IMMUNE SYSTEM MODULATION IN THE CENTRAL NERVOUS SYSTEM; A POSSIBLE ROLE FOR ENDOCANNABINOIDS

Adel R. A. Abd-Allah

The immune system is designed to protect the body from infection and tumor formation. To perform this function, cells of the immune system can be dangerous for the survival and function of the neuronal network in the brain under the influence of infection or immune imbalance. An attack of immune cells inside the brain includes the potential for severe neuronal damage or cell death and therefore impairment of the CNS function. To avoid such undesirable action of the immune system, the CNS performs a cascade of cellular and molecular mechanisms enabling strict control of immune reactions "immune privilege". Under inflammatory and pathological conditions, uncontrolled immune system results in the activation of neuronal damage that is frequently associated with neurological diseases. On the other hand, processes of neuroprotection and neurorepair after neuronal damage depend on a steady and tightly controlled immune surveillance. Many immunosuppressants play a role to rebalance the immune reaction in the CNS and other organs which presents an important therapeutic target. It has been reported recently that endocannabinoids are secreted in abundance in the CNS following neuronal insult, probably for its protection. There are at least two types of cannabinoid receptors, CB1 and CB2. Both are coupled to G proteins. CB1 receptors exist primarily on central and peripheral neurons. CB2

Department of Pharmacology, College of Pharmacy, King Saud University, P.O BOX 2457 Riyadh, 11451, Saudi Arabia.
E-mail: arabdallah@hotmail.com

receptors are present mainly on immune cells. Endogenous agonists for cannabinoid receptors (endocannabinoids), have been discovered, the most important being arachidonoyl ethanolamide (anandamide), 2-arachidonoyl glycerol (2AG), and 2-archidonyl glyceryl ether. Following their release, endocannabinoids are removed from the extracellular space and then degraded by intracellular enzymic hydrolysis. Therapeutic uses of cannabinoid receptor agonists/antagonists include the management of many disease conditions. They are also involved in immune system suppression and in cell to cell communication.

**Key words:** Central nervous system (CNS), immune system, endocannabinoids.

**Introduction**

*Cannabis*: Cannabis plant or hemp means any plant of the genus *Cannabis sativa* (*Cannabinaceae*). It is a single plant species, but exists in many different biological, chemical and/or morphological varieties. It is a cosmopolite, annual plant, bush growing widely throughout the world (1).

*Cannabis sativa* (*C. sativa*) grows as common weed in many parts of the world, and drug preparation vary in potency, cultivation and methods of preparation.

*Cannabis indica* is a shorter, harder variety with rounded bluish-green leaves cultivated in India and Afghanistan for hashish. Most marijuana grown in the United States of America (USA), since the late 1980s are hybrids of the two plants to yield a much more potent product than marijuana in the past. The resin found on flower clusters and top leaves of the female plant, is the most potent drug source used to prepare Hashish, the highest grade of the cannabis. The bud of female sinsemilla, is the part most often smoked marijuana (2).

Cannabis may also mean the flowering or fruiting tops of cannabis plant. Moreover, it may be known as a tobacco-like greenish or brownish material consisting of the dried flowering, fruiting and leaves of *Cannabis sativa* plant.

**Common illicit forms of cannabis:**

Cannabis may be one of the following shapes; loose herbal material, blocks of compressed herbal material, corn-cob shaped herbal material wrapped in coarse vegetable fiber; herbal material tied using twine around a central bamboo cane and finally herbal material in a small roll wrapped in paper (3).

**Certain common street names:**

It may have any of the following names; Bango, Hemp, Marihuana, Marijuana, Sinsemilla, Marie-Jeanne, Ganja, Grass, Kif...etc. Historically, Cannabis has been used as an agent for achieving euphoria since ancient times. Its use spread from China to India and then to North Europe at least 500 years ago. A major crop in Colonial North America, marijuana (hemp) was grown as extensively cultivated during World War–II, when Asian sources of hemp were cut off (4).

The use of cannabis herb for medical purposes was described by Egyptian and Chinese as early as 2700 BC, revealing that the drug was used for rheumatic pains and other conditions (5). The herb contains more than 60 active compounds; the most psychoactive one is Δ⁹- tetrahydrocannabinol (Δ⁹-THC) that was first isolated in 1964 by Gaoni and Mechoulam (6). Other natural compounds such as Δ⁸- tetrahydrocannabinol (Δ⁸-THC), cannabinol and cannabidiol were also identified. The Δ⁹-THC content is highest in the flowering tops, declining in the leaves, stem and seeds of the plant.

**Pharmacological actions of cannabinoids:**

Ingestion of *Cannabis sativa* preparations such as marijuana (leaves and flowering tops) or ganja (resin) results in an intoxication characterized by sedation, cognitive dysfunction, failure to consolidate short-term memory, alteration in time assessment, perceptual changes, motor incoordination and poor executive function (7).

Cannabinoid compounds isolated from the plant *C. sativa* comprise a family of tricyclic ring structures most actively Δ⁸-THC. Other cannabinoid compounds including cannabinol and cannabidiol, fail to elicit the same psychoactive effects of Δ⁹-THC, but can exhibit anticonvulsant activity and induce hepatic metabolic enzymes (8). The pharmacological effects of cannabinoids have been attributed to the activation of cannabinoid receptors CB1 and CB2 (9).
Cannabinoid receptors:
* The CB1 receptor:
  In 1988, Devane et al. (10) were the first to demonstrate the existence of specific cannabinoid binding sites in the rat brain. Definitive proof of the existence of the cannabinoid receptor came from the study of Matsuda et al. (11), when they isolated the cDNA of a cannabinoid receptor from the rat cerebral cortex. This receptor was named CB1, and its 472 amino acid sequence revealed that it is a member of the G-protein coupled receptors [GPCRs] (12). Activation of CB1 leads to inhibition of adenyl cyclase with consequent reduction of cyclic AMP levels. One of the cyclic AMP-dependent cannabinoid effect is the enhancement of voltage-sensitive outwardly rectifying K⁺ channels, which occurs as a result of decreased phosphorylation of the K⁺ channel protein by A-kinase (13). Apart from inhibition of adenyl cyclase, CB1 utilizes several additional effector systems (intracellular mediators) involving G-proteins such as the inhibition of N-type Ca²⁺ channels and the activation of mitogen-activated protein kinase (14). Other cannabinoid-induced cellular effects include activation of inwardly rectifying K⁺ channels and possibly activation of phospholipase-A, C and D (15).

Different G-proteins or second messengers may couple to CB1 in different brain regions and may mediate different physiological effects. Utilization of diverse effector systems by CB1 may explain how the response to cannabimimetics varies across different types of cells. Understanding which physiological responses are mediated by each of the above intracellular signaling systems is of great significance, and may provide new grounds for the design of selective cannabimimetic agents.

* The CB2 receptor:
  Homology cloning revealed the existence of a second cannabinoid receptor, CB2 (16). It is present in the periphery and mainly in tissues and cells of the immune system. Localization of CB2 in the immune system suggests an immunomodulatory role for this receptor. Thus, CB2 may be the mediator of the long-known immunosuppressive properties of marijuana. CB1 and CB2 share some common signal transduction pathways, such as inhibition of adenyl cyclase and stimulation of mitogen-activa-

Distribution of cannabinoid receptors:
CB1 is a ubiquitous receptor found in the CNS and the periphery, and in both neural as well as non-neural tissues. CB1 is one of the most abundant G-coupled receptors in the brain (18). As shown by various mammalian brain autoradiographic studies (19), CB1 density is highest in the basal ganglia, substantia nigra pars reticulata, entopeduncular nucleus, and the external segment of the globus pallidus than in different brain regions.

In the periphery, CB1 is found in the adrenal glands, bone marrow, heart, lungs, prostate, testes, thymus, tonsils, spleen, lymphocytes, phagocytes, smooth muscle, vascular endothelium, peripheral neurons (e.g., in the gut), kidneys, uterus, and sperm (20).

The CB2 receptor has a more limited distribution. It is principally found in cells associated with the immune system, such as leukocytes, spleen, thymus, and tonsils (various amounts of CB1 are found in some of these cells as well) (21). Among the human blood cells, B lymphocytes express the highest levels of CB2, followed in order by natural killer cells, monocytes, polymorphonuclear neutrophils, CD⁸⁺ lymphocytes, and CD⁴⁺ lymphocytes.

Fig. 1. Natural cannabnergic receptor ligands.
There is a similar distribution of CB1 receptors in humans (22). It is here, however, that the pattern of receptor localization within the cortex can be best ascertained within the more elaborate human cortex. The highest densities are found in association and limbic cortices, with much lower levels within primary sensory and motor regions, suggesting an important role in motivational (limbic) and cognitive (association) information processing. Moreover, combined with electron microscopy and electrophysiology studies, CB1 receptors have been shown to be localized presynaptically on GABAergic interneurons (23). This would be consistent with the proposed role of endocannabinoid compounds in modulating neurotransmission. Hence, the anatomy of CB1 receptors can provide clues to their function.

**Endocannabinoids:**

The discovery of the cannabinoid receptors and their G-protein-coupled nature strongly suggested the existence of one or more endogenous cannabinimetic ligands (Fig.3) that exert their physiological activity upon binding to these receptors. Initial efforts to identify a possible protein (24) or other water-soluble endogenous cannabinimetic ligands were unsuccessful (13). The hypothesis that such a putative endocannabinoid should be lipophilic, like the classical exogenous cannabinoids, led studies to search for such a ligand in the hydrophobic fractions of porcine brain extracts (25). Repetitive fractionations and purifications led to the identification of a substance that bound to CB1 in a saturable fashion. This compound was the ethanalamide of arachidonic acid [arachidonyl ethanolamide (AEA)]. The authors named this brain constituent anandamide from ananda, the Sanskrit word for bliss.

It has been shown later on that anandamide is found in the human brain at the following levels; 100 pmol/g in the hippocampus, 75 pmol/g in the thalamus, 60 pmol/g in the cerebellum, and 55 pmol/g in the striatum (26). Furthermore, AEA surges were observed when cerebellar granule cells are treated in hypoxic conditions. It may also occur in living tissue under certain conditions, e.g., hypoxia and brain injury (27).

In the periphery, anandamide is found in the spleen and heart at ~ 10 pmol/g (25). It is also found in rat testes and uterus in concentrations far exceeding those in the brain. Very low levels have been detected in serum, plasma, and cerebrospinal fluid, a fact that suggests that anandamide is not hormonal in nature, but is formed at or near its sites of action (28).

In addition to anandamide, several other endogenous polyunsaturated fatty acid derivatives were also found to act as cannabimimetics. They are now collectively referred to as endocannabinoids. This class of endogenous ligands includes two more fatty acid ethanolamides that bind to CB1 preparations with similar affinities to that of anandamide. All three N-acylethanolamine endo-cannabinoids were found to be CB1 agonists in the mouse vas deferens (29). A non-amic acid (AA) derivative was first isolated from canine gut and identified as 2-arachidonylethanolamine (2-AG) (30).

2-AG was found later also in the brain and the spleen. It was shown to be released in a Ca²⁺ - dependent manner, and to reach concentrations 170 times higher than those of anandamide in the brain (31).

**Biosynthesis and fate of anandamide and 2-AG:**

During the past few years, there has been Considerable progress in understanding of the physiological pathways that are involved in the synthesis and inactivation of endocannabinoids. Anandamide is currently believed to be formed from membrane phospholipids through a pathway that involves Fig [2]; [1] a transacylation of the amino group of phosphatidylethanolamine with arachidonic acid from the sn-1 position of phosphatidylcholine and [2] a D-type phosphodiesterase activity on the resulting N-arachidonyl-phosphatidylethanolamine[NAPE](32).

![Simplified schematic diagram for the synthesis of anandamide.](image)

**Fig. 2.** Simplicated schematic diagram for the synthesis of anandamide.
2-AG is biosynthesized by two possible pathways Fig [3]: [1] a PLC-mediated hydrolysis of membrane phospholipids, followed by a second hydrolysis of the resulting 1,2-diacylglycerol by diacylglycerol lipase, or [2] a PLA1 activity that generates a lysophospholipid, which, in turn, is hydrolyzed to 2-AG by lysophospholipase C (33).

It is now also believed that endocannabinoids are synthesized within the cell membrane and act on the same or neighboring cells as autocrine or paracrine mediators (32).

Experimental evidence to date indicates that anandamide and 2-AG, unlike other classical neurotransmitters, are not stored in vesicles (34). Therefore, it is believed today that anandamide and 2-AG are produced and immediately released from neurons upon demand. The poor water solubility of anandamide must preclude extensive free diffusion in the extracellular space. Thus, it is suggested that anandamide, once cleaved from NAPE, is immediately expelled out of the cell membrane with the assistance of a membrane transporter (such as a P-glycoprotein) or a lipid-binding protein (such as lipocalin). Such a lipid-binding protein may also facilitate the diffusion of anandamide through the aqueous extracellular medium to its sites of action. Anandamide is inactivated in two steps: first by transport inside the cell and second by subsequent intracellular enzymatic hydrolysis. The transport of anandamide inside the cell is a carrier-mediated process, as it was shown to be a saturable-, time-, and temperature-dependent process that involved some protein with high affinity and specificity for anandamide (35). Although the anandamide transporter (AT) protein is not molecularly characterized, its activity is well characterized and attenuated by specific transporter inhibitors.

Reuptake of 2-AG is likely mediated by the same facilitating mechanism. Once anandamide is inside the cell, it is hydrolyzed by the fatty acid amide hydrolase (FAAH) (36). This enzyme is membrane associated and shows significant specificity for anandamide (37). Less is known about the role and metabolic fate of 2-AG. It is possible that in many tissues, 2-AG is only an intermediate of a signaling pathway that generates 1,2-diaclyglycerol and AA, two well known signaling molecules. In the brain, however, 2-AG may have regulatory roles, since it escapes immediate metabolism and accumulates in response to stimuli-generated Ca²⁺ surges (38). This may arise from differences between metabolizing isoenzymes or their levels of expression from tissue to tissue. Anandamide amidase recognizes and hydrolyzes 2-AG (32,33).

However, there is evidence for the existence of an additional specific hydrolase (monoacylglycerol lipase) that hydrolyzes 2-AG. In addition to this pathway, 2-AG diffuses rapidly into the cell membrane, where it could be either hydrolyzed to AA and glycerol or esterified back to phosphoglycerides (32).

The calcium dependency allows for regulation of anandamide and 2-arachidonoylglycerol synthesis, and there are several reports showing that their synthesis is increased both in physiological conditions (i.e. following depolarizing stimuli and receptor-mediated activation of the inositol phospholipid signalling pathway (39) and under conditions of cellular damage, such as is seen after neurotoxic insult, trauma or in models of multiple sclerosis (40). Anandamide is also produced in the periaqueductal gray region after nociceptive input (41).

There is also data to suggest that inflammation also results in an increased rate of anandamide synthesis, but this may in some cases be a consequence of the accompanying cell damage rather than the inflammation per se (42).

Endocannabinoids are released by an as yet unclear mechanism. After interaction with the receptors, their action is terminated by cellular reuptake, followed by metabolism, mainly by the enzymes fatty acid amide hydrolase and mono-acylglycerol lipase, but also by cyclooxygenase-2 and lipoxygenase enzymes (43).
Cannabinergic ligands:
The first specific antagonist to the CB1 cannabinoid receptor was SR141716 (rimonabant), a compound discovered in a high throughput screening program at Sanofi Recherche (44). Because rimonabant can block dysfunctional craving for food and drugs, it is currently undergoing clinical trials for obesity, smoking cessation and alcohol abuse (45). Also a specific CB2 receptor antagonist, SR144528 (46) may be clinically useful in immune modulation.

Pharmacological and therapeutic potential of cannabinoids:
Most known cannabimimetics today have very broad effects on organ systems, some of which are still unexplained. The ubiquitous pharmacology of cannabimimetics is one of the reasons why the clinical application of these drugs has not yet reached its full potential. This review is a trial to summarize the effects of cannabinoids on the mutual communication between the central nervous system and the immune system modulation with the possible therapeutic uses that may emanate from these actions.

In the brain, the endocannabinoids behave as neurotransmitters or neuromodulators in a variety of processes, such as the regulation of motor behavior, cognition, learning and memory, and antinociception. They also play a role in neuronal development (47). The involvement of the endocannabinoids in these functions has been proposed based on the distribution of cannabinoid CB1 receptor binding and mRNA levels in the brain and on the well-known pharmacological effects of plant derived and synthetic cannabinoids (32).

The endocannabinoids have been shown to be synthesized, released, taken up, and degraded in neuronal elements by mechanisms similar in part to those for other neurotransmitters, although their lipid structure implies some differences with respect to classical amino acid, amine, and peptide transmitters (30-33). For instance, AEA is known to be formed upon demand by receptor-stimulated phospholipase D-mediated cleavage of a membrane precursor (N-arachidonoylphosphatidylethanolamine). However, instead of accumulating in synaptic vesicles, it is immediately released into the extracellular milieu and taken up by a specific carrier-mediated system that is present in both neurons and glial cells (34) and that also works for 2-AG. Once within the cell, it is degraded by the action of an amidohydrolase enzyme [fatty acid amide hydrolase (FAAH)], to form its two basic components, arachidonic acid and ethanolamine (32). This enzyme has been located in neuronal elements. It has also been suggested that this last reaction may be reversed to increase the synthesis of AEA under special circumstances (33-38). Nevertheless, the precise distribution of AEA-producing neurons is still poorly known, thus limiting our knowledge of the role that this neuromodulator plays in the normal functioning of the brain.

Some of the lines of research focus on the synthesis of novel agonists that provide: [1] higher metabolic stability than anandamide, such as methanandamide (AM356) (48); [2] better water solubility, which will improve the mode of administration of cannabinoids for therapy, such as O-1057 (17); [3] selective affinity for the different receptor subtypes, such as some recent AEA analogs, which are potent CB1 receptor agonists, but which bind weakly to CB2 receptors (49), and particularly, compounds such as HU-308, JWH-133, or others (49), which mainly behaved as CB2 receptor agonists. The advantage of the latter compounds is that they do not produce any psychotropic effects, as those mediated by CB1 receptors, but are effective in some CB2 receptor mediated effects, such as reduction of blood pressure, inhibition of intestinal activity, inflammation and promotion of peripheral analgesic activity (48, 49).

Role of the endogenous cannabinoid system in the control of motor behavior:
The finding that the endocannabinoid system might be involved in the regulation of motor behavior is based on three lines of evidence. First, it has been well demonstrated that synthetic, plant-derived, and endogenous cannabinoids have powerful actions, mostly inhibitory effects, on motor activity (50). There are differences in both the magnitude and duration of the motor effects of the different cannabinoids, but these are attributable to differences in receptor affinity, potency, and/or metabolic stability. The second line; it is also well known that endocannabinoids and their CB1 receptors are abundantly distributed in the basal ganglia and the cerebellum, the areas that participate in the control of movement. The third line; an increasing number of studies have demonstrated that CB1 receptor binding was altered in the basal ganglia of humans affected by several neurological diseases.
Moreover, marijuana consumption affects psychomotor activity in humans, reflected by a global impairment of performance (especially in complex and demanding tasks) and resulting in an increased motor activity, followed by inertia and incoordination, ataxia, tremulousness, and weakness (51-53).


However, most of these effects rapidly developed tolerance when Δ9-THC administration was prolonged by several days (48).

The endocannabinoid uptake inhibitor AM404 (45) has been shown to mimic the behavioral effects of AEA on motor activity when administered alone in rats, as well as to produce similar neurochemical changes. Two key regions for the control of movement, the globus pallidus and the substantia nigra, deserve to be mentioned since they contain the highest levels of endocannabinoids, in particular AEA, in the brain, paralleling the highest densities of CB1 receptors (49-51). This strongly supports a functional role for the endocannabinoid system in the control of movement.

Effect on GABAergic transmission:

As expected from the location of CB1 receptors in striatal GABAergic projection neurons, the activation of these receptors seems to produce significant effects in GABAergic activity within the basal ganglia. Thus, electrophysiological studies indicated that cannabinoids may modulate GABA release in vivo in the globus pallidus and substantia nigra (51), although these effects were very modest. More recently, neurochemical studies demonstrated that the administration of cannabinoids did not affect GABA synthesis or release in the basal ganglia of naive rats, although cannabinoids were effective in increasing both parameters in animals with lesions of striatal GABAergic neurons, as produced in Huntington’s disease, HD (52).

In addition, the stimulation of CB1 receptors localized on axonal terminals of striatal GABAergic neurons has been shown to potentiate GABA transmission by inhibition of the uptake of this neurotransmitter in globus pallidus slices.

Effect on Dopaminergic transmission:

The administration of plant-derived, synthetic or endogenous, cannabinoids has also been reported to produce changes in the activity of nigrostriatal dopaminergic neurons (50-52). A decrease in the activity of tyrosine hydroxylase, the rate-limiting enzyme for dopamine synthesis, has been found in the striatum of rats acutely administered AEA (48-52). However, although these decreases were consistent and statistically significant, they occurred more probably as a consequence of modifications in the activity of striatonigral GABAergic neurons rather than as a direct effect of cannabinoids on nigrostriatal dopaminergic projections. In fact, nigrostriatal dopaminergic neurons do not contain CB1 receptors, at least in the adult brain, although these receptors co-localize with D1 or D2 dopaminergic receptors in striatal projection neurons (53), which supports a potential interaction between both receptor types at the level of G-protein/adenyl cyclase signal transduction mechanisms.

On the contrary, some glutamatergic transmission emerging data indicate that cannabinoids may also modulate glutamatergic activity in the basal ganglia by inhibiting the release of this neurotransmitter both in vivo and in vitro (50-52).

Neurodegenerative diseases and cannabinoids:

Beyond the changes observed in CB1 receptors in the basal ganglia during normal aging, several studies have also demonstrated changes in these receptors in the postmortem basal ganglia of humans affected by several disorders directly related to motor function, such as Huntington’s disease (HD) or Parkinson’s disease (PD), or not directly related to the control of movement, but exhibiting strong motor symptoms, such as Alzheimer’s disease (54).

In some cases, these changes appeared before changes in receptors for other neurotransmitters, even in presymptomatic phases, which might suggest an involvement of the endocannabinoid system.
in the pathogenesis of some motor disorders (50).

**Huntington’s disease (HD):**

HD is a genetic neurodegenerative disorder caused by an unstable expansion of a CAG repeat in exon 1 of the human Huntington gene. Translation through the CAG span results in a polyglutamine tract near the N-terminus of this protein, which leads to toxicity predominantly of striatal projection neurons (55). The symptoms of this disease are primarily characterized by motor disturbances, such as chorea and dystonia, and secondarily by personality changes and cognition decline (55). Several studies have clearly demonstrated that in HD, there exists an almost complete disappearance of CB1 receptor binding in the substantia nigra, in the lateral part of the globus pallidus, and, to a lesser extent, in the putamen (56). This loss of CB1 receptors is concordant with the characteristic neuronal loss observed in HD that predominantly affects medium-spiny GABAergic neurons which contain most of the CB1 receptors present in basal ganglia structures (55, 56).

This is also consistent with the fact that other phenotypic markers for those neurons, such as substance P, enkephalin, calcineurin, calbindin, and adenosine and dopamine receptors, are known to be also depleted in HD (57). However, recent data in postmortem tissue have revealed that the loss of CB1 receptors occurred in advance of other receptor losses, and even before the appearance of major HD symptomatology. This suggests that losses of CB1 receptors might be involved in the pathogenesis and/or progression of the neurodegeneration in HD (54).

Compounds able to directly or indirectly activate CB1 receptors in the basal ganglia may be useful in those diseases in which endocannabinoid transmission is hypofunctional, as is the case in HD. This is an important proposal, since HD is a motor disorder where the therapeutic outcome has been poor and there is a lack of novel pharmacological therapies with symptomatic and/or neuroprotective efficacy. Therefore, compounds like direct agonists of CB1 receptors, but, particularly, indirect agonists that through inhibiting endocannabinoid uptake and/or FAAH activity may elevate the levels of endogenous cannabinoids, might improve the motor deterioration seen in HD (55).

**Parkinson’s disease (PD):**

PD is a progressive neurodegenerative disorder in which the capacity of executing voluntary movements is gradually lost. The major clinical symptomatology in PD includes tremor, rigidity, and bradykinesia (slowness of movement). The pathological hallmark of this disease is the degeneration of melanin-containing dopaminergic neurons of the substantia nigra pars compacta, which leads to severe dopaminergic denervation of the striatum (58). Compared with HD, much less data exist on the status of CB1 receptors in the postmortem basal ganglia of humans affected by PD. Only recently, it has been found that CB1 receptor binding and the activation of G-proteins by cannabinoid agonists were significantly increased in the basal ganglia as a consequence of the selective degeneration of nigrostriatal dopaminergic neurons that occurs in PD patients. These increases were not related to the dopaminergic replacement therapy with levodopa (L-DOPA) that these patients underwent chronically, since they were also seen in 1-methyl 4-phenyl-1, 2, 3, 6 -tetrahydropyridine (MPTP)-treated marmosets, a primate PD model, and disappeared after chronic L-DOPA administration in these animals (57).

This is concordant with previous results in rats that showed that dopamine exerted a negative effect on CB1 receptor gene expression (48). Interestingly, as demonstrated for HD, the changes in CB1 receptors are also an early event in the molecular pathogenesis of PD.

Data obtained in humans and non-human primates are consistent with results found in PD rodent models, which also exhibited an overactive endocannabinoid transmission in the basal ganglia. Furthermore, using the PD rat model generated by acute treatment with reserpine, Marzo et al. (32) reported that an increase in the content of endocannabinoids in the basal ganglia was paralleled by hypolocomotion. This effect is strongly related to the decrease in dopamine transmission caused by reserpine, because the stimulation of dopaminergic receptors with selective D1 or D2 agonists was accompanied by a restoration of normal endocannabinoid contents and by stimulation of locomotion (32).

Therefore, it could be assumed that endocannabinoid transmission in the basal ganglia becomes
overactive in PD, which is compatible with the hypokinesia that characterizes this disease. This would support the suggestion that CB1 receptor antagonists, rather than agonists, might be useful in alleviating motor deterioration in PD or in reducing the development of dyskinesia caused by prolonged replacement therapy with L-DOPA (59).

In contrast to HD, where the endocannabinoid transmission is hypoactive, PD, in which nigrostriatal dopaminergic activity is greatly reduced, is associated with overactivity of endocannabinoid transmission, so hypokinetic signs in this disease might be ameliorated by blocking rather than activating CB1 receptors.

**Neuroprotective effects of cannabinoid-related compounds:**

Beyond the use of cannabinoid-related compounds to alleviate the symptomatology in motor disorders, these compounds also exhibit a potential usefulness as neuroprotectant substances in a variety of neurodegenerative diseases (60). Therefore, they could be used not only for ameliorating the motor deterioration, but also for delaying or arresting the progressive neurodegeneration occurring in motor disorders. Cannabinoids may play a neuroprotective role by means of three different mechanisms. Cannabinoids have been shown to be potent antioxidant compounds, although acting through a receptor-independent mechanism in vitro (61). Thus, they might be neuroprotective in disorders associated with oxidative stress. Of special interest is the effect of cannabidiol, a non-psychoactive cannabinoid that exhibits an antioxidant potency even superior to that of ascorbate and α-tocopherol (61). Furthermore, some cannabinoids have been shown to be effective neuroprotective agents in animal models of cerebral ischemia (62).

This antioxidant capability of cannabinoids might prevent neuronal death in various motor disorders, particularly in HD, where it has been demonstrated that production of free radicals, a consequence of mitochondrial dysfunction, is one of the major cytotoxic events that takes place during the pathogenesis of this motor disorder (57). Cannabinoids have the property of inhibiting N-methyl-D-aspartate (NMDA)-receptor-mediated glutamatergic neurotransmission (61). Excitotoxicity is known to be responsible for much of the cellular damages that take place in some neurodegenerative processes, mainly through the activation of NMDA glutamatergic receptors. Both Δ9-THC and AEA have been shown to inhibit the activity of those receptors in cortical and cerebellar neuronal cultures (60-62), probably by their ability to inhibit Ca2+ currents through the activation of CB1 receptors. Finally [3], the neuroprotectant effect of cannabinoids may also be based on their ability to increase the presence of neurotransmitters in the synaptic cleft in GABA synapses (48).

**Cannabinoids and pain:**

Preclinical studies in animals revealed that cannabinoids block pain responses in every pain model tested. Perhaps the earliest study of this type was performed by Nagayama et al. (62) who demonstrated that cannabinoids suppress canine reactions to pinpricks. Early studies by Bicher and Mechoulam (63) and Kosersky et al. (64) paved the way for many subsequent studies, which verified the ability of cannabinoids to profoundly suppress behavioral reactions to noxious stimuli, inflammation, and nerve injury. In models of acute or physiological pain, cannabinoids are highly effective against thermal and chemical pain (65). Typically, cannabinoids were comparable with opiates both in potency and efficacy (66).

In models of tonic or chronic pain, both inflammatory and neuropathic, cannabinoids showed even greater potency and efficacy (65, 66). Moreover, Cannabinoids altered neurotransmission through CB1 receptors by inhibition of P/Q-type Ca2+ channels and adenylyl cyclase and by activation of K+ channels and mitogen activated protein kinase (67). The overall effect is cellular inhibition. Cannabinoid receptors also occur in high density in many areas related to pain. These areas provide peripheral, spinal, and central targets through which cannabinoids modulate pain since, cannabinoids produced profound suppression of cellular nociceptive responses.

**Cannabinoid in feeding and appetite:**

The effect of cannabis and Δ9-THC on appetite in humans and in experimental studies has attracted some attention. The usual observation was of increased appetite, mostly for sweets, around 3 hr after cannabis use, but it could also be noticeable earlier. A typical report is that by Abel [68], who found increased consumption of marshmallows after cannabis smoking, compared with controls.
In rodents, the effect is dose-dependent. In a pre-fed rat paradigm, Δ⁹-THC increased appetite (69), although the animals subsequently compensated for their hyperphagia, so that 24-hr intakes were similar to controls. Interestingly, Williams and Kirkham (70) showed that, anandamide induced overeating in rats, which was mediated by the cannabinoid CB1 receptors and it was blocked by the CB1 antagonist SR141716A.

Nevertheless, the endogenous opioid system seems to be somehow associated with the endocannabinoid system in the modulation of feeding. Both the opioid (dynorphin) (71) and endocannabinoid systems may be stimulated following diet restriction, and there are possible cross interactions between them. On the other hand, 2-AG was found to be present in animal and human milk, which suggested that the endocannabinoid system could be involved in suckling and neonatal development (67-70). Anandamide is found in very low amounts in milk. However, 2-AG is present in microgram levels per gram of extracted milk lipids, and is accompanied by considerably higher amounts of 2-palmitoyl glycerol and 2-linoleoyl glycerol (66). The latter two fatty acid glycerol esters do not bind to the cannabinoid receptors and are not considered to be endocannabinoids. However, they have been found to enhance the activity of 2 AG in numerous in vitro and in vivo assays.

Clinical uses:

The effects of marijuana in stimulating appetite have justified the use of Δ⁹-THC in cancer cachexia (71). Dronabinol (Marinol) is an oral form of Δ⁹-THC that is used clinically in the treatment of anorexia and weight loss in HIV infection and in the control of nausea and vomiting associated with cancer chemotherapy (72). Since the onset is gradual and its effects sometimes cause anxiety and dysphoria, there is little risk of abuse (73).

Problems of cannabimimetic treatment are mainly CNS side effects, including confusion, anxiety, dizziness, emotional lability, euphoria, thinking abnormalities, and hallucinations. Δ⁹-THC has also been tried in anorexia nervosa, but with little success. Dronabinol has also been tried, apparently with success, in managing the appetite and behavioral problems associated with Alzheimer’s disease (74).

The Sanofi-Synthelabo Research Group recently has presented their results on the effects of SR141716 (a CB1 antagonist) in obese male patients (75). Obese male patients in a double-blind crossover study (20 mg vs. placebo) were treated for 7 days followed by a 28-day washout period. While SR141716 had no effect on taste, it induced a significant decrease of hunger, caloric intake, and weight. In a separate study, at doses of 5, 10, and 20 mg, the drug significantly reduced body weight in obese patients in comparison with placebo. It was also reported that the tolerability of the drug of SR141716 was excellent and that the observed decrease in weight did not reach a plateau during a 4-month study. These results point out that SR141716 may become a therapeutic drug in obesity (75).

Cannabinoids and migraine:

Cannabis indica is probably the most potent remedy which is at our command. Its effects are most decided, and many cases of hemicranial pain have been cured by this means alone. It must be given for a long time, and in some instances it is necessary to give gradually-increasing doses up to the physiological effects (76).

Cannabinoids and epilepsy:

Endocannabinoids have been found produced under conditions of neuronal excitability and specific intercellular signaling. For example, an epileptic seizure, with its large swings in transmembrane voltage, increases in intracellular calcium, and marked release of neurotransmitters, such as acetylcholine and glutamate that prominently release endocannabinoids. In deep seizures, kainic acid (a glutamate agonist) induced an increase in hippocampal levels of anandamide. This simply means that seizures-induced releaser of endocannabinods, occurs for normal neuroprotection (77).

Moreover, cannabinoids have complex actions on seizure activity and exert both anticonvulsant and proconvulsant effects. In one single case report and two anecdotal reports, smoking cannabis appeared to alleviate seizures in patients with generalized, partial or absence seizures. With scanty human data, the role of cannabinoids in epilepsy remains speculative. Cannabidiol may have a therapeutic potential, as it does not interact with cannabinoid receptors and has a different profile of anticonvulsant activity in animal models (75-77).
Anti-ematic use of cannabinoids:

Cannabinoids have been used in the prevention of nausea and vomiting caused by anticancer drugs. Nabilone and dronabinol ($\Delta^9$-THC in sesame oil) have been shown to be as effective as or more effective than phenothiazines, metoclopramide and domperidone for this indication, although they have not been tested against the 5-HT3 antagonist ondansetron (67-73).

Immunomodulatory effect of cannabinoids:

$\Delta^9$-THC exerts its immunomodulatory effects that alter the normal functions of T and B-lymphocytes, NK cells, and macrophages in humans and animals. These modulatory effects have been observed during both in vivo and in vitro cannabinoid treatment. In addition, the molecular and cellular mechanisms for these effects are not fully defined; however, it appears that receptor as well as non-receptor mechanisms may be involved (78).

The broad spectrum of action of $\Delta^9$-THC on the immune functions is thought to result in decreased host resistance to bacterial and viral infections as observed in various experimental animal models (79).

Studies in the early 1970s using human peripheral blood mononuclear cells (PBMCs) from marijuana smokers showed a tendency for heavy use to result in suppression of lymphocyte proliferation in culture as well as alterations in PBMCs immune cell subsets (80). Serum immunoglobulin (Ig) levels were also modulated by marijuana use, with IgG protein levels decreasing and IgE protein levels increasing. These functions vary from lymphocyte proliferation and antibody production to cytotoxic activity (81). Other studies have demonstrated that $\Delta^9$-THC enhances certain functions. B-cell proliferation increased in the presence of $\Delta^9$-THC at nanomolar concentrations and the production of the chemokines and interleukin-8 (IL-8); increased at micromolar concentrations (82). The latter group also observed decreases in the levels of other cytokine after $\Delta^9$-THC treatment. Therefore, the data that have accumulated over the past three decades indicate that $\Delta^9$-THC and cannabinoids are immunomodulatory. One of the important risk factors of marijuana use is its suppression of host resistance to infections. This aspect has been studied in both humans and animals, and the results have suggested that cannabinoids have a moderating effect on various infection paradigms (83).

A correlation between marijuana smoking and herpes-virus infection was observed to increase the risk of mortality in HIV positive marijuana smokers (Sidney et al. [84]. Furthermore, alveolar macrophages from marijuana smokers were found to be deficient in several functional properties including phagocytosis and bactericidal activity (85).

Neuro-immune cell communication:

The global function of the immune system is designed to protect against infection and/ or tumor formation. The way by which cells of the immune system perform this function can be dangerous for the survival and function of the neuronal network in the brain. When the immune cells attack the brain due to infection or inflammation, it starts the potential for severe neuronal damage or cellular death and in turn, impairment of CNS functions. To overcome such harmful action of the immune system, the CNS performs an impressive arsenal of cellular and molecular mechanisms enabling strict control of immune reactions what so-called immune privilege. Under inflammatory and pathological conditions, loss of control of the CNS and activation of the immune system result in induction of neuronal damage cascades that are frequently associated with neurological diseases. On the other hand, processes of neuroprotection and neurorepair after neuronal damage depend on a steady and tightly controlled immune surveillance. Many compounds are reported to protect against uncontrolled immune reactions in different organs such as l-carnitine (86), alpha-lipoic acid (87) and Q10-coenzyme (88). One thing observed in all these agents is their ability to counteract reactive oxygen species (ROS), produced by the immune cells, through strong antioxidant mechanisms.

The immune system serves a highly specialized function in the CNS including negative feedback mechanisms that control the immune reactions. Recent studies have revealed that endocannabinoids participate in one of the most important actions of the brain’s negative feedback system. The CNS endocannabinoid system consists of cannabinoid receptors, their endogenous ligands and enzymes for the synthesis and degradation of endocannabinoids (82-85). It participates crucially in neuronal cell-cell-communication and signal transduction, e.g., by modulating synaptic input and protecting neurons
from excitotoxic damage. On other hand endocannabinoids show a strong antioxidant action (89). Over the last decade, it has also become evident that endocannabinoids play an important role in the communication between immune cells and in the interaction between nerve and immune system during CNS damage. Thus, therapeutic intervention in the CNS endocannabinoid system may help to restore the well-controlled and finely tuned balance of immune reactions in pathological conditions (90). Moreover, ziring et al., (91) reported that formation of B and T cell subsets requires the cannabinoid receptor CB2. Another study introduced new players in the activity of endocannabinoids on synaptic transmission, and immune cell function through distinct actions away from the CB1 and CB2 receptors (92).

Cannabinoids and multiple sclerosis:

Multiple sclerosis is a disorder of the nervous system in which the ability of neurons to conduct impulses becomes impaired through the loss of myelin, which normally forms the outer covering of many nerve fibers, and through axonal loss. These changes may result from inappropriate immune responses by patients. The nature of the resulting symptoms depends on where the demyelination and axonal loss have occurred. The signs and symptoms of multiple sclerosis fluctuate unpredictably, and tend to worsen with age. They can include painful muscle spasms, tremor, ataxia, weakness or para- lyisis, difficulty in speaking, constipation, and loss of bladder control. Some of these signs and symptoms can also be experienced by patients with spinal cord injury (93).

The clinical evidence for the effect of cannabinoids in such cases comes from a study involved eight clinical trials performed with a rather small number of multiple sclerosis patients and from a study of one patient with spinal cord injury [94]. Five of these investigations were carried out with orally administered Δ⁹-THC; the results obtained suggesting that this treatment can reduce the intensity of several signs and symptoms of multiple sclerosis or spinal cord injury. In particular, objective testing has provided evidence that Δ⁹-THC can decrease spasticity, rigidity, and tremor and can improve walking ability, performance in a handwriting test, and bladder control as well as improving mobility, and quality of sleep; relieves pain; and induces a sense of well-being. In one double-blind trial with a single patient with spinal cord injury in which the effects of Δ⁹-THC and codeine were compared, it was found that oral Δ⁹-THC (5 mg) reduced pain and spasticity, whereas oral codeine (50 mg) had only an analgesic effect (95).

In addition, Notcutt et al. (96) have reported the clinical outcome of giving nabilone orally to 60 patients. These included 16 with advanced multiple sclerosis, of which, 6 experienced analgesia, muscle relaxation, and/or sleep improvement after nabilone.

Conclusion

It is really interesting to report that cannabinoid research has yielded much information and has taken us toward a better understanding of the molecular mechanisms of cannabinoid action, especially with the discovery of anandamide and 2-AG. Currently, there are multiple known endocannabinoid proteins (at least two receptors, CB1 and CB2; an enzyme, FAAH; and a transport protein, AT) as potential therapeutic targets for developing useful medications in the treatment of a multitude of ailments, such as drug addiction, pain, and motor disorders as well as immuno-suppression in autoimmune disorders and graft rejection.

References

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