Review

The Endocannabinoid System of Animals

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Simple Summary: The recent discovery of the Endocannabinoid System, and its ubiquitous presence in nearly all animals, has opened the door to novel approaches targeting pain management, cancer therapeutics, modulation of neurologic disorders, stress reduction, anxiety management and inflammatory diseases. Endocannabinoid-related molecules, both endogenous and exogenous, are able to function as direct ligands or otherwise influence the ECS. This review article introduces the reader to the endocannabinoid system in animals, and documents its potential as a source for emerging therapeutics.

Abstract: The endocannabinoid system has been found to be pervasive in mammalian species. It has also been described in invertebrate species primitive as the Hydra. Insects apparently are devoid of this otherwise ubiquitous system that provides homeostatic balance to the nervous and immune systems, as well as many other organ systems. The endocannabinoid system (ECS) has been defined to consist of three parts: 1. Endogenous ligands, 2. G-protein coupled receptors (GPCRs), and 3. Enzymes to degrade and recycle the ligands. Two endogenous molecules have been identified as ligands in the ECS to date. These are the endocannabinoids: Anandamide (arachidonoyl ethanolamide) and 2-AG (2-arachidonoyl glycerol). Two G-coupled protein receptors have been described as part of this system, with other putative GPC being considered. Coincidentally, the phytochemicals produced in large quantities by the *Cannabis sativa* L plant, and in lesser amounts by other plants, can interact with this system as ligands. These plant-based cannabinoids are termed, phytocannabinoids. The precise determination of the distribution of cannabinoid receptors in animal species is an ongoing project, with the canine cannabinoid receptor distribution currently receiving the most interest in non-human animals.

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1. Introduction

Common to nearly all animals except the Phyla *Protozoa* and *Insecta*, the endocannabinoid system arose in the phylogeny concurrently with the development of the nervous system as multicellular animals developed increasing complexity. This system was unknown to scientists until the mid-1990's, but research into this fascinating and clinically useful system is advancing rapidly, especially with the use of state-of- the-art LC-MS analyzers, and immunohistochemical and polymerase chain reaction (PCR) analytic technologies.

For over 70 years scientists have been hobbled by the legal and regulatory prohibitions related to research into cannabis and its associated molecules. Phytocannabinoids from *Cannabis sativa* L. and the naturally occurring endocannabinoids can serve as ligands in the ECS.

2. The Endocannabinoid System

The endocannabinoid system (ECS) has been identified in nearly all animals, from complex mammals like primates to phylogenetically primitive animals such as the cnidarians. The near universal presence and early emergence of the ECS, evolutionarily, is a strong indicator of its biological importance. Cannabinoid receptors are expressed in most animals, including vertebrates (mammals, birds, reptiles, and fish) and invertebrates (sea urchins, leeches, mussels, nematodes, and others).

The most primitive animal with an ECS is the Hydra (*H. vulgaris*), a cnidarian in the class Hydrozoa, which is the first animal to develop a neural network. A study of the ECS in the Hydra determined

that the major function of the ECS in this primitive organism is to control its feeding response [1]. It is evident from this data that all veterinary species contain an ECS. Therefore, an understanding of the ECS in these species is critical to the development of clinical applications for endocannabinoids and the phytocannabinoids, terpenes, and flavonoids derived primarily from *Cannabis sativa* L.

The ECS was discovered during the search for the biological targets for the recently described structure of the psychotropic phytocannabinoid, -Δ-9-tetrahydrocannabinol (THC) [2]. THC is the only psychotropic cannabinoid found in *Cannabis sativa* L and is responsible for some of its biomedical activity along with the non-psychotropic cannabinoids such as cannabidiol (CBD), Cannabigerol (CBG), Cannabichromene (CBC), other minor cannabinoids, terpenes and flavonoids.

Research sponsored by the National Institute on Drug Abuse in Bethesda, MD led to the discovery of the GPCR which was named CB_1. Two years following the discovery of the CB_1 receptor, Makriyannis characterized a second GPCR named CB_2 [3]. Both the CB_1 and the CB_2 receptors play important roles in many essential biological processes, including neuronal plasticity, pain, anxiety, inflammation in general and especially neuroinflammation, immune function, metabolic regulation, reward, craving and bone growth [4].

Following the discovery of the membrane receptors that accept plant-based ligands, researchers quickly identified the endogenous ligands that bind to the cannabinoid receptors (CBR). These endogenous ligands are the endocannabinoids (eCB), arachidonoyl ethanolamide (AEA), a long-chain fatty acid amide, was described in 1992, and was named: “Anandamide” by Mechoulam, after the Sanskrit “Ananda” meaning bliss, as this endogenous molecule is responsible for feelings of well-being. The ester of this fatty acid amide, 2-arachidonoyl glycerol (2-AG), was discovered shortly thereafter in 1995 [5-7]. Both of these compounds are able to activate both CB_1 and CB_2 receptors, and were found to produce effects similar to THC, which is the only phytocannabinoid that binds orthosterically to the cannabinoid receptors.

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eCBs are produced ad hoc by enzymes within the cell membrane that are activated by calcium ion elevation. The level of endocannabinoids is termed the "Endocannabinoid tone" and that level varies based on the specific tissue in which they are found. These levels are dependent upon their rate of production minus the rate of their enzymatic degradation by another set of enzymes, fatty acid amide hydrolase (FAAH) and monoacyl-glycerol lipase (MAGL). Respectively, these enzymes deactivate AEA and 2-AG.

By definition, the endocannabinoid system consists of these three parts:

1. Endogenous ligands
2. Membrane receptors
3. Deactivating enzymes

The regulation of endocannabinoid tone is modulated by a transport mechanism that carries released eCBs retrograde from the post-synaptic cell membrane to bind with the cannabinoid receptors present on the pre-synaptic membrane, and then carry them back to the post-synaptic neuron for degradation by their respective enzymes, FAAH or MAGL.

Endocannabinoids act on the presynaptic endocannabinoid receptors following their release from the post-synaptic neuronal membrane. The eCBs modulate neurotransmitter release by inhibiting the influx of intracellular calcium which in turn inhibits the release of neurotransmitters. eCBs undergo rapid re-uptake by the cells and are then degraded. Their half-life is quite brief. Following release, they undergo rapid reuptake by the cell and are then degraded. The production of eCBs can be stimulated by injury or excessive neuronal discharges [8]. ECS signaling comes in two forms—tonic and phasic. Tonic signaling establishes eCB tone or a basal level of signaling, while phasic signaling involves changes over time of eCB levels.

Omega-3 fatty acids are essential for the proper regulation of the ECS tone, since polyunsaturated fatty acids feed directly into the eCB signaling pathways [9]. These studies into the ECS were able to characterize the two cannabinoid receptors, the proteins that modulate their function and the eCB

family of compounds that encompass the ECS system. The ECS plays a major role in the regulation of many aspects of animal physiology. Today we know that the CB1 cannabinoid receptor is the most abundant GPCR in the human brain but is also present in many other organs such as the heart, blood vessels, liver, lungs, and the digestive system, as well as fat and sperm cells [10].

The CB1 receptor belongs to the Class A rhodopsin-like family of GPCRs. It is found primarily in the central nervous system with concentrations found in the cortex, hippocampus, outflow of the basal ganglia, and cerebellum. There can be both intra- and interspecies differences in the anatomical location of cannabinoid receptors in the ECS. It's important to note that CB1 in humans is not prevalent in the brain stem or medulla oblongata, the organs responsible for controlling vital autonomic functions such as breathing and heartbeat. This is a strong contributing factor to the safety profile of cannabinoids in humans and the main reason that it is nearly impossible to overdose on THC [11].

For dogs this is not true. Government studies in the 1970's determined that dogs have a very high density of cannabinoid receptors in the cerebellum and brain stem and medulla oblongata [12]. This concentration of CB receptors in the cerebellum explains "Static Ataxia", the neurologic reaction to THC that is specific to dogs naïve to THC. Static ataxia was first described in the literature by Dixon in 1899 [13]. Cannabinoid receptors are found to a lesser extent in the periphery of cardiovascular, immune, gastrointestinal, and reproductive tissues. CB2 receptors are located primarily in immune cells, among them leukocytes and those of the spleen and tonsils [14]. The CB1 and CB2 receptors share a significant degree of homology despite being located primarily in the CNS and immune system, respectively.

One of the functions of cannabinoid receptors in the immune system is the modulation of cytokine release. Activation of B- and T- cell CB2 receptors by cannabinoids leads to inhibition of adenyl cyclase in these cells and to a reduced response to immune challenge [15]. Both CB1 and CB2 are cause a decrease in adenyl cyclase activity and the cAMP pathway. They also stimulate mitogen-activated protein kinase (MAPK) cascades, modulate ion channels, and modify intracellular

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calcium levels and subsequent neurotransmitter release [16-19]. Potassium channel activation can also serve as a signaling mechanism for the CB2 receptor [20,21].

Cannabinoid action is not limited to signaling outside of the cell. Fatty acid-binding proteins (FABP) are essential for the transport of cannabinoids into the cell. Once inside the cell they bind to cannabinoid receptors on the outer mitochondrial membrane and have several pathways: 1. Recruit nuclear transcription factors which modify gene expression [22] and 2. Regulate neuronal energy metabolism [23]. CRIP1a, another cannabinoid signaling protein, inhibits constitutive eCB signaling [24]. Mitochondrial CB1 receptors modify cellular respiration through inhibition of soluble adenyl cyclase and reducing activity in the electron transport chain. Mitochondrial receptors may also play a role in the pro-apoptotic mechanisms of cannabinoids upon cancer cells, via the release of ceramide and its role in creating ER stress, leading to autophagy and if the cell cannot correct itself, apoptosis ensues [25].

3. Veterinary ECS: Our Current State of Knowledge

Most of what we know about the medical and health benefits of cannabis relates to humans and not animals. Many of the biological interactions of the endocannabinoid system occur across most of the animal species. Therefore, describing the medical and health benefits of cannabis as relates to humans, are starting points for evaluating cannabis as a therapeutic agent in veterinary species.

The CB1 receptor is highly conserved across all mammalian species, but there are significant primary sequence differences that have been discovered between the human and rat cannabinoid CB2 receptors and the newly cloned canine cannabinoid receptor, CB2. It was found that the binding affinities for canine CB2 receptor were 30 times less than those measured for human and rat CB2 receptors. The functional properties of the cannabinoid CB2 receptor are highly dependent upon the level of receptor expression and the nature of the selected signaling pathway [26].

3.1 Anatomical Localization of Cannabinoid Receptors in the Dog

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3.1.1 Cannabinoid Receptor 1

Immunohistochemistry was used to anatomically localize the CB1 receptor in the normal canine nervous system. Nervous systems from a healthy 4-week-old puppy, three 6-month-old dogs, and one 10-year-old dog were evaluated. Strong "dot-like immunoreactivity" was found in the neutrophils of the cerebral cortex, cornu ammonis (CA), and dentate gyrus of the hippocampus, midbrain, cerebellum, medulla oblongata, and gray matter of the spinal cord. Dense CB1 expression was found in fibers of the globus pallidus and substantia nigra surrounding immunonegative neurons.

Astrocytes were consistently positive in all examined regions. In the PNS, CB1 immunohistochemistry stained neurons and satellite cells of the dorsal root ganglia and myelinating Schwann cells in the PNS.

The younger dog in this study had lower CB1 expression in the brain. The density of receptor expression in human fetal and neonatal brain tissue was greater when compared to the younger dog examined. Lower CB1 expression has been found in aged rats in specific regions, most prominently in the cerebellum, cerebral cortex, and basal ganglia and less prominently in the hippocampus. This reduction in CB1 density with age in these rats was consistent with the findings in the older dog examined in this study [27]. Previous studies have identified CB1 receptors in salivary glands [28], hair follicles [29], skin, and hippocampus in dogs [30].

Immunohistochemistry was used to study the localization of CB1 receptors on developing canine embryo (30 days old) with a commercially available antibody. CB1 receptor immunoreactivity was found primarily in epithelial tissues and included most structures of the central and peripheral nervous system, inner ear, olfactory epithelium and related structures, eye, and thyroid gland [30].

3.1.1.2 Canine CB_1 Receptor Localization

- Cytoplasm of basal and suprabasal layer cells
- Hair follicle inner epithelial root sheaths and arrector pili muscles
- Undifferentiated sebocytes at the periphery of sebaceous glands
- Mast cells and fibroblasts
- Upregulated in atopic dermatitis

3.1.2 Cannabinoid Receptor 2

Clinically normal dogs have a homogeneous distribution of CB1 and CB2 receptors in all epidermal layers. This is different than in human epidermis where the CB1 receptor is mainly detected in epidermal spinosum and granulosum layers CB2 is detected mainly in basal keratocytes. Both CB1 and CB2 receptors have been found in the skin of healthy dogs and dogs with atopic dermatitis. The epidermis of dogs is thinner than that of humans (2–3 nucleated layers in the dog versus 6–7 in the human), which might account for this difference. Hyperplastic epidermal changes were observed in dogs with atopic dermatitis. Strong CB1 and CB2 immunoreactivity was identified in suprabasal keratinocytes. Weak CB1 and strong CB2 immunoreactivity was found in basal keratinocytes indicating upregulation of these receptors during inflammation. CB1 and CB2 agonists decrease mast cell degranulation. To summarize, cannabinoid receptor localization on the skin of the dog was found in the cytoplasm of epidermal and follicular keratinocytes, sweat and sebaceous gland epithelial cells, and the mesenchymal dermal cells [31].

3.1.2.1 Canine CB2 Receptor Localization

- Epidermis.
- Cytoplasm of cells in the basal and suprabasal layers.
- Hair follicles in the basal and suprabasal cells of the outer and inner epithelial root sheaths.

- Mild immunoreactivity in cells of arrector pili muscles and secretory and ductal cells of sweat glands.
- Sebaceous glands in the cytoplasm and peripheral reserve cells.
- Mast cells, fibroblasts, and endothelial cells.
- Lymph nodes.
- Strong B-cell zone immunoreactivity mainly in germinal centers of secondary follicles.
- Upregulated in atopic dermatitis.

3.2 Invertebrate ECS

The two cannabinoid receptors, CB1 and CB2, have been found in mammals, birds, reptiles, and fish. In a study of seven representative species of invertebrates, McPartland used tritiated ligand binding assays to characterize the cannabinoid receptors in *Ciona intestinalis* (Deuterostomia), *Lumbricus terrestris* (Lophotrochozoa), *Peripatoides novae-zealandiae* (Onychophora), *Jasus edwardi* (Crustacea), *Panagrellus redivivus* (Nematoda) [the beer mat nematode], *Actinothoe albocincta* [white striped anemone] (Cnidaria), and *Tethya aurantium* (Porifera) [Orange Puffball sponge] [32].

Cannabinoid binding was detected in all species studied except for the sea anemone (*A. albocincta*) and sponge (*T. aurantium*). The receptors were consistent with CB1 receptors but not CB2 receptors. Three of the organisms tested, earthworm (L. terrestris), velvet worm (*P. novae-zealandiae*), and mat nematode (*P. redivivus*), were compared to a standard CB1 ortholog in rat cerebellar tissue. A high affinity binding interaction was observed at various concentrations characteristic of CB1 receptors.

The authors of this study hypothesize that cannabinoid receptors evolved in the last common ancestor of bilaterians, with secondary loss in insects and other clades. After conducting a systematic literature review, the authors found that cannabinoid receptors have been identified in sea urchins,

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leeches, earthworms, hydra, lobster (*H. americanus* and *J. edwardi*), and the beer mat nematode (*P. redivivus*), but not the nematode (*C. elegans*). No binding was observed in sponges (Porifera).

In a separate study, McPartland found that insects (*Apis mellifera* [western honey bee]), *Drosophila melanogaster* [common fruit fly], *Gerris marginatus* [water strider], *Spodoptera frugiperda* [fall armyworm moth larva], and *Zophobas atratus* [darkling beetle]) are devoid of cannabinoid receptors. This loss of CB receptors is unique to comparative neurobiology, in that no other known mammalian neuroreceptor has been found to be missing in insects (Ecdysozoa). The authors suggest that the lack of cannabinoid receptors in insects is due to their lack of ligands, in that insects produce little or no arachidonic acid, the precursor to the biosynthesis of endocannabinoids [33].

3.3 The Endocannabinoid System and Disease [11]

Most of the work that has been done to delineate the effect of the endocannabinoid system on various diseases, has focused on the human animal and experimental models with laboratory animals. The assumption can be made that comparable benefits can be achieved in veterinary species via comparable mechanisms of action. Clinical studies are needed in veterinary species to further delineate applications for phytocannabinoids and for endocannabinoid molecules.

3.3.1 Modulation of Anxiety and Stress

Deficiencies in eCB signaling have been implicated in the etiology of a variety of conditions including PTSD, migraine, and fibromyalgia. Circulating levels of eCBs have been found to be markedly decreased in these disorders. The decline in circulating eCBs is correlated with anxiety-like behaviors. Chronic environmental stress leads to a down-regulation of CB_1 receptors combined with reduced levels of AEA but increased levels of 2-AG [34] .

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CBD in animal models was shown to have anxiolytic properties. Healthy human subjects who had to perform a stressful public speaking test (SPST), were given a 300 mg dose of CBD isolate which reduced their subjective anxiety comparable to anxiolytic benzodiazepam [35]. CBD has also been shown to ameliorate some of the undesirable effects of THC and when administered concurrently helps to temper the psychoactivity of THC.

3.3.2 Modulation of Inflammatory Conditions

Inflammation is a common condition that underlies the development and progression of many diseases and health conditions. The ECS has been shown both *in vivo* and *in vitro* to be involved in regulating the immune system through its immunomodulatory properties. Cannabinoids have been found to play a key role in several experimental models of autoimmune disorders such as multiple sclerosis, rheumatoid arthritis, colitis and hepatitis.

The cannabinoid receptor CB_2 is primarily found on the surface of immune cells. The primary function of these receptors is to modify the inflammatory response. These receptors protect the host from the pathogenesis of these conditions through the induction of multiple anti-inflammatory pathways such as suppression of T-cell-mediated immune responses. They induce apoptosis of T-cells and suppress pro-inflammatory cytokines and chemokines and also inhibit T-effector cell proliferation at the same times as stimulating proliferation of T-regulatory cells.

The cannabis plant contains many molecules that reduce inflammation. THC and CBD both have strong anti-inflammatory properties, while CBC, CBG, and THCV have also demonstrated anti-inflammatory properties. The apoptotic mechanism of phytocannabinoids upon immune cells is to activate of CD95 which induces both Bcl-2 and caspase cascades. Cannabinoids have also been demonstrated to promote the production of anti-inflammatory interleukins such as IL-10 while

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inhibiting the production of pro-inflammatory cytokines such as TNF-α in a CB_1 - dependent fashion [36].

Non-steroidal anti-inflammatory drugs (NSAIDs) produce their anti-inflammatory response through interactions with the ECS. Acetaminophen is metabolized in the liver resulting in *N*-arachidonoylphenolamine (AM-404) which acts as both a cannabinoid receptor agonist and eCB reuptake inhibitor [37]. These interactions also block the conversion of arachidonic acid into inflammation and pain promoting prostaglandins [38].

Terpenes can also have anti-inflammatory activity. Among the terpenes, α-pinene, β-myrcene, β-caryophyllene bind to the prostaglandin receptors (PGE1 and/or PGE2) to produce an anti-inflammatory effect. β-caryophyllene is the only terpene known to bind to cannabinoid receptors thus attenuating inflammation in a CB_2 - receptor dependent fashion [39].

3.3.3 Modulation of Pain

Endocannabinoids modulate neural conduction of pain signals by both reducing the nociceptive neural signal of pain, and by reducing inflammation by means of activation of cannabinoid receptors, either by endogenous ligands or Δ-9-THC.

CB_1 receptors modulate neurotransmitter release in the brain and spinal cord. CB_1 receptors are also present in nociceptive and non-nociceptive sensory neurons of the dorsal root ganglion and trigeminal ganglion, as well as macrophages mast cells and epidermal keratinocytes.

CB_2 receptors are found in cells of hematopoietic origin. There are few CB_2 receptors in the brain, spinal cord, and dorsal root ganglion. CB_2 receptors will up-regulate in response to peripheral nerve

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damage. These cannabinoid receptors regulate neuroimmune interactions and interfere with inflammatory hyperalgesia.

The endocannabinoids anandamide and 2-AG are produced in tissue that has been injured, and activate cannabinoid receptors to suppress the sensitization of the nerve to nociceptive signals and/or to suppress inflammation. The anandamide modulates pain by: 1. Inhibiting nociceptive signals at the synapse by activating CB_1 receptors; 2. Transformed by COX-2 enzymes into pain-relieving molecules (prostamides). 3) Reduces inflammation through activating CB_2 and other receptors. 2-AG plays a role in the descending modulation of pain during acute stress. Both molecules are produced as the body's first response to tissue injury [40,41].

3.3.4 Metabolic Effects

Satiety in part, is modulated through the hypothalamic pro-opiomelanocortin (POMC) neurons. Activation of the CB_1 receptor inhibits the POMC neurons and results in appetite increase. This reduction in satiety can be attributed to the inhibitory effects of cannabinoids on the release of the appetite suppressant α–melanocyte-stimulating hormone (α-MSH). There is an inverse correlation between levels of orexin-A and α-MSH. Orexin A induces hyperphagia by increasing levels of 2-AG [42].

Cannabis users who have hyperexia, also known as "the munchies", eat more calories due to this inhibitory effect of cannabinoids. Paradoxically, users of cannabis are slimmer than non-users of cannabis. The prevalence of obesity is lower in regular cannabis users compared to non-users, even after adjusting for important variables such as age, sex, and tobacco smoking status. [43]. Cannabis users display lower levels of fasting insulin and better insulin sensitivity than their non-using counterparts [44]. These are the metabolic effects of the endocannabinoid system, due in part to the presence of cannabinoid receptors on the mitochondria.

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3.3.5 Cancer

The ECS plays a key role in modulating cell differentiation, cell proliferation, and cell death. Additionally, cannabinoids such as THC and CBD stimulate appetite and reduce the emetic responses seen in chemotherapy. These qualities make the ECS an attractive target for use in cancer therapy. Cannabinoids can also down-regulate certain cancers through modulation of gene expression.

In lung cancer the administration of CBD results in an upregulation of the expression of the intracellular adhesion molecules (iCAM) which in turn prevent metastasis [45].

In gliomas the administration of CBD results in reduction in the expression of pro-angiogenic factors in a dose-dependent fashion. THC, when administered in conjunction with CBD, has been found to be synergistic to inhibit proliferation and survival of human glioblastoma cells [46].

The *in vitro* addition of CBD to breast cancer cells was found to down regulate the expression of ID-1, a large contributor to metastasis of breast cancer cells. [47].

3.3.6 The Role of Antioxidants and Neuroprotection

Cannabinoids act as antioxidants and neuroprotectants. The US National Institute of Health (NIH) holds a patent on these compounds for this purpose [48]. As antioxidants, cannabinoids can neutralize reactive oxygen species. Cannabinoids inhibit voltage-gated calcium channels resulting in the inhibition of the release of glutamate. This neurotransmitter stimulates neuronal depolarization. Glutamate is released during periods of ischemia and other traumatic brain events. In excess, glutamate itself is toxic and can lead to neuronal cell death through excitotoxic stress [49].

Compounds with antioxidant properties are often neuroprotective, for instance through reduction of toxic reactive oxygen species (ROS) produced during ischemic metabolism. Both THC and CBD have been shown to have antioxidant properties [50]. THC and CBD are both able to prevent

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glutamate induced neurotoxicity. The neuroprotective effect of these compounds is independent of their CB receptor binding activity. THC and CBD reduce ROS in vitro, similar to known antioxidants such as ascorbate and butylated hydroxytoluene (BHT). CBD has been shown to protect against cerebral ischemic injury [51], and also attenuates Alzheimer's-related neuroinflammation in animal models [52].

Terpenes have strong antioxidant properties and can also serve as neuroprotectants. β-myrcene, a common terpene in high THC cannabis but not low THC cannabis (hemp), protected against oxidative stress and histological damage induced by ischemia-reperfusion in a mouse model of cerebral ischemia. Terpenes in cannabis with antioxidant properties include β-caryophyllene, limonene, and β-myrcene [53]. Nearly all of the cannabinoids have been found to have potent antioxidant properties. [54,55].

The ECS is involved in the development of many neurodegenerative conditions. Cannabinoids have been shown to have neuroprotective properties, possessing the ability to reduce neuroinflammation, and promote neurogenesis [56-58].

In Alzheimer's Disease (AD) cannabinoids are able to clear the toxic beta amyloid (Aβ) plaques associated with this disease. AD has been found to be associated with a loss of the body's natural production of eCBs, which has been defined as a clinical endocannabinoid deficiency syndrome (CEDS), described later in this article.

CBD has also been shown to reduce the expression of genes implicated in the phosphorylation of the tau protein the hyperphosphorylation of which leads to the formation of neurofibrillary tangles that further contributes to the progression of the disease [59]. Furthermore, cannabinoids have been demonstrated to enhance the clearance of Aβ from the brain as well as prevent the inflammatory cascade that is produced by the accumulation of these mis-folded proteins intracellularly [60,61].

3.3.7 Cardiovascular Modulation by the ECS

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Cannabinoids modulate blood pressure and heart rate, either increasing or decreasing blood pressure and heart rate, depending upon local conditions [62-64].

CBD has a direct effects on arteries, causing vaso-dilation, and subsequent hypotension as a result. This is a mild hypotensive effect, but could be problematic in uncompensated cardiac disease. CBD has a protective effect against vascular damage due to hyperglycemia from Type 2 diabetes, diabetic angiopathies and also with systemic inflammatory processes. The antioxidant and anti-inflammatory effects of CBD mediate these cardiovascular effects [65,66].

CBD demonstrated anti-arrhythmic effects following coronary artery occlusion in rats. This study found that these anti-arrhythmic effects were mediated through non-receptor pathways that did not involve the CB_1 receptor.

CB_1 antagonists increased blood pressure and left ventricular contractile performance in a group of rats bred to have spontaneous hypertension. Reductions in blood pressure, cardiac contractility and vascular resistance were mediated via the inhibitory effects of CBD on the enzyme FAAH by increasing the serum half life of anandamide in normotensive rats. CB_1 antagonists inhibited these effects. CB_1 antagonists lowered blood pressure better in the hypertensive rats versus normotensive rats. This may be due to the upregulation of CB_1 receptors in heart and aortic endothelium in hypertensive rats, but not the normotensive cohort [67].

3.3.8 Modulation of Pulmonary Function

Inhaled and oral THC can create bronchodilation up to two hours following administration. CB_1 receptor activation inhibits cholinergic contraction, which allows it to inhibit bronchospasms. This may be why asthma sufferers find some comfort from cannabis use [68]. CBD decreased pulmonary inflammation and improved pulmonary function tests in murine models of inflammatory lung disease, as well as improving pulmonary function in a model of COPD [69]. THC resulted in a reduction of allergen-induced mucus production [70].

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3.3.9 Clinical Endocannabinoid Deficiency Syndrome (CEDS)

Russo has postulated that several chronic conditions may be due to deficiencies in eCB signaling. PTSD, migraine, IBS, PTSD and fibromyalgia are of particular note in this Clinical Endocannabinoid Deficiency Syndrome [71,72]. There are known mutations in ECS genes that contribute to such deficiencies, which helps to explain the genetic component of these diseases. Other causes that can affect the constitutive tone, or systemic level of eCBs include certain pharmaceuticals and diseases that can deplete eCB levels or interfere with eCB production. Human patients with mutations in CNR1 and DAGLA genes show signs of CEDS [73]. Human IBS patients with mutations in the CNR1 gene, were found to also have altered rates of colonic transit [74]. With PTSD, fear extinction is impaired in patients who were homozygous for a CNR1 mutation [75].

These disorders all have in common markedly decreased systemic levels of eCB. Circulating eCB deficiencies are also inversely correlated with anxiety-like behaviors. Environmental stressors when chronic will down-regulate CB_1 receptors and reduce levels of both AEA and 2-AG [76].

3.3.10 Problems associated with the Endocannabinoid System

The psychoactive effects of THC, which is a CBR agonist, are undesirable in veterinary species. Dogs in particular will suffer from Static Ataxia upon exposure to THC at doses > 0.5 mg/kg IV [13]. As a result, states with medical or adult use marijuana laws, have found an increase in Animal ER admissions for THC toxicosis [77]. A report of the summary of calls to the Pet Poison Hotline found a relative good safety profile for CBD, and THC when given in moderate amounts [78]. Oral tolerance to THC can be achieved in the dog following 7-10 days of a low, sub-psychotropic of 0.05-0.1 mg/kg BID PO. Concurrent use of CBD in equal or greater amounts than the THC will assist in this process [12]. ECS stimulation that is excessive and prolonged can create memory deficits in humans. Upon cessation of prolonged ECS stimulation withdrawal symptoms will develop.

3.4 The Safety of Cannabidiol in Dogs

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Two university studies, published in 2018 found CBD, when present in a full-spectrum hemp oil extract, to be safe when given at a high dose of 10 mg/kg/day for 6 weeks to Beagles, with only a mild and transient elevation in serum alkaline phosphatase, diarrhea, and no interference with anti-epileptic drugs. As CBD is metabolized via the P450 enzyme system, there is concern that the concurrent use of CBD with drugs that are also metabolized through that pathway may have their pharmacokinetics altered, which could alter their therapeutic value for a given patient [79].

Efficacy studies were performed for osteoarthritis at a dose of 2.0 mg/kg BID and clinical benefits were seen in the 16 client owned dogs with osteoarthritis who were recruited for this study [80]. Lower doses of 0.5 mg/kg BID have anecdotally been found to be effective for most dogs with osteoarthritis. The second efficacy study evaluated the ability of cannabidiol to reduce the number of breakthrough seizures in dogs with refractory epilepsy. At 2.5 mg/kg BID, the study found only a partial success for that pilot work. A larger study, funded by the AKC Canine Health Foundation is now being conducted using 4.5 mg/kg BID as the test dosage to evaluate whether it will be better able to extinguish these breakthrough seizures in these dogs [81].

4.0 Discussion

From the research presented in this paper, its obvious that the endocannabinoid system in not just present in nearly all animals, but plays an integral role in maintaining homeostasis for a number of organ systems. The endocannabinoid system modulates the nervous and immune systems and other organ systems through a complex system of receptors and chemical signaling molecules to relieve pain and inflammation, modulate metabolism and neurologic function, promote healthy digestive processes, support reproductive function and embryologic development.

The future looks bright as cannabinoid research, in the post-cannabis prohibition era, is finally able to search for additional discoveries regarding the role the endocannabinoid system plays in the pathogenesis of disease, and the maintenance of health.

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