University of Veterinary Medicine

Department of Physiology and Biochemistry

Central and Peripheral Cannabinoid Receptors and their Roles in Different Situations of Inflammation

Claudia Barbara Kemenesi

Supervisor: David Sandor Kiss, PhD

Budapest, Hungary

2022

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LIST OF ABBREVIATIONS

2-AG	2-Arachidonoylglycerol
AEA	Anandamide
AgRP	Agouti-related protein
AKC	American Kennel Club
CART	Cocaine-amphetamine-regulated transcript
CBD	Cannabidiol
CGRP	Calcitonin gene related peptide
CNS	Central nervous system
CRP	C-reactive protein
DAGL	Diacylglycerol lipase
ECS	Endocannabinoid system
FAAH	Fatty acid amide hydrolase
GABA	Gamma-aminobutyric acid
IL-6	Interleukin-6
MAGL	Monoacylglycerol lipase
MAPK	Mitogen-activated protein kinase
MCH	Melanin-concentrating hormone
NAPE	N-acyl phosphatidylethanolamine
NPY	Neuropeptide Y
NTS	Solitary tract nucleus
PBN	Parabrachial nucleus
PEA	Palmitoylethanolamide
PI3K	Phosphatidylinositol 3-kinase
POMC	Pro-opiomelanocortin
PPAR-α	Peroxisome proliferator-activated receptors alpha
SP	Substance P
THC	Tetrahydro-cannabinol
TNF-α	Tumor necrosis factor-alpha
TRPV	Transient receptor potential
TRPV1	Transient receptor potential vanilloid 1
VTA	Ventral tegmental area

INTRODUCTION

With the increasing legalized sales and use of marijuana worldwide, there has been a raising awareness about its impact on animals. Limited research on the use of CBD in animals has been carried out, although several veterinary collages and research institutes all around the world, are in the process of conducting research on the effectiveness of CBD to treat a variety of symptoms in animals.

CBD, unlike THC, is non-psychoactive and so far, appears to be safe to be used on pets. Numerous studies have shown CBD having a variety of effects for animals including managing pain, weight management, controlling seizures, as well as acting as an anti-inflammatory, and influencing homeostasis on different levels

It can be generally said, endocannabinoids help in managing stress and anxiety disorders as well as having many important key roles in maintaining homeostasis. In case homeostasis is disturbed, inflammation and activation of the immune system almost always happens, this thesis will be built on this foundation and concept.

A growing list of evidence points to the links between stress, anxiety, and inflammatory responses. An increase in stress can start a vicious circle, as anxiety causes an increase in inflammatory response, which, if left unchecked, may lead to further increased stress and anxiety.

Dogs and cats are prime targets for naturally occurring cannabinoids in cannabis, including CBD. Veterinary medicine tends to be quite conservative, but there is a clear trend in interest in the effects of CBD in animals.

Numerous cannabis products are toxic for animals because of the levels of THC in plant or plant-based edibles. It is believed that canines are 4 to 6 times more prone to THC toxicity than humans. Clinical signs of canine intoxication include depression, hypersalivation, mydriasis, hypermetria, vomiting, urinary incontinence, tremors, hypothermia, and bradycardia. Higher dosages may additionally cause nystagmus, agitation, tachypnea, tachycardia, ataxia, hyperexcitability, and seizures. [1]. Treatment of marijuana ingestion in animals is largely supportive. Vital signs including temperature and heart rate and heart rhythm must be continually monitored. Stomach content and urine can be tested for cannabinoids [1].

There is no official research on CBD use in dogs or scientific evidence to support the effects of CBD in dogs. However, the American Kennel Club (AKC) Canine Health Foundation (CHF) is currently sponsoring a study by the University of Colorado's College of Veterinary Medicine and Biomedical Sciences that will evaluate the use of CBD in treatment-

resistant epileptic dogs. CHF hopes this will be the first study to provide/ scientific evidence on the use of CBD in dogs with this condition [2], [3].

Given the potential benefits of increasing anandamide levels in dogs and cats, hemp products could be used to reap and utilize their benefits.

As cannabinoids and their receptors are found throughout the body, they carry out a modulatory effect on various physiological processes. In this literature review, I will only focus on the most commonly occurring clinical conditions occurring in feline, canine and equine patients, namely:

- Endocannabinoid system's function in inflammatory processes related to the skin tissue
- Endocannabinoid system's function in pain management and cutaneous tissue related disorders
- Endocannabinoid system's function in obesity.

All these conditions involve the activation of the immune system as well, thus have the potential to cause, acute or chronic inflammation locally or systemically.

CANNABINOIDS

OVERVIEW OF THE ENDOCANNABINOID SYSTEM

Cannabinoids are a large group of biologically active substances present in the cannabis Sativa plant (common hemp; other synonyms: marijuana, anasha, hashish). Cannabinoids have long been known as psychoactive substances with a pronounced effect on the central nervous system, causing hallucinatory and psychedelic effects. The earliest reference to the medicinal properties of cannabis dates to 2700 BC when it was used in China to treat obstipation, malaria, rheumatic pain, and menstrual irregularities. Since then, cannabinoids have been used throughout history in India, the Middle East, South Africa, and South America [4], [5].

Formed at the early stages of evolution, the endocannabinoid system is a universal signaling structure that controls many physiological functions of the body, including the regulation of the nervous and immune system, energy metabolism and reproduction, cell growth, and differentiation. The main components of the endocannabinoid system are:

1. cannabinoid receptors CB_1 and CB_2,
2. endogenous cannabinoids (AEA, 2-AG, noladin ether, virhodamine, N-arachidonoyl dopamine) [6]
3. enzymes involved in the process of their biosynthesis and degradation (FAAH, DAGL, MAGL, NAPE) [7]

All links of the system are present not only in humans and highly organized representatives of the animal kingdom, but also in the most primitive organisms that inhabited the planet as early as 700 million years ago: mollusks - Mytilus edulis, snails - Aplysia californica, hydras - Hydra Vulgaris, sea urchins - Paracentrotus lividus, etc. [8].

CANNABINOID RECEPTORS

DISCOVERY OF CB_1 AND CB_2 RECEPTORS

By origin, cannabinoids can be exogenous (obtained from plant materials or synthesized chemically) and endogenous (produced by the body). The discovery of exogenous and endogenous cannabinoids has an interesting history. In 1964, an Israeli Professor, Raphael Mechoulam for the first time isolated a psychoactive substance from Cannabis sativa, trans-9tetrahydrocannabinol [9]. In subsequent years, its pharmacological properties were thoroughly studied at the level of organs and tissues. The possible presence of endogenous ligands in the body, have also been hypothesized, which causes similar pharmacological effects.

In the nineties of the last century, the search for endogenous cannabinoids began in organs and tissues of the human body, which were based on hypotheses about the biosynthesis of cannabinoids from lipid components of cell membranes.

For a long time, it was thought that cannabinoids performed their action at the same level - through nonspecific interactions with membrane lipids, however research has discovered mechanisms of more refined receptor interactions. In 1990, Matsuda had identified cannabinoid receptors in the brain and at the level of peripheral neurons [10]. In 1992, Lumir Hanush isolated the first endogenous ligand from the brain – anandamide [11]. In 1993 Munro et al., identified cannabinoid receptors in peripheral tissues (CB_2 receptors).

CB_2 receptors act at the level of cells of immune origin, including lymphocytes, mast cells, and macrophages, as well as the basal layers of the epidermis and hair follicles in humans [12], [13] but we shall discuss this more in depth in the next chapter.

In parallel to all this, another type of cannabinoid receptor, CB_1, was discovered. CB_1 receptor agonists have shown antinociceptive effects in acute pain and neuropathic pain [14] and the journey of cannabinoid research has begun.

Cannabinoid Receptor Types and Their Localization

As having mentioned, until today, two different subtypes of endocannabinoid receptors CB_1, and CB_2 have been discovered and studied. The detection of specific cannabinoid receptors indicates the existence of endogenous substances that affect these receptors in normal or pathological conditions (endogenous cannabinoids or endocannabinoids). This system of receptors, together with endogenous ligands, formed the so-called endogenous cannabinoid system, or the endocannabinoid system (ECS) [14].

“Endocannabinoidome” is a term that has been made to describe an expanded definition of the ECS, involving other ligands and receptors and their interactions with the ECS, as well as with receptors outside the ECS. The endocannabinoidome’s overall homeostatic functions involve glucose metabolism (weight gain/weight loss), gut immunity, circulating ligands and blood pressure. It can be modulated by vitamin D levels, diet, exercise, and many different types of drugs [15].

As seen on figure 1. the localization and distribution of CB_1, and CB_2 receptors is measurably very different. The fact that CB_1 receptors are found in mainly in the CNS, while CB_2 receptors are mainly found on immune cells and occur only in a small amount in the CNS, which indicates that CB_1 receptor activation is the primary cause for the psychological actions of endocannabinoids in the CNS. [14].

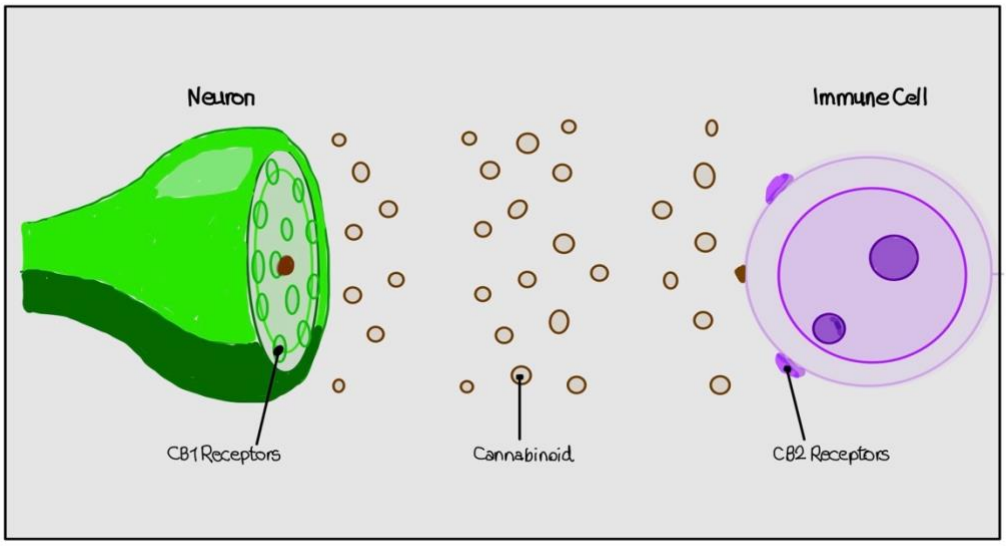

Figure 1. Simplified overview of Cannabinoid receptor types and their location

CB_1 RECEPTORS

CB_1 receptors are associated with pain sensitivity at both central and peripheral levels - the effect of endocannabinoid ligands on them inhibits somatic (or protopathic) and neuropathic pain. In addition, CB_1 receptors, when activated by endogenous ligands, can affect the growth, differentiation, and apoptosis of connective tissue and epithelial cells [16].

CB_1 receptors are localized in the brain and spinal cord, peripheral nervous system, and many other organs and tissues. In the brain, the concentration of CB_1 receptors is higher than any other receptors involved in neuromodulation processes; the main places of concentration are the cortex, hippocampus, basal ganglia , nucleus accumbens, cerebellum, amygdala, hypothalamus [14]. These regions of the brain are responsible for cognitive functions, emotions, motor responses, sensory processing, memory, and homeostasis. In minimal amounts, CB_1 receptors are also found in the brain stem, however, ligand binding is nearly absent in the lower brain stem areas that control cardiovascular and respiratory functions. This could explain why even high doses of cannabinoids are not lethal in many but not all species. Published evidence indicates that cannabis may not be completely safe for all veterinary patients, such as dogs, due to the high density of CB_1 receptors in the canine cerebellum and medulla oblongata [17].

In addition to the nervous system, CB_1 receptors are found in the heart, lungs, endothelium, gastrointestinal tract, prostate, as well as in the bone marrow, tonsils, spleen, and thymus gland. This type of receptor is present in all organs of the endocrine system:

hypothalamus, pituitary gland, thyroid gland, adrenal glands, pancreas, gonads; in addition, a high expression of CB_1 receptors in adipose tissue was noted [18], [19] which is why it can play an important role in glucose metabolism and obesity as it shall be discussed further in the last chapter.

CB_2 RECEPTORS

The key role in the reactive response to tissue damage is played by the activation of CB_2 receptors, which are found in the parenchymal and connective tissue structures of organs, in the skin, blood cells - macrophages, basophils, lymphocytes, and neutrophils.

These receptors, when activated by endogenous ligands, similarly to CB_1 receptors, can regulate the processes of proliferation, differentiation, and -after programmed cell death- apoptosis [7], [11].

CB_2 receptors are concentrated mainly in organs and tissues that provide an immune response – such as in the spleen and its macrophages/monocytes, thymus gland, tonsils, bone marrow, blood leukocytes [7], [11]. Although much lower concentrations than in immune system related organs, small amount of CB_2 expression in the brain has also been mentioned, mainly in microglial cells, and has been found to be upregulated in response to chronic pain [7].

To date, no convincing information has been obtained on the possible localization of CB_2 receptors in the nervous tissue; nevertheless, their presence has been established in the microglia of the brain (Dewey et al. 1972).

Cannabinoid receptors CB_1 and CB_2 are members of the G protein-coupled receptor superfamily (Gi / o family), therefore play a role in adenylate cyclase modulation and activation/inhibition of kinases related to its signal transduction pathways. The differences between the two types of receptors are determined by the sequence of their constituent amino acids, signaling mechanisms, localization features, as well as the nature of interaction with some agonists and antagonists [6], [21].

The amino acid sequence of the CB_1 and CB_2 receptors is 48% similar. Under experimental conditions, it has been determined that the transmission of signals that are activated when interacting with receptors of endogenous and exogenous agonists (either of plant origin or synthetic cannabinoids) can be carried out with the participation of many regulatory mechanisms: adenylate cyclase, A-, D-, C- types of potassium channels; L-, N-, P / Q– types of calcium channels, mitogen-activated protein kinase, protein kinases A, B and C; phosphatidyl-inositol-3-kinase, focal adhesion kinase, nitric oxide synthetase, and ceramide.

As we know, signal pathways are determined by the type of cells (tissue type) and experimental conditions. During neuromodulation, both receptors, mediated by Gi / o -protein, interact mainly with adenylate cyclase and MAPK, and in addition CB_1 receptor also interacts with various types of calcium and potassium channels, gaining significance especially in the cardiac tissue, making cannabinoid activated signal transduction pathways an even more multiplex network, and exactly due to its complexity not always easy to decipher.

CANNABINOID RECEPTOR LIGANDS -ENDO AND EXOCANNABINOIDS

DISCOVERY OF SOME IMPORTANT ENDOCANNABINOIDS AND THE SIGNIFICANCE OF PEA DURING INFLAMMATION

The discovery of the cannabinoid system took place thanks to the integration of the results of scientific research in the fields of molecular biology, chemistry, structural biology, pharmacology, biochemistry, and neurophysiology [22].

The presence of receptors for exogenous cannabinoids in mammals indicated the existence of natural, endogenous ligands. The first endogenous cannabinoid was discovered in 1992 [14]. To the surprise of researchers, regarding its structure, it turned out to be not a protein, as was initially assumed, but a lipid, and was an amide of arachidonic acid and ethanolamide. The first endocannabinoid was called anandamide (AEA), translated from Sanskrit "ananda" - inner bliss) [23]. A second natural cannabinoid receptor ligand, 2-AG, was discovered three years later. The first scientific studies to study the cannabinoid receptor antagonist rimonabant began in 1993. [21].

In 1957,– PEA was discovered and isolated from soy lecithin, peanut meal and egg yolk and was later investigated [24], following the suggestions, how this substance could be accumulated in inflamed tissues and having immune system modulating properties.

The pronounced anti-inflammatory and anti-ischemic properties of PEA molecule has also been demonstrated in many different areas [25]. First it has been thought, PEA is a strong CB_2 receptor agonist [26], however later it has been proved that as a matter of fact, PEA has a low affinity for CB_2 receptors, explaining, why some of its anti-inflammatory effectors were not blocked by CB_2 antagonists. [27]. PEA is an endogenous universal tissue-specific cannabinoid, a structural analog of AEA, into which and from which it can be converted during redox reactions [28].

PEA is found in various organs and tissues only during a pathological process, in contrast to its structural analog anandamide, which can be isolated from tissues in normal conditions as well [29]. PEA has great significance in regards its relationship with the ECS. Decades of research on this molecule, has lead scientists to come up with the so-called term, "entourage effect", which brings up the possibility that PEA could produce indirect receptor-mediated effects (Costa et al. 2008), for example, as studies have shown it can enhance AEA-induced relaxation through TRPV1 receptors in rat isolated mesenteric arteries which has been reported by Ho et al. [30] - the significance of TRPV1 receptors will be mentioned more in depth in a later chapter, when itching and pain related signaling linked with the endocannabinoid system shall be discussed. Another example for its entourage effect, is how it carries out agonist effects on GPR55 receptors (an atypical cannabinoid receptor), and can alter mesolimbic dopaminergic activity states in the ventral hippocampal regions [31].

Furthermore, it can also be noted that an other important property of PEA is that it can inhibit anandamide hydrolyses by FAAH: in rat brain membranes for example it has been shown to inhibit FAAH by 40-50% [32]. A simplified overview of PEAs effects can be seen on Figure 2.

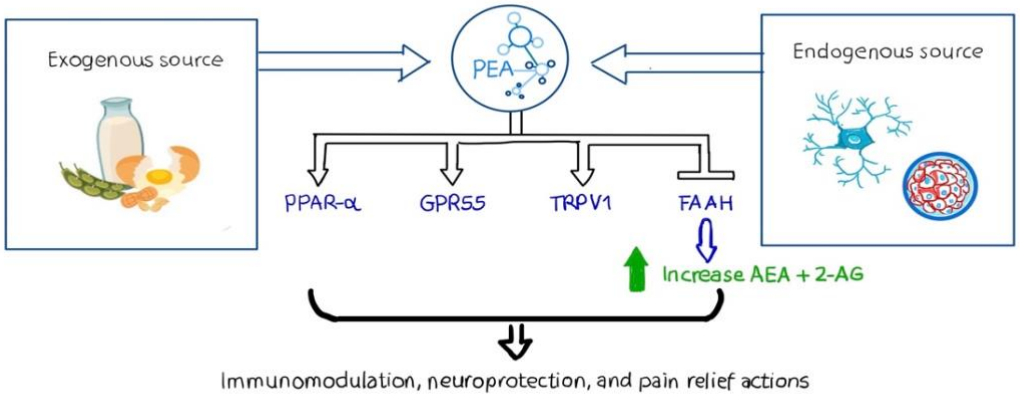

Figure 2. Simple overview of different types of sources and components that participate in establishing the entourage effects of PEA which contribute to modulate endocannabinoid effects during inflammation, and in painful conditions.

The most studied pathobiochemistry of PEA seems to be related to the skin tissue, at the level of the granular layer of the epidermis. With various types of damage (mechanical, thermal, ultraviolet, ionizing, chemical, microbial, etc.), PEA is released from N-palmitoyl phosphatidylethanolamine - a natural lipid component of cell membranes. PEA as mentioned

earlier accumulates exclusively in inflamed tissues [33]. In skin tissue it has been shown that PPAR-α mediates the anti-inflammatory effects of PEA, as this amide serves as its endogenous ligand.

For us to see PEA's role more clearly, a establishing some sort of categorization might be helpful: we can state there are two principal groups of PEA effects in regards of inflammation:

- effects associated with the neutralization of free radicals in organs and tissues.
- effects mediated through binding to CB_2 receptors of cells and nerve endings.

To understand more how PEA works, let us briefly revise the effects of free radical oxidation first.

Free radical oxidation is a constant companion of skin lesions of various origins and nature (skin photoaging, dermatoses, wounds). It has been established that within the foci of inflammation and hypoxia, free radical oxidation reaches its maximum. Chemical bonds of phospholipids, ceramides, triglycerides, and higher fatty acids, as well as membrane proteins (glycoproteins), are destroyed with the formation of many free bonds (radicals) that enter into the oxidation of other, intact biomolecules [34].

However, when PEA and other tissue-specific cannabinoids bind to CB_2 receptors of nerve endings, the concentration, and activity of SP - a universal neurotransmitter that promotes the activation of pain sensations especially when inflammation increases- so that vasodilation, migration of cells, and liquid part of the plasma into tissues and many other pro-inflammatory effects may happen. In other words, we can also say PEA downregulates mast cell activation, and decreases tissue inflammation by potentiating effects of AEA on TRPV1 inotrope receptors [23], [35], furthermore through PPAR-α receptors it is able to increase expression of CB_2 receptors.

In addition to all this another important effect of PEA is mediated directly through CB_2 receptors on the surface of immunocompetent cells, primarily tissue basophils (mast cells), as part of the prevention of their degranulation in tissue. In these cells, the processes of granule accumulation and subsequent degranulation of biologically active substances, namely, histamine, serotonin, cytokines, leukotrienes, nitrogen oxide, and superoxides, are blocked. Thus, the release of these biologically active compounds in the tissue does not occur, and therefore, they do not affect specific receptors to help decrease tissue inflammation [36].

To summarize we can state, PEA being a compound of the endocannabinoidome carries out rather complex biological and pharmacological effects. It is not a true endocannabinoid,

despite some structural and metabolic similarities with those signaling molecules. Due to its low selectivity, it is preferred and has been given the potential to treat certain diseases (for example skin related diseases which shall be discussed later) where multiple-target molecules have advantages over the classical single-target drugs. This is probably the main significance, and the big potential PEA carries within itself.

NATURE AND FUNCTION OF CANNABINOIDS

In the last century, as search was carried out, for medicinal substances which control different inflammation related diseases, within this aspect naturally occurring molecules, or biomolecules, formed in the body under either normal conditions or during unfavorable environmental factors, have attracted attention. These molecules were discovered to be the previously introduced endocannabinoids – which are fatty acid amides in nature [37].

Fatty acid amides are a group of lipid bioregulators formed from long-chain saturated and unsaturated fatty acids of complex lipids by amidation with appropriate amines.

The most studied are ethanolamides of fatty acids, for which an alternative pathway of biosynthesis has been established - through the hydrolysis of N-acylated phosphatidylethanolamines by phospholipase D. This process is observed during inflammation or oxidative stress, when lipids of biological membranes are destroyed [37]. An increase in the endogenous synthesis of cannabinoids under the influence of unfavorable factors can be considered as a protective reaction that allows one to take control of alteration processes at the molecular level [36].

Fatty acid ethanolamides bind to cannabinoid receptors of the central nervous system (CB_1) or peripheral tissues (CB_2) and are considered endogenous ligands of these receptors [37]. They exhibit pharmacological properties characteristic of cannabimimetic. Simple fatty acid amides are also endogenous bioregulators, acting as sleep-inducing (oleamide) or angiogenic (erucamide) [37].

Endocannabinoids are formed inside neurons and in several other cells from membrane phospholipids with the participation of phosphodiesterases. The formation of new molecules occur during membrane depolarization and a subsequent increase in the level of intracellular Ca^{2+}. Given the high lipophilicity, endocannabinoids do not accumulate in vesicles and after synthesis, are quickly released into the intercellular space. As a rule, endocannabinoids move away from the site of synthesis at extremely short distances [38], [39]. After binding to the receptors, the secondary messenger system is activated; transmission of signals are carried out

either by reducing the activity of adenylate cyclase via blocking calcium channels or by stimulating potassium channels [40].

In a more extensive point of view cannabinoid and endocannabinoid compounds can also activate a more widespread range of receptors, including nuclear PPARs that act as transcription factors also for regulating lipid metabolism and that can be activated by prostaglandins, leukotrienes, N-acylethanolamine and Δ9-THC. Another example is, how ECS compounds can activate ionotropic receptors of the family of TRP channels, which generate a cationic current in mechanic, thermic, nociceptive and chemical sensory neurons and that can be activated by anandamide, PEA, and also by synthetic cannabinoids [41], [42].

CB_1 and CB_2 receptors are classical G_i protein coupled receptors, which inhibit the activity of adenylyl cyclase by $G_{i\alpha}$, additionally, through the βγ complex which can close K^+ and Ca^{2+} channels, modulate release of Ca^{2+} from intracellular compartments, and activate phosphatidylinositol 3-kinase (PI3K) and the mitogen-activated protein kinase (MAPK) pathways and consequently mediate signaling to the nucleus (Sanchez-Aparicio et al., 2020). By their coupling to G_i protein, CB receptors decrease neurotransmitter release from terminals. Almost all kind of neurotransmitters are regulated by endocannabinoids as well and their receptors. Regulation of neurotransmitter release can be acute or in a long-term fashion. Short- and long-term depressions, neuronal forms of plasticity related with learning and memory are modulated by CB_1 receptors. CB_1 receptors also modulate neurotransmitter uptake [43]. The location of CB_1 receptors in CNS is closely related with the brain areas related with symptoms of cannabis intoxication and in consequence with their functions, for example, increase of appetite produced by cannabinoids is related with the expression of receptors in nuclei related with eating behavior including hypothalamus, solitary nucleus tract, and accumbens nucleus. Increased locomotor activity and/or incoordination of movement induced by cannabis in patients and experimental animals, is related with location of CB_1 receptors in basal ganglia and cerebellum. This knowledge was what lead to the proposal of using cannabinoid compounds for the management of eating disorders such obesity and anorexia, disorders of the control of movement, and as antiemetic by their effects related with the location of the receptors in the centers controlling these reflexes [44] (This topic shall be discussed more in depth in Chapter “Cannabinoids and Obesity”).

Among the endocannabinoids, which include anandamide, 2-arachidonylglycerol (2-AG), noladine ester, O-arachidonic-ethanolamine (virodgamin), N-arachidonyl-dopamine, and

oleamide, the most important and best-studied to date are anandamide (AEA) and 2-arachidonylglycerol (2-AG)

Despite the similarity of the structure and the main spectrum of biological effects, the metabolic processes of the main endocannabinoids have significant differences. Anandamide is formed as a result of hydrolysis of its precursor N-phosphatidylethanolamine by phospholipase D [45]. Anandamide is cleaved to arachidonic acid and ethanolamide by using FAAH a microsomal enzyme found in postsynaptic neurons and some other cells [40].

Anandamide is a natural ligand and has a higher affinity for CB_1 receptors, while 2-AG activates both types of cannabinoid receptors [46].

There is an interesting possibility, how a functional relationship seems to exist between CB_1 receptors and receptors for 5-hydroxytryptamine, dopamine, orexins, as well as the interaction of anandamide with vanilloid receptors located in several nociceptive neurons (Cravatt et al., 2001), such sort of relationship can contribute or even enhance analgesic and anxiolytic effects.

As having mentioned earlier, it can be stated the endocannabinoid system manifests its modulating effects only in those organs where and when, it is in demand and various mechanisms are involved in carrying out the effects of endocannabinoids. In the central nervous system, endocannabinoids act as neurotransmitters and neuromodulators. Under the influence of neuronal impulse, classical neurotransmitters are released from the vesicles of the presynaptic terminals, diffuse through the synaptic cleft, and interact with receptors located on postsynaptic neurons. Another mode of action is via stimulation by exocannabinoids (phytocannabinoids or synthetic cannabinoids) as shown in Figure 3.

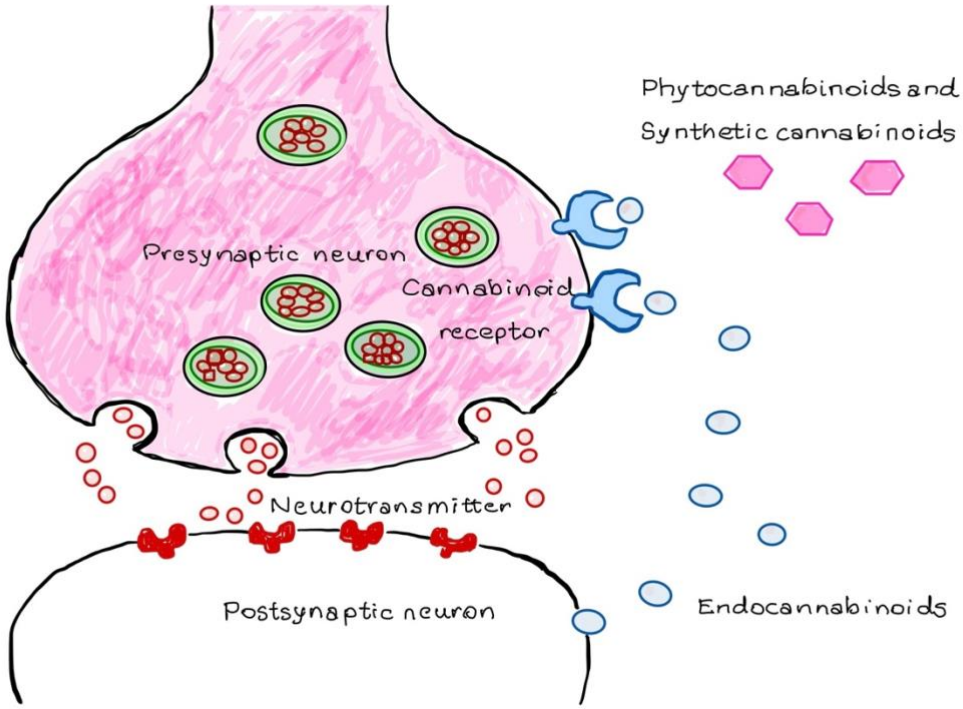

Figure 3. Endocannabinoids are produced postsynaptically and act in a retrograde manner on the presynaptic neuron. Phytocannabinids and synthetic cannabinoids also carry out their modulatory effects on postsynaptic neurons.

Endocannabinoids, lipophilic in nature, are released from the postsynaptic neurons. Immediately after synthesis, endocannabinoids are directed to the presynaptic terminals, where CB_1 receptors are localized, this way of activating a receptor is called retrograde signaling. The main effect will be the suppression of the production of neurotransmitters such as glutamate and GABA [38], [46], [47].

Furthermore endocannabinoids, besides modulating other neurotransmitter release, can also potentiate autocrine signals of mediators that inhibit the activity of cells of the nervous system, as it has been shown with the example of GABA-ergic neurons in the cerebral cortex. Both para- and autocrine effects can be exerted (without the involvement of synaptic transmission). This effect is observed mainly in glial cells, adipocytes, and the liver [47], [48].

An interesting, thought inducing fact is how endocannabinoids, along with CB_1 receptors, can also be located intracellularly, and a possibility of the existence of corresponding intracellular signals also cannot be ruled out [49].

DEACTIVATING ENZYMES

After postsynaptic release endocannabinoids are rapidly deactivated by uptake into cells and enzymatic hydrolysis. Endocannabinoid system reuptake happens via a carrier-mediated mechanism, which has not been molecularly characterized yet. Endocannabinoid reuptake however has been demonstrated in discrete brain regions and in various tissues and cells throughout the organism [37], [46].

After release, AEA and 2-AG can be eliminated by a two-step mechanism consisting of a carrier-mediated transport into cells, followed by enzymatic hydrolysis. As there is a rapid deactivation process, endocannabinoids may primarily act near their sites of synthesis by binding to, and activating cannabinoid receptors on the surface of neighboring cells [14], [40].

FAAH is an intracellular, membrane-bound protein. It's primary structure displays significant similarities with a group of enzymes known as "amidase signature family" [40], [50].

FAAH may act as a general hydrolytic enzyme not only for fatty acid ethanol amides (such as previously mentioned AEA) but also primary amides , such as oleamide (a biologically active lipid of unclear function) and even esters, like 2-AG [40]. There are three major challenges that can lie before a researcher, or pharmacist interested in the mechanisms of endocannabinoid inactivation.

1. The need for a deeper molecular understanding of these mechanisms (for example finding the identity of endocannabinoid transporter and the existence of additional hydrolytic enzymes for AEA and 2-AG).

2. The second challenge lies in the development of potent and selective inhibitors of endocannabinoid inactivation (FAAH inhibitors should combine the potency of those currently available with greater pharmacological selectivity and biological availability).

3. The third challenge is the validation of endocannabinoid mechanisms as targets for therapeutic drugs.

The points listed above are of course linked with the need to be able to gain further knowledge and understanding on endocannabinoids roles in physiology, on which much research is currently focused on.

ENDOCANNABINOID SYSTEM'S FUNCTION IN SKIN RELATED INFLAMMATORY PROCESSES, PAIN AND ITCHING

INTRODUCTION

The skin is the largest mammalian organ and the primary interface between the body and the environment. It is composed of the epidermis, dermis and hypodermis. Epidermis, of ectodermal origin contains cells like, keratinocytes, melanocytes, Langerhans cells and Merkel cells. Dermis, of mesodermal origin, includes cells such as collagen-producing fibroblasts, macrophages, mast cells, plasma cells and different types of T cells. Last but not least, the hypodermis is made of adipocytes, fibroblasts and macrophages, and is well supplied by vessels and nerve fibers [23], [51].

The skin has seven main functions namely:

1. Provides first line defense system against invading pathogens and trauma ("outside-in barrier") by both physical barrier properties and by cellular protective mechanisms, provided by bidirectional interactions between epithelial and immune cells
2. Regulates body temperature
3. Prevents excessive water and electrolyte loss ("inside-out barrier")
4. Major sensory organ which senses changes in the environment through cutaneous nerve endings (nociceptors), which contribute to regulating local homeostasis through the neuroimmune system.
5. Exocrine (sweat and sebum) function
6. Endocrine (synthesis of different hormones e.g., vitamin-D, steroids, peptide hormones)
7. Regenerative function (wound healing) [23], [52]

As it can be seen healthy skin tissue plays an essential role in maintaining homeostasis on many different levels therefore it is noteworthy to dissect this topic further as endocannabinoid system activation goes hand-in-hand with restoring homeostasis whichever tissue it may be.

PAIN

Pain (dolor) is one of the four cardinal signs of inflammation defined by the greatest Roman medical writers Celsius in the 1st century AD., in his work "De Medicina", which definition is still valid and used today, both in human and in veterinary medicine (the other three signs being: rubor-redness, calor-warmness, and tumor-swelling).

The sensation of pain is associated with the activation of the receptors in primary afferent fibers, such as unmyelinated C-fiber and myelinated Aσ-fiber. Both of these receptors are otherwise silent during the absence of pain and are activated when there is a potential of noxious stimulus [53]. Endocannabinoids modulate neural conduction of pain signals by both reducing the nociceptive neural signal of pain, and by reducing inflammation through activation of cannabinoid receptors. CB_1 receptors present on nociceptor terminals mediate anti-nociceptive and anti-inflammatory actions of locally produced AEA through its inhibitory influence of the release of excitatory neuropeptides [54]. Both CB_1 and CB_2 are expressed in the dorsal ganglia, and their stimulation at this level can contribute to decrease nociceptive transmission, however immune cells and keratinocytes -which topic I shall discuss more in depth in the Chapter on ECS function in Skin tissue- are also involved in the peripheral CB_2 receptor analgesia as CB2 receptor reduces the release of pro-nociceptive molecules from these cells [55], [56].

To be able to see better, how these elements are interrelated and interconnected, it is important for us to know and to understand all underlying mechanisms by which CB_2 receptors may alleviate chronic degenerative-related symptoms such as pain. By obtaining such knowledge and approaching with such point of view, veterinary practitioners and veterinary researchers could establish specific protocols to improve the quality of life of a selected group of patients, who for several reasons otherwise, may not reach a satisfactory therapy.

There is much information and evidence which supports the idea of taking advantage of the pharmacologic properties of CB_2 receptors and selective agonists which could carry out a protective action against pain but without all the psychoactive and undesired side effects of CB_1 agonists. The primary aim of this would be to incorporate cannabinoid receptor agonists, to provide reliable alternatives for painful conditions in veterinary patients, and in many cases also to try to decrease some side effects of other more conventional treatments [57], [58].

As discussed earlier in Chapter 2 in greater detail, CB_1 and the CB_2 receptors participate in numerous essential biological processes. To mention only a few, some of these are: neuronal plasticity, pain, anxiety, inflammation, neuro-inflammation, immune function, metabolic regulation, and finally bone growth and bone remodeling. [42], [57].

It is believed that the use of selective CB_2 receptor agonists represent a potential field for veterinary patient treatment, especially in those who are going through some sort of painful disease. Upregulation of CB_2 receptor expression in the dorsal horn and DRG is seen in both neuropathic pain and inflammatory pain. It is well documented that the peripheral expression of CB_2 receptors in cells and tissue related to the immune system is upregulated during immune mediated inflammatory events. The upregulation of this receptor is an important therapeutic target for inflammatory pain, but also for the neuroinflammatory based neuropathic pain. Antinociception to thermal stimuli is also seen via CB_2 receptor activation with synthetic cannabinoids [59], [60].

It has been shown that cannabinoids suppress C-fiber evoked responses in the spinal dorsal horn, recorded in normal and inflamed rats. Spinal Fos protein (product of c-fos gene, a proto-oncogene, also expressed when subjected to peripheral noxious stimulation) expression is also suppressed by cannabinoids, in animals going through persistent pain, which occurs through CB_1 and CB_2 receptor selective mechanisms [58], [61].

Besides CB_1 receptors, nociceptor nerve terminals express not only ligand gated but also voltage gated ion channels, such as TRPV1 and TRPA1 [62]

TRPV1 is involved in transmission of pain through primary sensory afferent perivascular neurons. The activation of TRPV1 by endogenous AEA leads to a release of SP and of CGRP, which have allogenic, local vasodilatory and pro-inflammatory effects as well as anti-hypertensive effects. [62].

Endogenous ligands using TRPV1 receptors for inter-and intra-cellular signaling have been termed as endovanilloids, anandamide being the first. TRPV1 receptors otherwise can be activated both by exogenous (capsaicin) and endogenous agents (e.g.: heat, low p.H., PEA, anandamide, etc.) [63].

As an example stimulation of TRPV1 receptors in the periaqueductal grey (PAG) inhibits pain by :

- acting on rostral ventromedial neurons that mediate analgesia by sending descending projections to the dorsal horn spinal cord neurons, or by
- desensitizing the activity of other neurons involved in inducing hyperalgesia [64]

Besides TRPV1 receptors AEA can also mediate pain via:

1. inactivating nociceptive signals at the synapse by activating CB_1 receptors
2. reducing inflammation by activating CB_2 receptors
3. allowing COX-2 enzymes to transform them into prostamides, prostaglandin ethalonamides (pain-relieving molecules) [64].

To summarize these findings: administration of endocannabinoids or exogenous cannabinoids can produce anti-nociception in different pain models. These antinociceptive effects however may be different depending on the cannabinoid employed and/or the mechanism used to modify endocannabinoid levels (for ex.: degrading enzymes etc.). Inhibition of endocannabinoid re-uptake, exogenous administration of endocannabinoids (AEA, 2-AG) and the use of FAAH inhibitors, produce anti-nociception in different models through CB_1 receptor activation. CB_1 receptor independent mechanisms -such as TRP channels and potassium channels which are involved in the modulation of pain processing- also participate in AEA's function in anti-nociception. CB_2 receptors also participate in endocannabinoid antinociception via the upregulation of these receptors during the activation of the immune system.

ITCHING (PRURITUS)

Itch and, the previously discussed, pain are closely related, however are different sensations. They share overlapping mediators and receptors, and it has also been proven neurons associated with the modulation of itch, are also sensitive to pain stimuli.

Pruritus, is the term for itch and in the past was often mis-spelt 'pruritis', making it into an inflammation. Pruritic is preferred to prurient for itchy, indicating the complex nature of the subject, also indicating that itching is a sensation intertangled with emotion.

Chronic itching lasts for more than 6 weeks of time. Amongst dermatological patients, pruritus is one of the most common reasons both dog and cat owners seek for veterinary care therefore it seems to have great clinical significance and why it feels it should have a place in this discussion.

Itching is -usually- a short unpleasant sensation that makes one want to scratch its own skin. The original purpose of itching is to eliminate the action of pruritogens by scratching. Itching occurs in primary skin diseases, but it can also be a symptom of systemic diseases, which comprises approximately 10-50% of all cases [65], [66].

Over the past 20 years, fundamental research has been carried out in the field of pathophysiology and biochemistry of pruritus, as well as its pharmacological treatment. In practice however drugs that specifically affect the mechanisms of itching is significantly limited: in clinical practice widespread glucocorticosteroids and antihistamines are administered which carry out non-specific effects on the symptom of itching, and this, in turn, results in poor symptom control and furthermore more importantly causes many side effects [67]. Due to all this there would be a need for other more specific treatment options to choose from.

ECS has a fundamental role in the control of skin derived sensations such as pain and itch. Synthetic CB agonists and/or endocannabinoids carry out potent analgesic effects in both humans and animals by activation of CB_1 and/or CB_2 receptors and other receptors as well e.g.: TRPV1, at sensory nerve terminals and/or inflammatory cells [23], [68] which I shall discuss more in depth at the end of this chapter.

DIFFERENT TYPES OF CATEGORIZATION SYSTEMS FOR ITCHING

There is a distinguishment between the neuroanatomical and clinical classification of pruritus. Within the neuroanatomical classification, pruritus is further differentiated based on its main possible causes:

1. proprioceptive - realized in the skin.
2. neuropathic - is the result of pathological changes in peripheral nerves.
3. neurogenic - mediators realize itching in the central nervous system without damaging the nerves.
4. psychogenic [67], [69]

Clinical classification, on the other hand, differentiates pruritus, considering the presence or absence of an inflammatory process in the skin:

I. Based on the clinical picture:

- itching of initially inflamed skin;
- itching of primary non-inflamed skin;
- itching with chronic secondary excoriation.

II. Based on possible diseases:

- dermatological diseases;

- systemic diseases;
- neurological diseases;
- psychosomatic/psychogenic diseases;
- neoplastic diseases;
- mixed diseases;
- other (reasons unknown). [35], [69], [70]

Regarding its location and level of severity itching can be generalized or localized. As a potential manifestation of systemic diseases, generalized itching deserves special attention as it may accompany- or may be accompanied by- many other, serious diseases, and in most cases, it is the symptom that first draws owner's attention to discovering a disease with a more serious underlying pathologic condition, on their pet animal.

In case of generalized pruritus, the cause lying in the background of itching can be different such as:

- metabolic and endocrine disorders (parasitic invasions, hyperthyroidism/ thyrotoxicosis, pregnancy, chronic renal failure, hematological diseases, drug reactions, tumor diseases, CNS tumor, latent (occult) carcinoma;)
- psychopathic/psychogenic itching
- senile itching [70], [71].

CAUSES OF PRURITUS

Special attention should be paid to understand skin diseases in order to be able to understand their causes and the pathophysiology mechanism behind, and thus to be able to find the most appropriate treatment for them. In case of inflammatory skin diseases, itching is a persistent condition eliminated with difficulty and more effort from the veterinarian/owner's side is needed. Such conditions include atopic dermatitis, allergic contact dermatitis, urticaria, , bullous diseases, eosinophilic skin diseases, certain types of medication, parasitic reactions, mastitis, itchy papules. In addition, skin infections - bacterial, viral, and fungal -; neoplastic diseases – cutaneous epitheliotropic T-cell lymphoma; as well as several other skin diseases - xerosis, senile pruritus, anogenital pruritus, primary cutaneous amyloidosis, post-traumatic and post-burn itching, pruritic scars, aquagenic itching can also be added as a cause [35], [69], [71].

In canine species atopic dermatitis is probably one of the most common disease associated with pruritus and besides that, flea allergy dermatitis, seasonal allergy dermatitis,

contact dermatitis (caused by for e.g., soaps, different fabric materials etc.) and sarcoptic mange (mites) are some of the most frequent causes of pruritus in dogs [67], [69].

In cats, the most common causes of itching are parasites, infections, and allergies, such as flea or other insect bite related allergy, food allergy, and the previously mentioned atopic dermatitis which we shall also discuss more in depth later in this chapter. There are many skin diseases that do not initially cause itching. However, itching may develop with these diseases due to secondary bacterial or yeast infections [72].

Independent nerve endings and receptors responsible for conveying the sensation of itching towards the CNS are found only in the skin. The nerve fibers conducting the itching signal are localized mainly at the site of the dermal-epidermal junction and the proportion of unmyelinated C-fibers conducting the itching signal is only 5% of all nerve endings in the skin. Endogenous substances that can provoke itching on such nerve endings are therefore important. [71], [73]. After various nonspecific trigger factors listed earlier, as well as after scratching, the free nerve endings in the skin release neuropeptides. Itching mediators are amines (histamine, serotonin, tryptamine), neuropeptides, proteases, opioid peptides, eicosanoids, leukotrienes, cytokines, interferon-gamma, to mention a only few, but also cellular elements such as neutrophils, eosinophils, basophils play a role in mediating itching sensation. [73], [74]. The number of sensory fibers in such cases increase and the number of adrenergic independent fibers decrease, which determines the role of primary afferent and autonomic nerve fibers in the pathophysiology of pruritus. [73], [74].

As mentioned earlier, in the beginning of this chapter, exactly because of such complex mechanism lying in the background of itching, it makes it very difficult to search for its real inhibitors and still seems to be waiting for further research to be able to use alternative treatments, such as involving the activation or deactivation of the ECS. Topical cannabinoids for itch and pain seem to be already on the online market, however, are not available as prescription medications from veterinarians yet.

ATOPIC DERMATITIS

In one way or another dermatitis and itching go together with each other and are often discussed together in the same context. Dermatitis is a universal term describing inflammation of the skin. Some forms of dermatoses occur with itch which sometimes seems to be unbearable for the animal.

As most skin diseases, dermatitis can be induced by various factors, such as allergens (allergic dermatitis), infections, atopic dermatitis, external compounds (contact dermatitis) or

caused by specific parasitic skin lesions: scabies, lice, arthropod mites, cercarial dermatitis (aka. summer's itch). Itching in such cases stops after the elimination of the invasion [67], [69]. Often, inflammatory dermatoses are accompanied by severe itching, and this special, mainly dermatological symptom only, further worsens the quality of life of veterinary patients, leading to secondary infection.

Atopic dermatitis (also called as "eczema" in humans) is a common allergic skin disease of complex etiopathogenesis. Immediate-type hypersensitivity to environmental allergens that arise as a result of environmental and genetic factors is a major part of the pathogenesis in most, but not all patients [69].

One of the chronic diseases with excruciating chronic itching in both humans and companion animals is atopic dermatitis, therefore it also feels to have a higher clinical significance. Most patients subjectively determine the severity of atopic dermatitis by the intensity of itching, which affects the quality of life, rather than only the appearance of rashes. Nowadays it seems research into the cause of pruritus in atopic dermatitis has deepened. Some researchers have found that in atopic dermatitis, the number of nerve fibers in the skin changes [73], [75], and it is also a well-known fact that mutations in FLG gene are common, which codes for the structural protein filaggrin that plays an important role in the pathogenesis of atopic dermatitis. Filaggrin binds keratin fibers in epithelial cells and it is essential for the correct function and formation of skin barrier [76]. It has also been proved that Th2 cytokines play a key role in driving these skin related inflammation processes and that many other chemokines also play a part in the inflammatory pathways of atopic dermatitis [77].

Many elements of atopic dermatitis contribute to pruritus including drying of the skin, histamine release and sensory nerve fiber hypertrophy. As we might have known from earlier, dry skin itself aggravates pruritus by causing release of pruritogenic mediators from keratinocytes.

Relationship between Skin Tissue and The Endocannabinoid System

As having mentioned in the beginning of this chapter, cutaneous tissue, as the largest organ of the body, is an important part of the pacemaker for maintaining homeostasis. Recent studies allow us to make a separate concept of the endocannabinoid system (ECS) in the skin [23], [63] and can be important in the previously mentioned inflammatory skin diseases, such as pruritus and atopic dermatitis, as cannabinoids are potentially antipruritic.

ECS is represented by cells in which (and from of which) various endocannabinoid ligands and, correspondingly, CB_1 and CB_2 receptors of various structures of the skin and its

appendages are formed, on which they act. The result of such an effect of agonism, are due to certain modes of action that are realized both in physiological and in pathological cases [23], [63]. The latter adds to the protective effect of ECS under the influence of unfavorable environmental factors (mechanical, radiation, chemical, microbial and other types of damage).

The key endocannabinoid ligands in healthy skin are AEA and 2-AG. The sources for the synthesis for these substances are mainly laminar-folded lipids of the epidermis and sebum (higher concentration of fatty acids) and phospholipids [36], [45].

There is a certain pool of these endocannabinoids in the skin, which, by binding to CB_1 receptors, CB_2 receptors or different isoforms of TRP inotrope receptors of epidermal cells, dermal fibroblasts, sweat glands, sebaceous apparatus, immunocompetent cells, and free nerve endings, cause the onset of specific effects. The specified pool of ligands involved, is self-regulating and replenished when needed [63], [67].

"C(UT)ANNABINOID SYSTEM"

The endocannabinoid system plays an important role in skin homeostasis to the extent that has been recently termed as "c(ut)annabinoid system". In the last decades, due to its clinical significance, increased attention, and therefore more research has been focused on the possible roll of cannabinoids in dermatology, and the term skin "endocannabinoidome" has been made, which influences:

1. skin general homeostasis [23]
2. epidermal permeability [78]
3. hair growth [79]
4. inflammation, dermatitis [80]
5. wound healing [81]
6. itch, pruritus [63]
7. pain [63]
8. skin tumours [82]

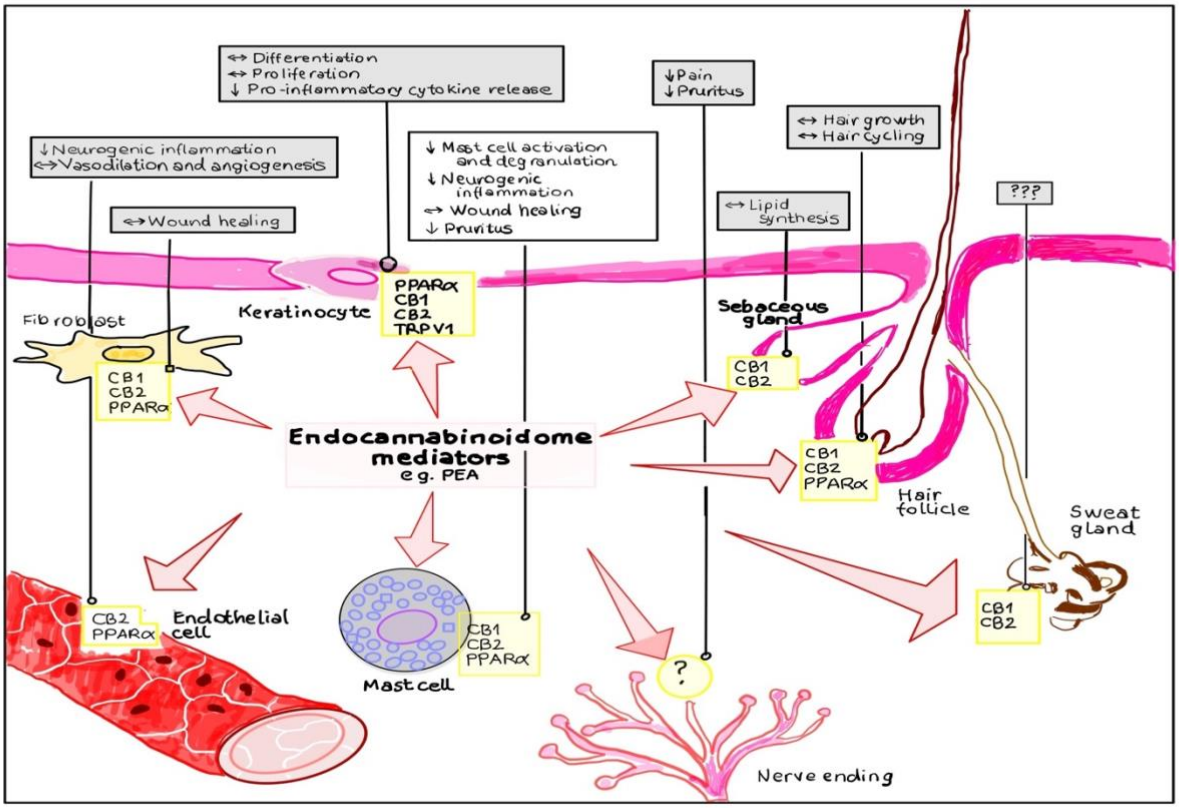

Figure 4. A simplified overview of the skin endocannabionoidome in domestic animals

To conclude it can be stated that a functional c(ut)annabinoid sysem exists in animals which could potentially extend the therapeutic area in veterinary field. A simplified overview of the skin endocannabinoidome in domestic animals is depicted in Fig 4.

TRP CHANNELS

TRP channels were first discovered in the fly eye, where light-activated rhodopsin stimulates phospholipase C (PLC), to hydrolyze a plasma membrane lipid, phosphatidyl-inositol bisphosphate (PIP_2). This in turn promotes gating of TRP channels to depolarize the photoreceptor cell.

Functional TRP channels consist of four subunits surrounding a central channel pore. In most cases, TRP channels are homo-tetramers of a given subunit, while others, subunits of two different TRP channel subtypes contribute to a heterotrimeric channel [83].

All known TRP channels are selective cation channels, with little or no anion permeability. Their relative selectivity for cations however may be different, thus most TRP channels are therefore, so called, “non-selective cation channels” that show permeability to

both monovalent cations (e.g.: Na^{+}) and divalent cations (e.g.: Ca^{2+}, Mg^{2+}) , a few however are highly selective for either calcium or sodium ions [83].

Cannabinoids as mentioned earlier, are ligands not only to CB_1 and CB_2 receptors but also to the TRP ion channels with individual differences in affinity and selectivity. These receptors have high expression in the skin, and are also present on terminal nerve endings of cutaneous fibers that extend to the dermis and epidermis [74].

TRP ion channels are a group of membrane proteins widely expressed in the skin and nervous system which mediate sensory responses such as nociception and pruritus, and play an important role in cutaneous nerve fiber activation and respond to various stimuli [83].

Most mammals express at least 28 different TRP channels which can be divided into six subgroups based on their primary amino acid structure, which all play roles in itch sensation and have been reported to interact with both endogenous and exogenous cannabinoids. [65]

1. TRP vanilloid : TRPV1, TRPV2 TRPV3, TRPV4
2. TRP ankyrin: TRPA1
3. TRP melastatin: TRPM1
4. TRP Canonical: TRPC1
5. TRP Mucolipin: TRPML1
6. TRP Polycystin: TRPP1

AEA behaves as a partial agonist at both CB_1 and CB_2 receptors, and also as an endogenous ligand for TRPV1, therefore it can activate both the endocannabinoid and endovanilliod systems [84].

Cannabinoid compounds disrupt neurogenic inflammation either through antagonism or stabilization of the ion channel of the closed confirmation, which prevents the neuronal activation by pruritic mediators [63].

PEA on the other hand - unlike AEA, or 2-AG- does not act directly at CB_1, CB_2 receptors nor on TRPV1 ion channel associated receptors, but it can significantly increase the effects of AEA, and also directly activates PPARα [85]. Cannabinoids can therefore modulate pain through activation of TRP channels and can induce anti-hyperalgesia in the peripheral nerves and alter perception after modification of other sensorial structures.

It seems that the effect of PEA and other endogenous cannabinoids on skin CB_2 receptors can somewhat control pruritus and are able to prevent the development of an inflammatory process, therefore may stop further tissue damage. If the nonspecific effects of

PEA are also considered as an indirectly potentiating antioxidant molecule, we could talk about a potentially new therapeutic and prophylactic approach in veterinary dermatology as well.

CANNABINOIDS AND OBESITY

INTRODUCTION

In parallel with the human obesity epidemic, there is increasing recognition that obesity is a common phenomenon in many domestic animal species as well. Moreover, the reasons that domesticated animals develop obesity are broadly like those reasons that have been attributed to obesity in humans.

Obesity is a disease of rapidly increasing prevalence in dogs and cats, with significant and often lifelong implications for animal welfare.

The American Veterinary Medical Association (AVMA) has recently approved a recommendation for a uniform definition, whereby obesity is present when a dog or cat is >30% above its ideal weight [86]. Body condition scoring (BCS) is the most used method to document adiposity in companion animals, and 30% above the ideal weight corresponds to a score of 8/9 using the preferred nine-point system; this is equivalent to a score of 4.5 using the five-point system with half units. Dogs and cats are considered overweight when their weight is more than 10–20% above their ideal weight, and their corresponding BCS is either 6/9 or 7/9 (3.5/5 and 4/5). Using BCS, 54% of dogs and 59% of cats in the United States are estimated to be overweight or obese, while a recent study in the United Kingdom classified 65% of adult dogs and 37% of juvenile dogs as overweight or obese [86].

In horses, significant interest is centered on the recognition that insulin resistance, a condition which can be triggered in obese patients, plays a role in the pathogenesis of laminitis, a potentially severe and debilitating cause of lameness in the equine species. Other equine medical conditions that are more likely in obese, insulin-resistant individuals include hyperlipemia (hepatic lipidosis) and developmental orthopedic disease (osteochondrosis). Furthermore pituitary pars intermedia dysfunction (equine cushing's syndrome) represents another common endocrinopathic condition of mainly geriatric horses associated with insulin resistance [87].

ENERGY BALANCE REGULATION

The constancy of body weight in a healthy individual is maintained, thanks to to the interaction and coordination of many central and peripheral mechanisms. The appetite-stimulating effects of marijuana on humans for example, have been known since ancient times.

Given that the focus of early scientific research was on the brain, it was assumed that cannabinoids have an extremely central effect on the regulation of body weight. A study by D. Cota et al. (2003) showed that the endocannabinoid system influences energy metabolism both through central orexigenic effects and through peripheral regulation of lipogenesis [88].

As currently established, endogenous cannabinoids regulate the energy balance in the body at key functional levels, which involve:

1. the limbic system (the hedonic effect of food),
2. the hypothalamus (integrative functions),
3. the gastrointestinal tract,
4. adipose tissue (Cota et al., 2003).

CENTRAL MECHANISMS OF ENERGY BALANCE CONTROL

Interpreting the results of early scientific studies which have been investigating the role of cannabinoids in the regulation of appetite and body weight, researchers have faced many difficulties due to the wide range of activities of various marijuana derivatives, dosages, and modes of administration.

In experimental animals, for example, the use of THC at a dose exceeding 10 mg/kg is accompanied not by an increase, but by a decrease in food intake [18]. As it turned out, large dosages lead to the manifestation of predominantly sedative properties of the drug, leveling orexigenic effects; the increase in appetite under the influence of cannabinoids was noted only in 3 out of 25 experimental studies.

With the standardization of research methods, it was proved that the activation of CB_1 receptors under the influence of phyto- and endocannabinoids stimulates food intake even in well-fed animals [89]. Prescription of the CB_1 receptor antagonist SR141716 (rimonabant) suppresses appetite and reduces body weight in experimental animals and humans. Mice with the knockout of the CB_1 gene, compared to controls, tend to eat much less food after a period of food deprivation [16], [90].

It has been observed that pharmacological blockade of CB_1 receptors in newborn mice using rimonabant on the first day of the postnatal period leads to the termination of milk sucking and starvation [91].

Injections of AEA into the mesolimbic and hypothalamic zones stimulate food intake in rats. In addition, levels of endogenous cannabinoids in these brain structures are increased

during fasting and decreased during meals. Such fluctuations are not registered in the parts of the central nervous system, which are not involved in the regulation of energy exchange [92].

To summarize these findings we can state, appetite control under the influence of cannabinoids is provided by a double mechanism either by the activation of the mesolimbic system, which is responsible for the emotional state (related with food hedonism), or via the interaction with hypothalamic structures that regulate energy balance. In the fallowing we shall dissect these two mechanisms further.

REGULATION OF APPETITE AT THE HYPOTHALAMIC LEVEL

The hypothalamus is the most important coordinator of energy metabolism. The arcuate nuclei of the hypothalamus contain two discrete pools of neurons. One pool of neurons producing NPY and AgRP stimulates appetite and increases body weight, i.e. has an anabolic effect, another pool of cells secreting POMC and CART, suppresses the processes of food consumption, i.e. has a catabolic effect. These groups of cells of arcuate nuclei primarily receive and transform information about the state of the energy balance coming from the periphery, and therefore they are referred to as first-order neurons. Both groups of neurons are associated with the paraventricular nuclei, ventromedial nuclei, dorsomedial, lateral, and perifornical regions of the hypothalamus, which contain second-order neurons that are also involved in the regulation of eating behavior and energy expenditure.

ENDOCANNABINOIDS POTENTIATING EFFECT ON THE LEPTIN PATHWAY

The functional relationship between the endocannabinoid system and leptin has been noted on many levels [93].

Leptin is a hormone secreted by adipocytes. In the arcuate nuclei of the hypothalamus, leptin inhibits the activity of NPY / AgRP -containing neurons and stimulates POMC / CART-producing neurons, which leads to suppression of appetite.

Leptin reduces food intake by increasing the release of neuropeptides that reduce appetite and by suppressing the release of factors that stimulate hunger. The decrease in leptin levels is accompanied by an increase in the levels of endocannabinoids in the hypothalamus [90], [94]. Leptin suppresses endocannabinoid synthesis by decreasing intracellular calcium and suppresses CB_1-dependent activation of MCH expressing neurons in the lateral hypothalamus. However, the effect of leptin is manifested only when the “pacemaker” for

restoring homeostasis is activated; otherwise (when the CB_1 receptor gene is knocked out), leptin does not reduce appetite in mice [90], [94].

There is an antagonism noted between leptin and glucocorticoids in the regulation of endocannabinoid synthesis in the PVN. Glucocorticoids through membrane receptor, trigger an endocannabinoid-mediated inhibition of synaptic excitation in the PVN, which allows a rapid decrease in the secretion of hypothalamic hormones. Leptin blocks the synthesis of endocannabinoids triggered by glucocorticoids [90], [94].

Relationship of Ghrelin and ECS

Ghrelin is another peptide hormone which is released mainly by the stomach, and in small amounts also by the small intestine, pancreas and brain.

Ghrelin receptors in the CNS are localized mainly in the arcuate, paraventricular, and ventromedial nuclei. When ghrelin is injected directly into the paraventricular nuclei in experimental rats, the amount of food consumed is doubled. The orexigenic effect of ghrelin is blocked by the administration of rimonabant at an extremely low dose, which does not affect appetite under normal conditions. These data indicate that the ECS is either directly or indirectly involved in the transmission of ghrelin signals [95]. Ghrelin promotes appetite via the paraventricular nuclei (PVN) and arcuate nuclei in the hypothalamus, both involved in appetite regulation. ECS and ghrelin work together to regulate energy balance. The action of ghrelin requires the appearance of AMPK in the PVN, which is realized through the activation of CB_1 receptors [96].

Activation of CB_1 receptors in the gastrointestinal tract may also be relevant for the pathogenesis of obesity. The response of ghrelin to fasting could be diminished with anorectic anti-obesity drug, also mentioned earlier, rimonabant, (an inverse antagonist of CB_1) suggesting that CB_1 receptors are involved in ghrelin secretion. [97].

AEA stimulates the synthesis and secretion of ghrelin in the stomach of rats. In normal-weight individuals, eating for pleasure is associated with increased levels of ghrelin and 2-AG [98] therefore also modulating the hedonic effects of food intake.

ENDOCANNABINOID SYSTEM'S ROLE IN THE REGULATION OF APPETITE

The endocannabinoid system as mentioned earlier plays a role in the development of eating disorders. It is a key modulator of the activity of brain regions responsible for reward processes -"hedonic food" [38], [97], [99].

One of the functions of the endocannabinoid system is participation in the regulation of metabolism. In pathological conditions, an increase in the tone of the endogenous cannabinoid system contributes to the development of obesity and associated different metabolic diseases [44]. Signaling of the endocannabinoid system is thought to be evolutionarily beneficial for survival in times of food shortages. It is also important to mention that the signal of the endocannabinoid system is enhanced by both hunger and excess nutrition.

This paradox explains the role of the endogenous cannabinoid system in both the initiation and endpoint of obesity [44]. It has been proved that CB_1 receptor is probably the best potential pharmacological target through which the ECS can be modulated in metabolic disorders and obesity. As discussed earlier in the first chapter, CB_1 receptor is one of the most expressed G protein-coupled receptors present in the brain, and can be found on axons, nerve terminals of neurons, and on astrocytes as well. In the brain, endocannabinoids are released immediately after being synthesized from cell membrane phospholipids from postsynaptic neurons, and activate CB_1 receptors presynaptically, in a retrograde manner, and modulate the release of different neurotransmitters such as GABA, glutamate, serotonin, noradrenaline, serotonin etc. [45], [62].

CB_1 receptors are also located on peripheral nerves, including the nerves that innervate the gastrointestinal tract, as well as on different organs such as adipose tissue, pancreas, liver etc. [88], [100], [101].

There is a positive correlation between the number of receptors in peripheral tissues and the degree of obesity. Obesity is generally associated with elevated plasma and adipose tissue originated endocannabinoid levels. It has been shown that the plasma level of 2-AG is increased in patients with visceral obesity and negatively correlates with insulin sensitivity. In addition, increased levels of both central and peripheral endocannabinoid receptors are found in obesity. For example, levels of endogenous cannabinoids and CB_1 receptors are elevated in nutritional obesity [44]. This is likely due to the higher availability of endogenous cannabinoid precursors and the dysfunction of their catabolism. It is believed that the expression of CB_1 receptors in the mesolimbic system is directly related to the motivation to consume food in response to reward.

For example, the increased appetite following cannabinoid consumption could be likely due to the rewarding effects of food intake and is the result of excessive dopaminergic transmission. That is why obese patients are more likely to develop depression than those with normal body weight, which significantly worsens the prognosis [40], [94], [102].

In the CNS, endocannabinoids act as retrograde neuromodulators, that is, they inhibit the release of excitatory and inhibitory neurotransmitters through presynaptic CB_1 receptors and by doing so, they modulate neuronal activity, including in the parts of the brain responsible for the regulation of energy balance: the hypothalamus, the trunk, the cortico-limbic system - the NA, and the VTA [40], [102].

It has been shown that the orexigenic or anorexigenic effect of endocannabinoids depends on the properties of the neuron on which the presynaptic CB_1 receptors are located.

The orexigenic effect of CB_1 receptor agonists on the body indicates a predominant inhibition of glutamatergic synapses. Endocannabinoids inform about instant changes in energy balance, as they are synthesized on-demand. Their concentration in the structures of the brain increases during fasting and decreases when the need for food is satisfied.

How Cannabinoids can potentiate alimentary hedonism

Both in animals and humans, under the influence of cannabinoids, the motivation for eating especially "tasty" food increases. The meaning of "tasty", varies amongst the different animal species: for carnivores it can be high protein content-activation of the so called, "umami" taste that can be attractive, while for herbivores it can be the high carbohydrate content.

Eating, as a form of pleasure, is a powerful orexigenic stimulus even in the absence of energy deficit in the body. Feelings of pleasure, satisfaction, and positive reinforcement are provided through the complex interaction of many signaling systems: opioid, dopaminergic, serotonergic signaling.

The endocannabinoid system is involved in behavioral responses associated with the activation of the gratification/reward system. This is confirmed by the high level of expression of CB_1 receptors in the corresponding parts of the brain. The gratification/reward system in mammals is represented by several synaptically interconnected brain structures, including the medial anterior brain bundle, the ventral tegmental region, the nucleus accumbens, and the globus pallidus [102]. These structures are involved in the processes of obtaining satisfaction from physiological stimuli aimed at the survival of the species (food intake, sex), and are also a neural substrate for developing addiction [102].

The most important morpho-functional basis of the satisfaction system is the mesolimbic dopaminergic system. It has been shown that the level of dopamine in the nucleus accumbens is increased when consuming appealing tasty food, while dopamine antagonists can reduce appetite [103] .

The presence of cannabinoids and CB_1 receptors was noted in the same zones of the forebrain limbic system, where the D_1 and D_2 dopamine receptors are concentrated. Psychoactive substances (marijuana, alcohol in humans), as well as stimuli of pleasure from eating tasty food, induce the production of dopamine. Considering the presence of a close correlation between the concentrations of dopamine, endocannabinoids, and the addiction to some sort of pleasant food, a hypothesis has been proposed that there is a relationship between the cannabinoid and dopaminergic systems in the regulation of eating behavior [104].

Cannabinoids have the capability to enhance the sense of pleasure in eating by increasing the release of dopamine in the NA. Activation of dopaminergic neurons found large number in the VTA- the center of the mesocorticolimbic circle, that plays a significant role in reward, motivation, and aversion- are mediated by the action of endocannabinoids on CB_1 receptors on glutamatergic terminals, which inhibit GABAergic neurons projecting from NA to VTA and by doing so, disinhibit dopaminergic neurons in the VTA [102].

ECS POTENTIATING FOOD INTAKE VIA GUSTATORY AND OLFACTORY PATHWAYS

Chemosensory stimuli (coming via the olfactory nerve or via gustatory perceptions) can also contribute to food hedonism, which either encourage or discourage eating. Endocannabinoid receptors colocalize with receptors responsible for different tastes, on the papillae of the tongue and enhance the sensory pleasure of foods. Endogenous cannabinoids furthermore also play a role in this processing of taste sensation in the CNS.

The gustatory sensation, carried by afferent fibers from the facial (VII), glossopharyngeal (IX) and vagal (X) nerve, are carried into the solitary nucleus (nucleus tractus solitarii), or in case of murine rodents and many other animals to the PBN (parabrachial nucleus). Afterwards the taste pathway bifurcates into two branches: thalamic branch and limbic branch.

Taste sensations while being processed in the PBN and/or the NTS integrate with signals from the gastrointestinal tract. The processed information determines the amount of food consumed and the intervals between meals. By stimulating CB_1 receptors in PBN, endocannabinoids increase the absorption of palatable food [44], [105]. An increase in food intake can also be achieved by increasing the concentration of endocannabinoids, that activate

CB_1 receptors in the terminals of the axons of the olfactory cortex, and inhibit granular cells in the olfactory bulb, which altogether increases the sensitivity to pleasant odors. Endocannabinoid signaling in the olfactory bulb is activated in fasting and by suppressing glutamatergic transmission, has the potential to encourage further food intake [106].

OBESITY TREATED AS AN INFLAMMATION

Obesity is the accumulation of abnormal or excessive fat that may interfere with the maintenance of an optimal state of health. The excess of macronutrients in the adipose tissues stimulates the adipose tissue to release inflammatory mediators [107].

The precise triggers of obesity-associated inflammation are poorly understood. Several potential mechanisms have been suggested, including intestinal antigens, various dietary components or metabolites, as well as signals associated with adipocyte death, hypoxia, mechano- transduction resulting from interactions between the cell and the extracellular matrix etc., [107]. Sustained inflammation is considered a strong risk factor for developing many diseases including, metabolic syndrome, diabetes, and cancer. As a risk factor, obesity predisposes to a pro-inflammatory state via increased inflammatory mediators IL-6 and TNF-α, and reduced levels of adiponectin, which has an anti-inflammatory function. TNF-α is overexpressed in the overweight state, while IL-6 is linked more to the obese state that influences the liver to synthesize and secrete CRP, which is a feature of systemic inflammation [108].

The facts listed presented above, show a complex relationship between different body condition levels and the concurrent activation-hyperactivation of the ECS. The current understanding of the activity of the endocannabinoid system in obese individuals is as follows: obesity is associated with adipose tissue-specific changes in gene expression that increase the synthesis of endogenous cannabinoids and reduce the degradation of endogenous cannabinoids, but dietary fat intake is not a mediator of these changes. That is why there is a need not only to correct the diet, but also to use drugs that affect CB_1 receptors [97], [109].

Clinical application, however, both in human and in veterinary medicine seems to be a long way off still: the methods of isolation and measurement of endocannabinoid concentrations have not been standardized, reference levels, and the influence of age, sex, interspecies differences, and influence of diseases on their values have not yet been established, which still awaits to be done and standardized.

CONCLUSION

To date, it can be stated, there is not enough scientific literature about the importance or the treatment protocol of using cannabinoids in veterinary patients, indicating to this group of molecules waiting to be used. The reason behind this situation is difficult to explain perhaps the main cause also relies on the facts, related to the use and abuse of cannabinoids in humans by itself and the stigmatization around the misuse of this drug in humans.

Cannabinoid receptors are extremely important for the normal functioning of the immune system, and maintaining homeostasis, but the exact mechanism of their action can hardly be called as generally known information. While it is starting to become more known, that cannabinoids have medical potential in various forms of inflammatory conditions, however the exact physiological processes that positively affect this field are not yet fully understood.

To present date it seems we still do not fully know the exact pharmacokinetics therefore all the effects of endocannabinoids on the body in the context of the phytocannabinoids from hemp or synthetic cannabinoids. From various studies it could be seen that targeting CB1 and CB2 receptors have clinical benefits, without doubt. It has also been noted and documented that receptor gene structure, and expression patterns differ between different animal species. These subtle differences between rodent and human disease models should be kept in mind as probably would have significance on canine and feline therapies.

In skin tissue it can be said that it forms a key part of the so called “c(ut)annabinoid” system. The biosynthetic and catabolic pathways of endocannabinoids are shared with other bioactive lipid mediators, making it become an extended endocannabinoid system, or so called, endocannabinoidome.

A great deal of evidence seems to exist showing a consistent improvement of skin health following dietary supplementation or topical use of PEA, in either experimental or in companion animals, and in the future, it would seem likely veterinary interest using products acting through the endocannabinoidome, including phytocannabinoids will continue to increase.

More research and clinical data however are still needed, but due to the relatively favorable safety profile of the active ingredients and their potential benefits, it seems appealing to incorporate them into clinical practice in various fields such as dermatology, or cases where modulating the metabolism, or immune system is needed, and could potentially be used in different autoimmune and neoplastic diseases as well. A better understanding, however, would

help us use cannabis more consciously to effectively regulate ECS while optimizing its beneficial effects.

The real progress however I believe would be to reach the level to establish treatment protocols for these diseases mentioned above, as probably we will only know its full potential after decades of use in clinical practice.

SUMMERY

The endocannabinoid system (ECS) is a network of receptors that are distributed throughout our body. Endocannabinoids affect physiological functions, consciousness and animal behavior. The body produces them naturally, and then they bind to so called 'pacemaker' receptors to regulate physiological processes and the functioning of the nervous system.

The pacemaker helps manage functions such as immunity, appetite, sleep cycles, pain response, etc. However, when endocannabinoid deficiency occurs, the body loses its stable state and ceases to function normally. In many cases, the use of plant cannabinoids (phytocannabinoids) helps restore balance and restore normal functioning.

Cannabinoid receptors themselves are a class of G-protein coupled receptors. They function as activators of intracellular signal transduction pathways, form part of the endocannabinoid system and are responsible for a wide range of physiological functions, as well as general homeostasis.

Cannabinoid receptors are activated by three main groups of molecules. These are endocannabinoids, which are produced by our own body, phytocannabinoids, which come from plants, and synthetic cannabinoids.

Currently, two subtypes of cannabinoid receptors are known and studied: CB_1 and CB_2, although many more are believed to be. They are found in virtually all parts of the body and are present in all mammals, fish, birds and reptiles. The most developed endocannabinoid system can be found in mammals. Each type of subtype of CB receptors found in different parts of the body has a different purpose.

CB_1 receptors are concentrated mainly in the brain (central nervous system, CNS), as well as in the lungs, liver and kidneys. Endocannabinoids bind to CB_1 receptors on presynaptic (mainly) glutamatergic and GABAergic neurons, resulting in decreased release of glutamate and GABA (gamma-aminobutyric acid). Limiting the release of glutamate induces relaxation, while limiting the release of GABA activates postsynaptic cells, which in turn affects memory and learning ability.

One of the main tasks of CB_1 is to maintain **homeostasis** (maintaining a stable state of the body through feedback), and by doing so its modulation plays an important role in the treatment of various diseases such as obesity, different skin related diseases and auto-immune system disorders. It can be stated that by acting on these receptors, it is possible to reduce anxiety, minimize pain, and thus to treat multiple inflammatory symptoms.

CB_2 receptors are found mainly in the immune system and hematopoietic cells. According to current research, by inhibiting adenylate cyclase, CB_2 receptor agonists cause a decrease in intracellular levels of cyclic adenosine monophosphate (cAMP). In simple terms, this means that receptors are associated with various functions of the immune system - regulation or suppression of immunity, induction of apoptosis, and cell migration.

CB_2 receptors may also play an important role in therapy. Changes in CB_2 receptors have been found in a variety of diseases that occur in multiple species. However more research is needed on this issue, in theory, CB_2 receptor activity could be a factor in the treatment of a range of disorders - gastrointestinal, bone, skin, autoimmune, pain syndromes, and even neoplastic lesions.

One of the main conclusions and 'take home message' from these findings, is that the body is composed of complex systems, where the interactions and signal transduction pathways are more compound than one might first think or assume.

Ultimately, the goal of all functions of a biological organism is by removing inflammation, pain, etc. **return to homeostasis** and, so far it seems, cannabinoids can play an important role in achieving this goal. This is done thanks to a complex and highly interconnected endocannabinoid system and the cannabinoid receptors with which it interacts.

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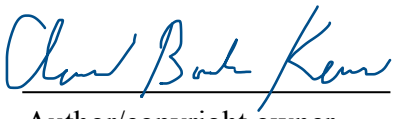

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Thesis progress report for veterinary students

Name of student:.....Claudia Barbara Kemenesi.....

Neptun code of the student:.....D59V4T.....

Name and title of the supervisor:....Dr. Dávid Sándor Kiss, PhD, associate professor.....

Department:.....Department of Physiology and Biochemistry.....

Thesis title:... Central and Peripheral Cannabinoid Receptors and their Roles in Different Situations of Inflammation .....

.....

Consultation – 1st semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2021	09	22	Consultion on general overview and chapters of the thesis	
2.	2021	10	08	Refinement of Thesis Structure	
3.	2021	10	20	Pages need to be added	
4.	2021	11	03	Additional advice on Reference handling	
5.	2021	11	23	Fixing coherency issues	

Grade achieved at the end of the first semester: .....5.....

Consultation – 2nd semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2022	02	22	Editing and format correction	
2.	2022	03	09	Overview too short	
3.	2022	04	07	Figure/diagram editing	

4.	2022	04	19	Conclusion too short	
5.	2022	05	03	Thesis final proofread and confirmation	

Grade achieved at the end of the second semester: .....5.....

The thesis meets the requirements of the Study and Examination Rules of the University and the Guide to Thesis Writing.

I accept the thesis and found suitable to defence,

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signature of the supervisor

Signature of the student: .....

Signature of the secretary of the department: .....

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