which the Endocannabinoid Signaling System has been Proposed to Play a Modulatory Action and to Serve for Developing Novel Pharmacotherapies

Brain process	Function attributed to endocannabinoids	Localization of CB_1 receptors	Potential therapeutic action
Motor activity	Inhibition of movement, although very low doses produced a stimulatory effect	Important presence of CB_1 receptors in the basal ganglia and cerebellum	Huntington's chorea (CB_1 agonists) Tourette's syndrome (CB_1 agonists) Multiple sclerosis (CB_1 agonists) Parkinson's disease (CB_1 antagonists) Diskynesias (CB_1 agonists/antagonists)
Nociception	Spinal and supraspinal antinociception	Presence of CB_1 receptors in brain regions involved in spinal and/or supraspinal analgesia	Treatment of pain, in particular chronic or neuropathic pain Reduction of the effective dose for morphine or other opioids
Memory and Learning	Modulation of processes of memory extinction or long-term potentiation	Important presence of CB_1 receptor in the hippocampus	Unknown
Cognitive processes	Alterations of motivational responses, emotionality and brain reward	Presence of CB_1 receptors in cortical and subcortical structures	Usefulness of some cannabinoid-based compounds, in particular CB_1 receptor antagonists, for the treatment of addictive states (i.e. alcoholism, cocaine and heroin relapse or nicotine addiction)
Neuroendocrine regulation	Changes in the secretion of several anterior pituitary hormones	Discrete presence of CB_1 receptors in hypothalamic nuclei	Unknown
Neurovegetative processes	Modulation of the brain structures involved in the regulation of emesis, body temperature and appetite	Presence of CB_1 receptors in area postrema and anterior hypothalamus	Increase of appetite in AIDS patients treated with antiretroviral therapy Reduction of nausea and emesis in cancer patients treated with antitumoral agents

2.2. Control of Movement

The abundant presence of CB_1 receptors in brain regions related to the control of movement, such as the caudate-putamen, the globus pallidus, the substantia nigra and the cerebellum, suggests that the endocannabinoid system might be strongly related to the control of movement (see recent reviews in [43,44]). Cannabinoids produce dose-dependent motor inhibition in both humans and laboratory animals. Thus, low doses reduce spontaneous activity while high doses may even produce catalepsia (for review, see [43,44]). By contrast, the administration of SR141716, a selective antagonist of CB_1 receptors, reversed these hypokinetic effects and, even, produced by itself a certain degree of hyperlocomotion due to its function as inverse agonist [51]. Another evidence supporting the involvement of the CB_1 receptor in the control of movement derives from the observation of motor anomalies in mice lacking CB_1 receptor gene [47,48], despite certain conflicting data between the two models of knock out mice developed.

The hypokinetic action of cannabinoid agonists has an explanation in the capability of these compounds to influence the activity of several neurotransmitters acting at the basal ganglia circuitry. Thus, the administration of cannabinoids to rats reduced the activity of nigrostriatal dopaminergic neurons which is compatible with a reduction in motor activity [52]. However, since nigrostriatal dopaminergic neurons do not contain CB_1 receptors [53], it is assumed that the dopamine-reducing effect of cannabinoids is indirect and produced through the activation of CB_1 receptors located onto striatal projection GABAergic neurons [53]. The activation of these receptors would reduce GABA reuptake [54] and/or increase GABA release [55], thus producing an increase in the activity of this neurotransmitter, which would result in a greater inhibition of nigral dopamine neurons. There is also evidence that cannabinoid agonists inhibit the activity of glutamatergic neurons in the basal ganglia circuitry [56]. CB_1 receptors located in GABAergic and glutamatergic neurons in the cerebellum have been also involved in motor effects of cannabinoids, in particular in their effects on posture and balance, but the neurochemical basis for these effects has been poorly explored (see [6] for review).

Based on these observations that strongly support that endocannabinoids modulate the activity of different neurotransmitters at the basal ganglia, it has been postulated that the pharmacological management of the endocannabinoid system might be useful to alleviate motor symptoms in various motor disorders (for review, see [43,44]). Preclinical studies have recently provided the first experimental evidence in rodent models (for review, see [44]). For instance, direct or indirect agonists of CB_1 receptors have been proposed as having therapeutic value in hyperkinetic disorders, such as Huntington's chorea [57,58] or Gilles de la Tourette's syndrome [59], whereas CB_1 receptor antagonists might be useful as coadjuvants in the treatment of hypokinetic syndromes such as Parkinson's disease [60,61]. The management of both CB_1 and CB_2 receptors has been proposed as clinically promising in multiple sclerosis, another neurological disease, whose origin is, in this case, immune but that progresses with several neurological deterioration that affects mainly the motor system [62,63]. Presently, there is a clinical trial with cannabis-based compounds in UK that will try to provide a scientific basis for numerous preclinical or anecdotal data suggesting alleviation of various symptoms, such as spasticity, in patients who self-medicated with marijuana (for review, see [62]). In addition, recent studies in animal

models of this disease have demonstrated the occurrence of marked alterations in the status of CB_1 receptors in motor regions [64] and that the administration of cannabinoids is able to reduce the magnitude and frequency of appearance of neurological signs [22].

2.3. Cortical Functions

CB_1 receptors are moderately abundant in the cerebral cortex, in particular they are located in superficial and deep layers, presumably onto GABAergic interneurons [17,65-67]. Endocannabinoids are also detected in the cerebral cortex [68]. These data agree with the observation that the administration of cannabinoids causes several changes in a variety of higher brain functions whose control resides mainly in the cerebral cortex, and suggest a role for the endocannabinoid signaling system in the control of sleep-waking cycle, performance of complex cognitive tasks, sensory perception, and other cognitive functions (for review, see [6]). In fact, these functions are related to many of the major subjective effects and cognitive impairments experienced by cannabis consumers and that have allowed several authors to suggest that long-term marijuana abuse might be associated with severe irreversible deficits in cognitive function and precipitation of psychiatric symptoms, such as psychosis, anxiety or depression, in particular when marijuana is consumed by young people (see a recent study in [69]), although the evidence is still confusing (see [6] for review).

2.4. Learning and Memory

Endocannabinoids have been also involved in learning and memory processes (see the chapter by Wotjak and coworkers in this Special Issue for details).

2.5. Appetite, Body Temperature and Emesis

Cannabinoid receptors and their endogenous ligands are present in several brain regions, such as the area postrema or certain hypothalamic nuclei, that participate in the control of body temperature, emesis, and appetite and food intake (see recent reviews in [70-72]). This anatomical observation allows to explain that the hypothermic, antiemetic and orexigenic effects exerted by cannabinoids are the result of the activation of CB_1 receptors in these brain regions [65-67], which has provided experimental support to some of the oldest therapeutic applications suggested for cannabinoid agonists, like the increasing effect on appetite and food intake. For instance, cannabinoids could be beneficial in situations where anorexia appears as a symptom: (i) the anorexia typical of older subjects, in particular if they are affected by demencia [73], or (ii) the cachexia developed by AIDS patients as a result of the chronic therapy with antiretroviral agents [74]. However, the data are more conflicting regarding the application of cannabinoid agonists to *anorexia nervosa* because, in these patients, the increased appetite might enhance the conflict leading to refuse food [75]. In a complementary way, it has been also postulated that antagonists of CB_1 receptors might be used to reduce obesity [76]. In fact, the compound SR141716 (rimonabant), patented by Sanofi-Synthelabo Recherche, is presently in a clinical trial (Phase III) for the treatment of obese patients with promising preliminary results [77]. Finally, based on their antiemetic and also orexigenic properties, cannabinoids are being recommended to reduce nausea and vomiting in patients subjected to anticancer chemotherapies [78].

2.6. Neuroprotection

It has been recently suggested that the endocannabinoid system might also develop an important function in the cellular decision death/survival (for review, see [79]), a fact particularly relevant for the brain tissue, considering the postmitotic characteristics of neuronal cells. This finding has derived from several experimental observations that indicate that cannabinoids combine at the same time neuroprotective [80-82] and antiproliferative [79,83] properties.

Cannabinoid agonists exhibited neuroprotection in models of acute injury, such as glutamatergic excitotoxicity, ischemic stroke, hypoxia, head trauma, oxidative stress, ouabain-induced secondary excitotoxicity, and others (see [80-82] for recent reviews). In addition, cannabinoids are also neuroprotective in several chronic neurological pathologies that also involve the occurrence of excitotoxicity and/or oxidative stress, such as Parkinson's disease, Huntington's chorea and multiple sclerosis (see [44] for review). In fact, it has been demonstrated that celular damage is accompanied by an increase in the production of endocannabinoids that has been related to an endogenous protector role [84].

The molecular mechanisms underlying these neuroprotectant properties of cannabinoids are diverse and might include mainly 4 options:

(i) antiglutamatergic properties: cannabinoids may reduce excitotoxicity by inhibiting glutamate release or by blocking NMDA receptors (see [80-82] for review),

(ii) anticalcium properties: cannabinoids would reduce Ca^{2+} entry by inhibiting several types of channels for this ion, then reducing the activation of Ca^{2+}-dependent cytotoxic cascades [80-82],

(iii) antioxidative properties: certain classic cannabinoids, that contain phenolic groups in their chemical structure, are able to reduce oxidative stress typical of brain injuries [80-82], although these properties would be CB_1 receptor-independent [85], and

(iv) antiinflammatory properties: cannabinoids are also able to reduce the inflammation that accompanies the states of neuronal injury and that is caused by local effects at the level of glial cells [80-82]; in part, this is the consequence of an effect of cannabinoids by protecting astrocytes and oligodendrocytes from death, which is also beneficial for neurons [86,87].

Despite the neuroprotectant properties that cannabinoids display, the clinical development is still poor and only dexanabinol (HU-211), a synthetic cannabinoid that does not have affinity for the CB_1 receptor, but exhibits properties as NMDA receptor antagonist, is being tested in a clinical trial to reduce brain damage caused by head trauma or cerebrovascular injuries [88].

In constrast with the protective properties of cannabinoids in non-transformed nervous cells, these

compounds are also able to elicit apoptosis in transformed nerve cells (C6 glioma, human astrocytoma U373MG and mouse neuroblastoma N18TG12 cells) *in vitro* [79,89], and to promote the regression of glioblastoma *in vivo*, through a mechanism that involves the activation of mitogen-activated protein kinase and ceramide accumulation [90]. In addition, cannabinoids have been recently reported to inhibit angiogenesis which represents a key process for tumorigenesis [91]. These antiproliferative effects of cannabinoids are also produced in tumors of peripheral organs such as breast, prostate, skin, and others (for review, see [92-94]) and represent a novel future potential utility of cannabinoid-based compounds in cancer treatment.

2.7. Brain Reward

The endocannabinoid system seems to also play a role in brain reward circuitry which is activated by different types of reinforcers, among them, the habit-forming drugs [50,95,96]. This role explains why cannabis consumption is associated with marked changes in endocannabinoid transmission, mainly CB_1 receptors, as numerous studies in laboratory animals have demonstrated (see [97] for a recent review), and it also explains the changes reported for the endocannabinoid system in states of dependence/abstinence for other drugs of abuse (see [50,95,96] and below). Different types of studies have provided evidence to support a role of the endocannabinoid system in brain reward. Thus, neuroanatomical studies have demonstrated that CB_1 receptors and their endogenous ligands are present in different limbic structures that form the brain reward circuitry [31,65-68]. In the same line, biochemical studies have proved the occurrence of changes in the density of CB_1 receptors and in the levels of their endogenous ligands in these regions of animals rendered dependent to one of the most important habit-forming drugs, such as opioids [98-101], cocaine [101,102], nicotine [102,103] or alcohol [96,101,102,104]. Finally, other studies have demonstrated that the pharmacological management of the endocannabinoid transmission might influence several signs indicative of drug addiction, such as the individual vulnerability, the craving, the degree of dependence, the intensity of abstinence, or the risk for relapsing [105-107], which has opened the possibility of using these substances in the treatment of different addictive states.

2.8. Neuroendocrine Regulation and Reproduction

Cannabinoids also modify the release of anterior pituitary hormones, in particular, the administration of Δ^9-THC or other plant-derived or synthetic cannabinoids decreased prolactin and gonadotrophin secretion, while increased ACTH release, in rodents [108-110]. These effects are produced in part through the activation of CB_1 receptors present in several hypothalamic nuclei, such as the ventromedial hypothalamic nucleus [66,111], which result in changes in the release of hypothalamic factors controlling anterior pituitary secretion [112]. However, a direct action of cannabinoids on anterior pituitary cells seems to be also possible since recent studies have demonstrated that CB_1 receptors are also expressed in the anterior pituitary [113]. Independently of the mechanism used by cannabinoids to alter anterior pituitary hormone secretion, it appears well-demonstrated that these compounds are able to control the circulating levels of these hormones and then to affect the activity of different peripheral glands. However, it has been also proposed that, in addition to their effects on hypothalamus-anterior pituitary axis, cannabinoids may also exert a direct action on some peripheral glands, in particular on reproductive glands [114]. In fact, cannabinoid receptors are located in some organs of the reproductive system, such as testes and uterus [115]. They also have capability to synthesize or metabolize anandamide [116] to exert different types of functions, such as the control of spermatogenesis and male fertility [117], and the regulation of the chemical communication between the embryo and the uterus that allows embryo implantation [116].

3. ADDICTIVE POTENTIAL OF CANNABINOIDS

Finally, we will address briefly some aspects of the addictive potential of cannabinoids, which represent the major brake for the development of therapeutic properties of these substances. In comparison with other drugs of abuse, *Cannabis sativa* derivatives have been considered as a "soft drug", in part due to the lack of data, until a few years ago, concerning their mechanisms of action in the brain, and in part due to the little evidence showing that signs of mild cognitive impairment in chronic cannabis users are irreversible and accompanied by neuropathological manifestations (see [6] for review). However, the recent description of the endocannabinoid system has provided, not only new targets to develop novel pharmacotherapies in various brain diseases, but also the bases to know the mechanisms of tolerance/dependence to cannabis (see [97,118] for review). In this sense, a long list of experiments, carried out mainly during the past decade, have addressed the analysis of the molecular changes underlying the pharmacological tolerance that occurs for a variety of pharmacological effects (motor inhibition, analgesia, ataxia, hypothermia, neuroendocrine effects and other effects) after a prolonged exposure to plant-derived, synthetic or endogenous cannabinoids in humans and laboratory animals (for review, see [97]). Most of these studies have provided robust evidence that this pharmacological tolerance is mainly linked to reductions in the availability and/or functionality of CB_1 receptors [119-121]. There exist some variations in the extent, onset and regional distribution of these reductions that can be attributed to the use of different types of cannabinoids (with different affinities and/or pharmacological potencies), doses and times for treatment, but all evidence is compatible with the involvement of pharmacodynamic events rather than to pharmacokinetic factors [97]. In addition, we have reported that cannabinoid tolerance is also accompanied by changes in the contents of anandamide and 2-arachidonoylglycerol [119].

In parallel to these studies, which strongly suggest that cannabinoid tolerance is consequence of region-dependent losses in CB_1 receptors, and also of adaptative changes of endocannabinoid contents, less data exist on the response of these adaptative changes when cannabinoid administration is abruptly terminated after a prolonged period of daily exposure (see [97] for a recent review). This is a relevant issue for cannabinoid consumption in humans, since there is no general agreement about whether cannabinoid tolerance

has elements of physical dependence, as it has been demonstrated for other drugs of abuse. For these drugs, the interruption of chronic administration results in the spontaneous occurrence of somatic and/or neurovegetative signs of abstinence in laboratory animals. However, this does not appear to be the case for cannabinoids, presumably because of their particular pharmacokinetic properties: (i) late onset, (ii) greater duration, and (iii) slow metabolic clearance (for review, see [11]). Thus, as it frequently happens with drugs that have a long duration of action, most of the studies with cannabinoids failed to elicit spontaneously any relevant signs of abstinence in laboratory animals (for review, see [97]). However, some signs, such as irritability, sleeplessness, anorexia, nausea and others, have been reported to occur in humans [122] and non-human primates [123], even though they are mild compared to those observed with opioids [124] and they do not appear in all individuals [125]. In laboratory animals, by analogy to the approach with naloxone in morphine-dependent rodents, cannabinoid action can be abruptly terminated, and abstinence elicited, by challenging chronically-treated animals with an appropriate antagonist. This is an effective means of demonstrating physical dependence in the absence of spontaneous abstinence. Thus, the blockade of CB_1 receptors with SR141716, a selective antagonist for this receptor subtype, was reported to elicit withdrawal signs in cannabinoid-tolerant animals [126-128], signs that were somatic (mainly of motor nature), with no relevant neurovegetative changes [97]. These somatic signs were accompanied by a series of molecular events, that also appear during the abstinence to other drugs, such as c-fos induction [129], increased CRF release in the central nucleus of the amygdala [129], changes in adenylate cyclase/cAMP signaling system [127,130] and decreased dopamine release in the nucleus accumbens [131]. However, these data have resulted conflicting in some aspects (for review, see [118]).

Another controversial aspect regarding cannabinoid addiction is the proposed enhanced vulnerability to consume other drugs of greater addictive power, such as morphine, alcohol or cocaine, that cannabinoid tolerance may generate, and that allowed some authors to develop the theory of "cannabis as a gateway drug" [132,133]. This theory, however, has been refused by other authors who did not find any cause-effect relationships between cannabis consumption and abuse of other drugs [134,135]. Only recently, this issue has been examined in laboratory animals (see [118] for review), but the results have been controversial. Even, some studies have reported that compounds acting at key steps of the endocannabinoid transmission might be susceptible of being used in the treatment of the addiction to other substances, as it has been discussed above in relation with the role played by this system in brain reward.

4. FUTURE PERSPECTIVES

Along this review article, we have demonstrated how the recent advances in the knowledge on the biochemistry, physiology and pharmacology of the endocannabinoid system, is providing the bases for explaining both the addictive potential of some cannabinoids and the therapeutic usefulness of different targets of this system. This development should lead in the mid term to the design and synthesis of novel molecules, more selective and with minimal side effects, which, through blocking or enhancing the function of the endocannabinoid system, might be tested in clinical trials to demonstrate their capability to alleviate a variety of symptoms of brain pathologies, some of them with a still poor therapeutic outcome. The time will say what will be the position of these promising cannabinoid-based molecules within the therapeutic arsenal against the neurological and psychiatric diseases.

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