Stephen Cital · Katherine Kramer ·
Liz Hughston · James S. Gaynor *Editors*

Cannabis Therapy in Veterinary Medicine

A Complete Guide

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Dedicated to my mother, grandmother, Mary Ellen Goldberg, and all the other powerful women in my life—S. Cital

Dedicated to August West, who initiated me into the world of veterinary cannabis, and to all my pets, clients, and patients from whom I continue to learn about the myriad benefits of this plant—thank you. This book would not exist without the tireless efforts of our lead editor, Stephen Cital. Thank you, Stephen, for your passion, for your dedication to our profession, and for your guidance throughout this process. You dragged me kicking and screaming into editorship and I appreciate you more than you know. Thank you for all of the opportunities you've given me, and for always having my back—L. Hughston

Dedicated to my beloved Alex and to my patients and their families, who have taught me so much about medicine. . .and life—K. Kramer

Dedicated to all my animal patients and colleagues from whom I have learned so much about cannabinoid medicine, and to my family and friends for their support and encouragement during the preparation of this book—J. Gaynor

Foreword

Cannabinoid Therapy in Veterinary Medicine: History and Perspective

As a human pediatric and adult neurologist, a 24-year student of cannabis and the endocannabinoid system (ECS), and the son and brother of veterinarians, I am delighted to have the opportunity to introduce this timely tome on veterinary "cannabinology."

The endocannabinoid system is a basic and fundamental homeostatic regulator of physiology (Russo 2011). It is hundreds of millions of years ancient, and has been phylogenetically widespread, including expression in all chordates from tunicates onward, notably missing a few major clades such as insects (McPartland et al. 2001; McPartland 2004), perhaps explaining why they lack a sense of humor.

In contrast, *Cannabis sativa*, the cannabis plant, is about 27.8 million years old (McPartland 2018), dispelling the anthropomorphic notion that it was placed on God's Green Earth for human diversion or veterinary applications; rather, its therapeutic utility in those areas is a happy accident of Nature for us, our pets, and domesticated work animals.

An examination of the therapeutic use of cannabis clearly reveals that, prior to its prohibition, it had always been part of the veterinary pharmacopeia. This is particularly the case in India, the archetypal base of cannabis medicine, where the ancient veterinary uses date from at least the twelfth century (Dwarakanath 1965) and have persisted into modern times (Indian Hemp Drugs Commission 1894; Russo 2005). By 1957, cannabis was still utilized to treat diarrhea in livestock, as an anthelmintic, for "footsore disease, increasing milk-flow in cows, and pacifying them, but also was administered to bullocks as a tonic, to relieve fatigue and to impart staying power" (Chopra and Chopra 1957, p. 9). The latter use of cannabis as a physical work aid parallels similar claims in human workers in Jamaica (Dreher 1982).

These traditional uses in India led directly to a watershed moment in the scientific investigation of cannabis, as William Brooke O'Shaughnessy, an Irish physician in India, applied the teachings of Ayurvedic medicine to the first modern systematic research on the plant's therapeutic properties (Russo 2017). He noted the narcotic effect of the electuary form of cannabis called majoon as reported by his informants and then proceeded to experiment on dogs and an expanded menagerie of other creatures to differentiate their reactions. The results affirmed both sedative and appetite stimulation effects of cannabis, along with static ataxia at higher doses (*vide infra*), all of which passed without notable sequelae after a few hours. He observed: "—while carnivorous animals and fish, dogs, cats, swine, vultures, crow and adjutants [military administrators], invariably exhibited the intoxicating influence of the drug, the graminivorous [grass eaters], such as the horse, deer, monkey, goat, sheep, and cow, experience but trivial effects from any dose we administered" (O'Shaughnessy 1838–1840, p. 363). These observations completed, O'Shaughnessy pressed forward with therapeutic applications of cannabis in recalcitrant human conditions ranging from rheumatism to tetanus, cholera convulsions, and even rabies. His teachings rapidly spread to Europe, where his pioneering work led to successful treatment of 4 of 5 tetanus cases in horses, provided an antidote to strychnine poisoning (Ley 1843), and subsequently set a foundation for our therapeutic cannabis knowledge base that persists after nearly two centuries.

Cannabis developed a strong foothold subsequently in veterinary practice in Europe, North America, and elsewhere. In France, the seed oil was utilized to treat chancres in dogs' ears, and as a purgative in cattle (Tabourin 1875). In Italy, the oil was suggested in veterinary practice for colic and urinary tract pain (Chiappero and Bassi 1879). In Scotland, human success with "Indian hemp" as an analgesic, hypnotic, and antispasmodic equal to opium was cited as evidence for veterinary application (Dun 1880). In South Africa, bowel inflammation, equine cough, and canine chorea were added to the indications (Gresswell et al. 1886). In England, the list expanded to include asthma, convulsions, cough, cystitis, and tetanus (Banham 1887). Across the pond in New York at Cornell University, besides tetanus and cystitis, cannabis was advanced as a treatment to control excitement in azoturia (Hassloch 1896), currently known as equine exertional rhabdomyolysis. At the University of Pennsylvania in Philadelphia, E. Stanton Muir performed extensive experiments with cannabis as a sedative in horses, finding it quite safe (Muir 1900), as well as analgesic, antispasmodic, and hypnotic (Muir 1904). In the Veterinary School of Harvard University, cannabis was observed to lead to survival in half the cases of tetanus in practice (Winslow 1901).

These same indications for cannabis persisted in the literature over the ensuing decades, with various new observations. Cannabis was noted as a powerful narcotic without constipation (Sayre 1907), as a treatment for melancholia in horses with pneumonia (Quitman 1912), as a liniment (Brumley and Snook 1913), for the relief of spasm and nervous irritability and a narcotic for equine operations (Milks 1917), for hobbling horses (Udall 1917), and for treatment of delirium associated with

parturient apoplexy (Winslow and Eichhorn 1919). Subsequent editions of these veterinary textbooks repeated similar observations in the USA (Milks and Eichhorn 1936, 1940), until the American prohibition of 1937 stopped supply. In Europe, cannabis usage continued a bit longer along traditional lines (Greig and Boddie 1942; Ironside 1946), adding indications such as volvulus and enteritis (Greig 1939).

This extensive utilization of cannabis in the veterinary context may have fallen out of vogue due to political misadventures, but supportive evidence remains not only in these moldering texts but also in preserved medicine bottles. The persistence of such products manufactured for decades is a testament to their likely efficacy: In centuries past, a farmer's good money would not likely be spent for sentimental reasons: either the medicine worked or a valuable animal was lost.

Patent medicines also existed for dogs, including "Security Cough, Cold and Distemper Remedy" which cost $1 in 1906 (equivalent to $28 today) containing *Cannabis indica*: "Will relieve the worst cough, chill or fever, Influenza or mucous membranes affections of the animal's throat, nose, eyes, mouth or air passages" (Wirtshafter 2016, p. 26). In some areas of the world, hemp seed persists as fish bait/fish food and remains a favorite seed of songbirds.

In 1938, Robert P. Walton published the definitive tome of the era on cannabis and its medical and veterinary applications (Walton 1938), citing many of the uses described above, just in time for the initiation of cannabis prohibition. He also summarized and expanded on the veterinary bioassays available to assess cannabis potency, most particularly in dogs. A gradual sedation without distress was an initial sign, followed by a progressive static ataxia that foreshadowed later knowledge of cannabinoid receptor density data (*vide infra*), and eventual sleep. When subjects were exposed too often or at elevated doses, tolerance to the intoxication was observed. The same phenomenon was observed later by a Greek veterinarian writing in French (Cardassis 1951) who related the case of a lamb that seemingly developed a compulsion to graze on cannabis presented after each feeding with gaiety and panic but repeating the exposure it continued developing and fattening normally. Readers may question how cannabis plants in the field would be psychoactive at all, since most would harbor non-intoxicating cannabinoid acids, but modern liquid chromatography techniques always show at least some neutral cannabinoids such as THC in fresh flower material (Lewis et al. 2018).

Beyond the patent medicines of the previous era, many mainstream pharmaceutical companies including Upjohn, Lilly, and Sharp and Dohme marketed their own products of *Cannabis americana*, a spurious appellation for a hybrid species of domestic agriculture (Wirtshafter 2016). These companies typically utilized canine subjects to titrate medicine batches and judge product consistency. United States Pharmacopoeia (USP) standard doses were developed (Walton 1938) but cannot be quantified with certainty today given our ignorance of the original concentrations of the preparations.

Walton also described effects of corneal anesthesia in rabbits, swaying and decreased tonus in cats, and profound and prolonged narcosis in frogs (Walton 1938). Finally, effects in mice included corneal anesthesia (analgesia), catalepsy, and hypnosis, providing three of the four components of the cannabinoid tetrad

(along with hypothermia) utilized today in that species for assaying cannabinoid activity of test compounds (Smith et al. 1994). Ultimately, Walton opined that lethal doses in animals were more likely attributable to alcohol content, stating, "When considered in terms of the minimally active doses, the drug has an extraordinarily high range of safety" (Walton 1938, p. 175).

The latter statement is supported by more recent reports of accidental poisonings with cannabis, particularly a review of 213 cannabis toxicosis cases after oral ingestion in dogs (Janczyk et al. 2004). Doses ranged from 0.5 to 90 g, with virtually all the patients demonstrating neurological effects such as sedation, ataxia and coordination impairment, and emesis in about 5% within 3 h, and lasting variable durations up to 4 days. With decontamination, fluid replacement, and diazepam (in some instances), all the animals completely recovered, however.

In all, aside from their increased susceptibility to ataxia, the effects of cannabis in dogs are closely related to those in humans. This is highlighted by the findings of one of the landmark studies of cannabinoid receptor CB_1 distribution in the brain shortly after the discovery of the endocannabinoid system (Herkenham et al. 1990). Throughout mammalian species, binding of cannabinoid ligands was greatest in the basal ganglia, hippocampus, and the cerebellum, the latter being particularly prevalent in the cerebellar molecular layer in dogs, highlighting their sensitivity to ataxia after exposure to tetrahydrocannabinol with doses as low as 0.5 mg/kg. As in humans, a paucity of receptor density in lower brainstem centers mediating cardiovascular and respiratory functions explains the relative safety of cannabis in even extreme overdoses. Observed K_i values and potencies of cannabinoid agonists in tests of canine ataxia and human subjective reports were highly correlated, supporting the similar effects of such drugs in the two species.

Considering this brief survey, what trends and suggestions may be put forward? Firstly, from the earliest modern scientific studies of cannabis, analogous conditions should be quite amenable to cannabis therapeutics across mammalian species. An examination of recent reviews (National Academies of Sciences Engineering and Medicine (U.S.). Committee on the Health Effects of Marijuana: an Evidence Review and Research Agenda 2017; MacCallum and Russo 2018), foremost among these indications would be treatment of chronic pain, whether neuropathic or cancer-related, emesis associated with chemotherapy, spasticity, sleep disorders, and epilepsy, especially with cannabidiol in the instance of the latter. However, the possibilities do not end there. The weight of history, basic research, and a considerable body of anecdotal evidence support many additional indications for cannabis in autoimmune conditions, obesity, neurobehavioral disorders, degenerative neurological conditions, and obstetrics and gynecology (Russo 2002, 2016, 2018).

American families spent $61.4 billion on their pets in 2011, 1% of total expenditures, or about $500 per household (Henderson 2013). This author and many of his cohorts expend considerably more in veterinary bills than on their own medical care. Modern trends indicate that cannabis has a large and increasingly important role to play in such treatment. It is equally clear that the disciplines of veterinary and human medicine have valuable insights to share and that a proper course of action would be a coordination of basic and applied clinical research efforts to produce a mutual

synergy that will expedite therapeutic advances and bring cannabis-based medicines of high quality, safety, efficacy, and consistency to our companion animals and the people who love them.

Ethan Russo

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About this Book

This text was written as a guide for veterinary practitioners interested in the use of phytocannabinoids as a therapy for companion animals. The pace at which research is being published on this topic and amid regulatory overhauls worldwide made the publication of this text critical and timely to practice veterinary medicine safely as it pertains to the therapeutic use of these compounds given client interest and the surmounting scientific evidence for their utility. Due to the speed at which regulatory changes are occurring in regard to cannabis (hemp and marijuana) and fear of publishing out of date material, this text focuses on the scientific and clinical aspects of cannabinoids in predominately dogs, cats, and horses. The authors of this text wrote their chapters to offer what evidence exists on their respective area of focus to help you make a more informed clinical decision. As with many things in veterinary medicine, we must be prepared to extrapolate information from other species, preclinical studies, and anecdote combined with our own clinical judgment to best care for our patients.

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Chapter 1
The Endocannabinoid System and Endocannabinoidome

Robert Silver

1.1 Introduction

The "Endocannabinoid System" (ECS) was not discovered until early in the 1990s, as a result of research to understand the actions of Δ^9-THC (THC) on the nervous system. Some of this work determined that THC works by binding to two endogenous membrane receptors that had not been identified prior. These endogenous membrane receptors were identified as G-protein coupled receptors (GPCR) and named Cannabinoid Receptor 1 (CB1) and Cannabinoid Receptor 2 (CB2). After the discovery of these receptors, it was a short path to find the endogenous ligands that pair with these receptors (Panagis et al. 2014).

The endogenous ligands are endocannabinoids, which are derivatives of, and manufactured from, the fatty acid arachidonic acid in the cellular membrane. The two endocannabinoids, arachidonoylethanolamide (AEA) and 2-arachidonoylglycerol (2-AG) were discovered following the discovery of the cannabinoid receptors in 1992 and 1995, respectively. Biosynthetic enzymes synthesize these endocannabinoids in the cell membrane, ad hoc, triggered by specific stimuli. Degradative enzymes play a role in endocannabinoid deactivation, with AEA deactivated mainly by fatty acid amide hydrolase (FAAH) and 2-AG deactivated by monoacyl-glycerol lipases (MAGLs) (Zou and Kumar 2018; Lu and Mackie 2016).

The defined *classic* ECS is the ensemble of the GPCR's CB1 and CB2 receptors and endogenous molecules involved with cannabinoid signaling along with catalyzing and hydrolyzing enzymes:

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1. Primary endogenous ligands, although others have been described, are endocannabinoids AEA & 2-AG
2. Endogenous cannabinoid receptors (CB1 & CB2)
3. Metabolic enzymes of biosynthesis and hydrolysis (DAGL; FAAH; MAGL; NAPE)

1.2 The Endogenous Ligands

Anandamide (AEA) and 2-AG are the primary endogenous triggers to activate cannabinoid receptor signaling, but other endogenous molecules such as palmitoylethanolamine (PEA) and oleoylethanolamide (OEA) also exert cannabimimetic effects (Veilleux et al. 2019).

AEA and 2-AG bind orthosterically to the cannabinoid receptors. They activate these receptors at the cellular level. Unlike the other neurotransmitters, like norepinephrine or acetylcholine which are hydrophilic and stored in intracellular vesicles, endocannabinoid ligands are lipophilic and produced on demand from arachidonic acid in the cellular membrane with the help of their biosynthetic enzymes. In the nervous system, both endocannabinoids are produced in the postsynaptic membrane and travel retrograde across the synaptic cleft to the presynaptic membrane where they bind to CB1 or CB2 receptors and modulate presynaptic neurotransmitter release. The evidence for 2-AG retrograde transmission is greater than for AEA, but these mechanisms have not yet fully been elucidated (Mechoulam et al. 2014).

This transient "on-demand" activity of endocannabinoids guarantees that endocannabinoid signaling is tightly controlled in the local area of activity and has a short duration of activity (Abramovici et al. 2018).

The production of endocannabinoids in the postsynaptic membrane is triggered by an intracellular calcium (Ca^{2+}) concentration increase secondary to either a depolarization signal, physiological, or pathological stimulus. These ligands can also signal other cannabinoid receptor-independent pathways, such as the TRPV1 and HT51 membrane receptor systems.

Allosteric antagonists or negative allosteric modulators, also known as "non-competitive" antagonists, can block activation of G-protein receptors such as CB1 & CB2 through binding to an allosteric site on the receptor. This allosteric affinity modulates the binding of the orthosteric ligand, which then blocks full activation of that receptor (Howlett et al. 2011).

AEA is a partial agonist of CB1 and CB2 with less affinity for CB2 than CB1. On the other hand, 2-AG shows greater potency and efficiency as an agonist for CB1 than AEA as well as greater potency than AEA for the CB2 as an agonist. Both of these endocannabinoids have also been shown to have actions with non-endocannabinoid receptors and ion channels.

Polyunsaturated fatty acids are known to directly influence input into endocannabinoid signaling pathways. Dietary intake of omega fatty acids is necessary for the regulation of ECS tone (Lafourcade et al. 2011). The ECS is intimately involved in the regulation of most aspects of animal physiology. The CB1

cannabinoid receptor is the most common GPCR in the human brain and many other organs. To date, these anatomical locations for the CB1 receptor include heart, blood vessels, liver, lungs, digestive system, fat, and sperm cells (Mackie 2008).

1.3 The Endogenous Receptors

The identification of the G-protein coupled receptor family, as well as the discovery of the endocannabinoid receptors, was made possible due to the advent of chromatographic analytical technology in the late 1970s to early 1980s. More specific examples of G-protein coupled receptors are the opioid, muscarinic, cholinergic, and α-adrenergic receptors, and they all hold in common the ability to inhibit the production of adenylyl cyclase. The newly discovered cannabinoid receptor (CB1) inhibited this enzyme as well, which identified it as a member of this family of G-protein coupled membrane receptors. The identification several years later of the CB2 receptor was accomplished through sequence homology (Elphick 2012).

Each of these two cannabinoid receptors has a distinct and unique anatomical spatial distribution with individual species variations. Both CB1 and CB2 are present in some of the same tissues simultaneously, although providing different but synergistic effects.

Generally, the CB1 receptor is located primarily in the CNS but is also present in much lower concentrations in most tissues and cell types peripherally. To date, the anatomical locations identified for the CB1 receptor include heart, blood vessels, liver, lungs, digestive system, fat, and sperm cells (Mackie 2008).

The CB2 receptor has the highest concentrations in the immune and hematopoietic systems. Identification of CB2 receptors were found in the brain, the gut, myocardium, endothelial, vascular smooth muscle, Kupffer cell, exocrine and endocrine pancreas, bone, reproductive organs and cells, and also in various tumors (Pacher et al. 2006; Gardner 2013).

CB2 receptors in the immune system can modulate the release of cytokines. Inhibition of adenylyl cyclase results from the activation of lymphocyte CB2 receptors by cannabinoids. In turn, this will reduce the cellular and humoral responses to an immune challenge (De Petrocellis et al. 1999). CB1 and CB2 receptors decrease adenylyl cyclase activity and down-regulate the cAMP pathway. Activation of lymphocytes results in mitogen-activated protein kinase cascades (MAPK), ion channel modulation, and modification of intracellular calcium levels. Potassium channel activation is also a signaling mechanism for the CB2 receptor (Griffin et al. 1999; Ho et al. 1999).

1.4 The Biosynthetic and Degradative Metabolic Enzymes

The formation of AEA is a two-step process that involves a Ca^{2+}-dependent N-acyltransferase transfer of arachidonic acid from phosphatidylcholine to phosphatidylethanolamine to yield N-arachidonoylphosphatidylethanolamine (NAPE) which is then hydrolyzed by a NAPE-specific phospholipase D (NAPE-PLD) into AEA. This process is the primary biosynthetic route, although there are alternate routes of synthesis available to manufacture AEA.

Multiple pathways with redundant precursors indicate the importance of this endocannabinoid to the physiology of homeostasis. Raphael Mechoulam has observed that most biochemical mechanisms in nature will use redundant precursors and pathways. Mechoulam calls this: "The stinginess of nature." He explains that "If nature knows how to do something, chances are it will do it again with small changes so it will not have to learn new things." (Gardner 2013).

2-AG is principally synthesized through phospholipase Cβ-mediated hydrolysis of phosphatidylinositol-4,5-bisphosphate, with arachidonic acid on the *sn-2* position, to yield diacylglycerol (DAG). DAG is then hydrolyzed to 2-AG by diacylglycerol lipase (DAGL). It is important to note that although both AEA and 2-AG are derived from arachidonic acid, their biosynthetic pathways are not the same as the biosynthetic pathways for the production of eicosanoids (Abramovici et al. 2018).

1.4.1 Degradation of Endocannabinoids

Fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) terminate the binding of AEA and 2-AG, respectively, to the endocannabinoid receptors. FAAH is localized primarily post-synaptically and preferentially degrades anandamide; MAGL is primarily localized pre-synaptically, near the presynaptic membrane, and degrades 2-AG. The rapid termination of the endocannabinoid signal guarantees that biological activities dependent upon these signals are appropriately regulated. With prolonged signaling activity, such as can be found with the use of exogenous cannabinoids (both synthetic and plant-derived), problematic adverse events may occur (Abramovici et al. 2018).

Located on the endoplasmic reticulum (ER), FAAH is in the cytoplasm of the cell. MAGL is found in the cell membrane and is also soluble. Because the extracellular space that bridges the synaptic gap and surrounds all cells is an aqueous environment, highly lipophilic molecules (such as the endocannabinoids) need to be transported to the cellular membrane and into the cytoplasm by specific molecules capable of carrying a lipophilic substance through an aqueous environment. The activity of these degradative enzymes is dependent on the transport of their lipophilic substrates (AEA and 2-AG) to the cell membrane where 2-AG can be degraded by

MAGL and into the aqueous environment of the cytoplasm to the ER where FAAH can degrade AEA.

1.4.2 Transport of Endocannabinoids for Activation and Degradation

Fatty Acid Binding Protein (FABP) is the transport protein molecule needed to carry lipophilic molecules such as fatty acids and endocannabinoids to their sites of activity and degradation. 2-AG uses FABPs for its retrograde transport to the CB1 receptor on the presynaptic membrane (Deutsch 2016).

FABPs also have an affinity for the highly lipophilic phytocannabinoids such as CBD and THC and are responsible for transporting them to the cannabinoid receptors. This molecule transportation mechanism, in part, is how CBD and THC can compete for anandamide uptake by FABP, and in so doing, can increase the serum half-life of the endocannabinoids. This is the mechanism that documents the ability of CBD/THC to boost the body's endocannabinoid signaling. FAPBs are also responsible for transporting the phytocannabinoids into the cell, where they can themselves be degraded enzymatically by P450 cytochrome enzymes (Elmes et al. 2015, 2019).

In biochemical studies it was found that CBD, and potentially other phytocannabinoids, can enhance AEA signaling indirectly by inhibiting AEA degradation catalyzed by FAAH. (Leweke et al. 2012, Papagianni and Stevenson 2019).

1.5 The Endocannabinoidome

The group of molecules and receptors that comprise the classic endocannabinoid system are part of a larger family of signaling molecules and receptor promiscuity termed the "Endocannabinoidome". These are compounds that are not specifically part of the endocannabinoid system but have a cross-signaling effect with the ECS. They are found to act on several receptor targets (GPR55, GPR18, GPR119, TRPA1, CB1, CB2, TRPV1, TRPA1, opioid, dopamine, and serotonin (5-HT) and glycine receptors) and non-receptor targets within the ECS. The individual molecules of the endocannabinoidome termed "entourage compounds" or "endocannabinoid-like molecules" include the acyl ethanolamides detailed below (Ngo, 2019).

Endocannabinoid-like molecules that have not been shown to bind to the cannabinoid receptors have binding affinity to the nuclear receptor/transcription factor peroxisome proliferator-activated receptor (PPAR α & γ). These molecules are fatty acyl ethanolamides such as palmitoylethanolamide (PEA) and oleoylethanolamide (OEA). These endogenous ethanolamides can potentiate anandamide's effect through the competitive inhibition of FAAH, similarly seen with CBD. These

ethanolamides have an allosteric modulatory effect on another receptor system, the transient receptor potential vanilloid (TRPV) channel. The effect these molecules have on the endocannabinoid system to potentiate the actions or serum levels of endocannabinoids has been termed the "Entourage Effect". The definition of the entourage effect extends to include the interaction of the active components of *Cannabis sativa* L., namely, the phytocannabinoids, terpenes, and flavonoids, and the endocannabinoid system (Abramovici et al. 2018).

Mechoulam, in his 2012 address to the International Cannabinoid Research Society in Freiberg, Germany (Gardner 2013), discussed the critical role that fatty acids bound to amino acids, "FAAA"s, play in intercellular signaling. These FAAAs are found in clusters that include their precursor molecules and their derivatives and are found in large numbers in the central nervous system, especially in the brain. AEA and 2-AG are just two of these types of molecules. These types of molecules are constituents of the endocannabinoidome, and Mechoulam believes these will be the subject of extensive study in the search for molecular therapies and pharmaceutical interventions in the future.

Further examples of these molecules include arachidonoyl serine (AraS), which can regulate vasoconstriction and the effects of brain trauma. Arachidonoyl glycine, on the other hand, can lower pain sensations. Oleamide is a sleep-inducing lipid, and oleoyl serine can be useful for osteoporosis. One of these entourage compounds, palmitoylethanolamide (PEA), concentrates in the brain when injured. There are hundreds of these entourage compounds in the brain, among other tissues. Mechoulam concluded that identifying and understanding the functions of the FAAAs will elucidate mechanisms of disease and provide both diagnostic and interventional tools for medicine in the future.

1.6 Endocannabinoid Tone

G protein-coupled receptors (GPCR) respond to activation by an agonist, such as AEA or 2-AG, by stimulation of protein activation, inhibition of adenylyl cyclase, activation of mitogen-activated protein kinase (MAPK), or regulation of an ion channel. Competitive antagonists can block the activity of these agonists through competition with binding sites but fail to activate a response from the GPCR.

Constitutive activity occurs when a receptor is activated without direct stimulation by an agonist. This results from other receptors that have been stimulated by their endogenous agonists and can be either autocrine (from the same cell as the GPCR) or paracrine (from a nearby cell) in nature. The sum total of this activity is considered to be the 'basal tone' of a tissue.

Allosteric or non-competitive agonists will block GPCR signaling by binding to an allosteric site which blocks the conformational change associated with the binding of the agonist at its orthosteric site, thus blocking activation of that GPCR. The constitutive activation of the GPCR can be blocked by an inverse

agonist, which, like the allosteric antagonist, blocks the constitutive activation of the receptor by the autocrine or paracrine signals.

These receptor interactions can be quite complex, and often overlapping in definitions and activity. "Tonic" signaling is the basal signaling, specific to a given tissue in the absence of agonist binding; "phasic" signaling results from the direct binding of the agonist at the orthosteric site. AEA is the endocannabinoid that regulates the basal synaptic signaling, and 2-AG is the phasic signaling agonist molecule (Matias et al. 2005).

The endocannabinoid tone, from a larger perspective, results from an individual's level of AEA and 2-AG, based on their synthesis, degradation, and the spatial density of endocannabinoid receptors in the body. The levels of these endocannabinoids maintain homeostasis and regulate pain, metabolism, and nearly every other process in the body. With decreased endocannabinoid tone, clinical problems can result.

1.6.1 Clinical Endocannabinoid Deficiency Syndrome (CEDS)

Russo has postulated (2004, 2016), and evidence exists to support his hypothesis, that several chronic conditions may be due to deficiencies in endocannabinoid signaling (Russo 2016a, b):

- Chronic Migraine
- Fibromyalgia
- Irritable Bowel Syndrome (IBS)
- Post Traumatic Stress Disorder (PTSD)

Like CEDS, many other neurological disorders are relative to neurotransmitter deficiencies:

- Acetylcholine (Alzheimer's Disease)
- Dopamine (Parkinson's Disease)
- Serotonin and Norepinephrine (Clinical Depression)

It is beyond the scope of this chapter to discuss the endocannabinoid system relative to specific diseases. However, it is worthwhile to note that the syndromes that have been most closely associated with an endocannabinoid deficiency (PTSD, Chronic Migraine, Fibromyalgia, IBS) have objective findings that support their inclusion in this syndrome.

The gene variants that encode endocannabinoid production, activity of ECS receptors, and gene variants responsible for enzyme production are only just beginning to be researched but help explain part of the genetic component of these diseases. Moreover, the constitutive tone or systemic level of endocannabinoids is affected by some pharmaceuticals, such as NSAIDs and acetaminophen, and diseases

that can deplete endocannabinoid levels or interfere with endocannabinoid production. Human patients with mutations in CNR1 and DAGLA genes show signs of CEDS. Human IBS patients with mutations in the CNR1 gene were found to have altered rates of colonic transit. Impaired fear extinction has been identified with PTSD patients who are homozygous for a CNR1 mutation (Smith et al. 2017; Camilleri et al. 2013; Heitland et al. 2012).

These disorders all have in common markedly decreased systemic levels of endocannabinoids. Circulating endocannabinoid deficiencies are also inversely correlated with anxiety-like behaviors. Chronic environmental stressors can down-regulate CB1 receptors and reduce levels of both AEA and 2-AG (Hill et al. 2009).

As of this publication, the CEDS has not been identified or defined in our veterinary species.

1.7 The Evolution of the Endocannabinoid System

Nearly all animals, including vertebrates (mammals, birds, reptiles, and fish) and invertebrates (sea urchins, leeches, mussels, nematodes, and others), have been found to have endocannabinoid systems. The ECS is found in nearly all animals, from mammals to the more primitive phyla. The early emergence of the ECS in the phylogeny indicates its biological importance. An understanding of the role of the endocannabinoid system in health and disease is crucial for developing pharmacotherapeutic interventions.

1.7.1 Invertebrate Endocannabinoid Systems

The Hydra (*H. vulgaris*), a cnidarian in the class Hydrozoa, is one of the first animals with a neural network. The primary function of the ECS in this primitive animal is to control its feeding response (De Petrocellis et al. 1999). Subsequent studies identified endocannabinoid receptors and the presence of a FAAH-like amidase in the Sea squirt and determined an association with a behavioral response (Matias et al. 2005).

A systematic review was conducted of existing published research detailing the presence of ECS receptors in invertebrates. This literature was employed in the identification of subgroups of invertebrates to conduct tritiated ligand binding assays in the group of invertebrates that had not been studied prior. Seven species of invertebrates were examined using a tritiated ligand binding assay. Cannabinoid receptors were identified in the following species:

- *Ciona intestinalis* Sea squirt (Deuterostomia)
- *Lumbricus terrestris* Earthworm (Lophotrochozoa)
- *Peripatoides novae-zealandiae* Velvet worm (Onychophora)
- *Jasus edwardi* Rock lobster (Crustacea),

- *Panagrellus redivivus* Beer mat nematode (Nematoda)
- *Actinothoe albocincta* White striped anemone (Cnidaria)
- *Tethya aurantium* Orange Puffball sponge (Porifera).

No evidence of cannabinoid binding was detected in either the sea anemone (*A. albocincta*) or the sponge (*T. aurantium*). In the other organisms tested, CB1 receptors were detected, but CB2 receptors were not detected. The earthworm (*L. terrestris*), velvet worm (*P. novae-zealandiae*), and mat nematode (*P. redivivus*) were compared to a standard CB1 ortholog in rat cerebellar tissue and were found to have a strong binding affinity.

From this data, it was concluded that cannabinoid receptors evolved in the last common ancestor of the bilaterians but had a second loss in insects and other clades. Cannabinoid receptors have been identified in sea urchins, leeches, earthworms, hydra, lobster (*H. americanus* and *J. edwardi*), and the beer mat nematode (*P. redivivus*), but not the nematode (*C. elegans*) (McPartland et al. 2006).

Insects (*Apis mellifera* [western honeybee]), *Drosophila melanogaster* [common fruit fly], *Gerris marginatus* [water strider], *Spodoptera frugiperda* [fall armyworm moth larva], and *Zophobas atratus* [darkling beetle]) have not been found to contain cannabinoid receptors. No other mammalian neuroreceptor has been found to be lacking in insects. This is the only case in comparative neurobiology that a mammalian neuroreceptor is absent in insects (*Ecdysozoa*). One hypothesis for this absence of cannabinoid receptors in insects is the very low levels of endocannabinoid ligands measured. However, 2-AG has been found in more significant amounts than AEA, and FAAH-like molecules were not identified in the fruit fly (*Drosophila melanogaster*). Historically, cannabis extracts have been found to create behavioral changes in insects, but from this study, it appears this effect is not mediated by endocannabinoid receptors. Endocannabinoid receptors have been identified in animals phylogenetically older than insects, such as the Hydra (McPartland et al. 2001).

1.7.2 Vertebrate Endocannabinoid Systems

Studies of the endocannabinoid system of vertebrates have found evidence that it is present in all species. From the evidence that the endocannabinoid system exists in invertebrate species as early phylogenetically as the Cnidarians, except for insects, one can infer that it is common to all life because of its vital role in many crucial biological activities.

The anatomical sites and spatial density of cannabinoid receptors have both interspecies and intraspecies differences in vertebrate species. For instance, endocannabinoid receptors in humans are sparse in the brainstem and medulla oblongata, which are responsible for the control of vital autonomic functions such as respiration and heart rate. This fact is why cannabis has such a safe profile in humans. The published evidence indicates that cannabis may not have as safe a

profile for veterinary species such as the dog, due to the high spatial density of CB1 receptors in the dog cerebellum and medulla oblongata (Dewey et al. 1972).

Differences in the protein sequences of the CB2 receptor have been identified in the human, rat, and canine receptors. These differences are a curious occurrence despite the highly conserved structure of the CB1 receptor among all mammalian species. Endogenous ligand binding affinities for the canine CB2 receptor are measured to be about 30 times less than human and rat CB2 receptors (Ndong et al. 2011).

1.8 Canine

Compared to humans, the number of CB1 receptors in hindbrain structures in the dog far exceed those found in the human animal. Radioligand studies found that large numbers of cannabinoid receptors are located in the cerebellum, brain stem, and medulla oblongata in the dog (Herkenham et al. 1930). "Static ataxia," which is a unique neurological reaction to THC in the dog, is explained by this high concentration of CB receptors in the cerebellum. Static ataxia was first described in 1899 by Dixon in his pharmacologic study of Indian hemp in a variety of species, including humans (Dixon 1899). CB1 receptors in salivary glands (Dall'Aglio et al. 2010), hair follicles (Mercati et al. 2012), skin, and the hippocampus in dogs have been also been described (Campora et al. 2012).

1.8.1 Cannabinoid Receptor Spatial Distribution in a Variety of Tissues and Organs

The anatomical localization of the CB1 receptor in the normal canine nervous system has been determined through the use of immunohistochemical analysis. Nervous systems from healthy dogs at 4 months, 6 months, and 10-year-old dogs have been evaluated post-mortem. Neutrophils of the cerebral cortex, cornu ammonis (CA), dentate gyrus of the hippocampus, midbrain, cerebellum, medulla oblongata, and gray matter of the spinal cord were found to have strong immunoreactivity. Dense CB1 expression was found in the fibers of the globus pallidus and substantia nigra. These immunoreactive locations were surrounded by neurons with no immunoreactivity.

A consistent finding of positive immunoreactivity in astrocytes was recorded in all of the examined regions. In the peripheral nervous system, CB1 staining was localized in the neurons and in the satellite cells of the myelinating Schwann cells and dorsal root ganglia.

In comparing the younger dog nervous system to the older dog nervous system, lower CB1 expression was found in the brain tissue of the older animal. This was less

than the expression of receptor immunohistochemistry found in human fetal and neonatal brain tissue. Reduced receptor expression has been measured in aged rats, localized to the cerebellum, cerebral cortex, and basal ganglia, but less prevalent in the hippocampus. The older dog in this study was also found, like the aged rats, to have reduced measurements of CB1 receptor expression compared to the younger dogs' nervous systems examined (Ndong et al. 2011).

Canine claustrum samples were obtained from necropsy specimens of neurologically healthy dogs. They were examined by immunohistochemical and morphohistological analysis for CB1 receptors and fatty acid amide hydrolase (FAAH). The results of this analysis revealed a spatial distribution of this receptor and enzyme consistent with previous studies reported for other animal species. The CB1 receptor was located on presynaptic membranes and the FAAH was found in the cellular body and dendrites of the neurons (Pirone et al. 2016).

In another study, samples of the cervical (C6–C8) sensory ganglia and related spinal cord were harvested post-mortem from neurologically healthy dogs. Immunohistochemical analysis of this tissue detailed the spatial distribution of the cannabinoid and cannabinoid-related receptors, CB1 CB2, GPR55, PPARα, and TRPV1 in the cervical dorsal ganglion tissue examined. 50% of the neuronal population had weak to moderate CB1 immunostaining and TRPV1 receptor immunoreactivity while 100% of the population was positive for CB2. Schwann cells, blood vessel smooth muscle cells, and pericyte-like cells also demonstrated CB2 immunoreactivity. Nearly 40% of cells had GPR55 receptors in this same neuronal population. Endothelial cells and 50% of satellite glial cells (SGC) were positive for PPARα. SGC cells were positive for TRPV staining, but this was only in older dogs (Chiocchetti et al. 2019).

A 30-day old canine embryo was examined using immunohistochemistry to localize its CB1 receptors. Immunoreactivity was identified mainly in epithelial tissues and included most structures of the central and peripheral nervous system, inner ear, olfactory epithelium, and related structures, eyes, and thyroid gland (Pirone et al. 2015).

Homogeneous distribution of both CB1 and CB2 receptors in clinically healthy dogs and cats are found throughout all layers of the epidermis. CB1 and CB2 receptors are present both in healthy dog epidermis and in dogs with atopic dermatitis (Campora 2012).

There is a fundamental anatomical difference between human and canine epidermal architecture, with canine epidermis containing 2–3 nucleated layers of cells. In contrast, the human epidermis contains 6–7 nucleated layers of cells. Dogs diagnosed with atopic dermatitis have a hyperplastic epidermis. Suprabasal keratinocytes possess strong immunoreactivity for both the CB1 and CB2 receptors, whereas basal keratinocytes have shown weak CB1 but stong CB2 immunoreactivity. This is a strong indication that these receptors are upregulated during epidermal inflammation. Agonists to both CB1 and CB2 receptors have been found to reduce mast cell degranulation, which is an important step in the development of hypersensitivity reactions.

In an ex vivo study designed to localize the distribution of both cannabinoid receptors, GPR55, and PPARα in the canine gastrointestinal tract, CB1 immunoreactivity was found in the lamina propria and epithelial cells. The CB2 receptor immunoreactivity was observed in lamina propria mast cells and immunocytes, blood vessels, and smooth muscle cells. There is notable faint reactivity of the CB2 receptor in neurons and the glial cells of the submucosal plexus. $PPAR_{\alpha}$ receptor immunoreactivity was located in blood vessels, smooth muscle cells, and the glial cells of the myenteric plexus. GPR55 receptor immunoreactivity was localized in the macrophages of the lamina propria and smooth muscle cells. A wide distribution of the cannabinoid receptor ensemble was found in several cellular types from all layers in the gastrointestinal tract of the dogs evaluated in this study (Galiazzo et al. 2018).

Canine Degenerative Myelopathy (DM) is considered to be a disease model for amyotrophic lateral sclerosis (Lou Gehrig's Disease or ALS). CB2 receptors have been found to provide neuroprotective effects in a mutant mouse model of ALA in part by their upregulation in that model. Ex vivo postmortem spinal cords of healthy dogs and dogs confirmed diagnosed with degenerative myelopathy were harvested. PCR analysis was used to examine the endocannabinoid gene expression for both the healthy and affected dogs. No difference between the two groups was noted for the CB1 receptor, confirmed by immunostaining. However, dogs with confirmed DM had significant elevations of CB2 receptor levels spatially localized to the astrocytes, confirmed via immunostaining (Fernandez-Trapero et al. 2017).

Currently, studies are underway using polymerase chain reaction (PCR) technology to localize and quantify cannabinoid receptors in canine tissue, but that data is, as yet, unpublished. Early, but limited, findings in this unpublished data used immunohistochemistry and compared that to PCR technology. Selective staining was verified by western blot testing from 35 tissue samples derived ex vivo, from adult dogs presented to the Auburn University College of Veterinary Medicine Surgery Department for procedures related to the tissues submitted.

Results included dark staining of CB2 receptors in the endothelial cell membranes of most tissues submitted. Other findings saw less significant staining localized microscopically to cell membranes from cells located in the tissue parenchyma. High expression of the CB1 gene was located in blood, brain, testicles, ovary, and uterus. There was low expression in the kidney, lung, liver, and lymph node. CB2 expression was limited but had high expression in blood and lymph nodes. This study confirmed many earlier findings using immunohistochemistry, such as a high concentration of CB1 in grey matter and CB2 in blood and lymph nodes. Unexpected findings, based on the prior immunohistochemical studies, were high quantities of CB2 in both male and female gonads and low levels of CB2 expression in lung and liver, as compared to human and mouse models (Miller 2017).

1.9 Feline

The immunohistochemical distribution of CB1 and fatty acid hydrolase were examined ex vivo in feline ovaries and oviducts harvested during spaying of healthy cats during diestrus. The ovaries had primordial, primary, secondary, tertiary, and pre-ovulatory follicles in addition to active corpora lutea. CB1 immunoreactivity was not observed in immature follicles but was seen in the tertiary follicle granulosa cells. Staining for FAAH distributed differently than CB1. Its presence was detected in ovarian pre-antral follicles, oocyte cytoplasm, and in granulosa cells of all stages of follicular development, and in thecal cells of secondary and tertiary follicles. Luteal cells were immunoreactive for both CB1 and FAAH. These findings suggest that late-stage follicles and corpora lutea (CL) could respond to endocannabinoid or entourage compound intervention.

Oviducts exhibited CB1 staining only on ciliated cells, whereas FAAH staining was found on both ciliated and non-ciliated cells. It is known that the ECS influences sperm-oviduct interaction. AEA has been found to inhibit bovine sperm binding and induces sperm release from oviductal epithelial cells. A study in ewes treated with a CB1/CB2 agonist negatively affected luteal progesterone secretion. Hypothetically, fertility could be reduced by targeting the CL with endocannabinoids or entourage compounds. Pregnant mice exposed to an AEA analog or THC experienced pregnancy loss with embryo retention in the oviduct. Another study found that systemic and local (oviduct) AEA levels positively correlated with ectopic pregnancy.

This information suggests that modification of the ECS during pregnancy by pharmacologic intervention or the use of phytocannabinoids may adversely affect fertility and pregnancy. Further studies are indicated, the data suggests it would be wise to avoid use of such agents in reproductive animals (Pirone et al. 2017).

Felines with hypersensitivity dermatitis were found to have a proliferation of CB receptors and PPAR-α receptors. Distribution throughout the healthy feline skin was mainly in the epithelial compartment. In allergic cats ECS receptor expression increased significantly with the main changes being suprabasal for CB1 and dermal for CB2 and marked expression of PPAR-α in hyperplastic epidermis and perivascular infiltrate (Miragliotta 2018).

1.10 Summary

From the research presented in this chapter, it is clear that the endocannabinoid system is present in nearly all animals and plays an integral role in maintaining homeostasis for several vital organ systems. The endocannabinoid system modulates the nervous and immune systems and other organ systems through an elegant and sophisticated system of receptors and chemical signaling molecules to relieve pain and inflammation, modulate metabolism and neurologic function, promote healthy digestive processes, and support reproductive function and embryologic

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

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Chapter 2
The Pharmacology of Cannabinoids

Greg Copas, Erik Amazonas, and Sarah Brandon

2.1 Introduction

Just over 20 years ago, cannabinoid research focused on single compounds, such as Delta-9-tetrahydrocannabinol (THC), cannabidiol (CBD), cannabinol (CBN), etc. This is a common research paradigm for compounds which can be inexpensively isolated from their parent material. However, over the last decade, the arc of cannabinoid research has shifted to investigation of full spectrum formulations containing the most abundant phytocannabinoids (CBD and THC), as well as less common cannabinoids like cannabinol (CBN), cannabigerol (CBG), and cannabichromene (CBC) among others. The terpene and flavonoid profiles of cannabis plants have also begun to garner recent interest from researchers. As the scientific community's understanding of endocannabinoid receptor functionality expands, this research field will continue to grow (Russo 2011; Booth et al. 2017).

Readers will recall the basic anatomy and function of the endocannabinoid system (ECS) from the previous chapter and only a brief review is provided here. ECS receptors, ligands, endocannabinoid synthesizing, and catabolic enzymes work in concert to provide a negative feedback mechanism and a retrograde neuronal signaling system. Ligands are synthesized in the postsynaptic neuron and bind with cannabinoid receptors located on the presynaptic terminal. Endocannabinoid membrane transporters (EMT) facilitate bidirectional endocannabinoid (eCB) movement across cellular membranes. Ligand production and receptor upregulation are directly and proportionally related to increased sympathetic tone, increased inflammatory

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mediators, and increased stress catecholamines. Bound and unbound ligands are rapidly catabolized, thus ensuring a tight regulation of ligand concentrations. Furthermore, this inherent catabolic activity promotes new ligand binding and prevents oversaturation of the receptors. Endocannabinoid signaling is tightly regulated at the level of synthesis, release, uptake, and degradation.

This internal regulatory mechanism is a critical aspect of the ECS. It dictates how the ECS discreetly responds to events impacting homeostasis and reveals system complexity within endocannabinoid signaling. Examples of this signaling complexity are demonstrated by the creation of heterodimers formed between CB1 receptors and a variety of other G-protein coupled receptor (GPCR) systems (Wager-Miller et al. 2002) such as opioid, COX/LOX, 5HT, dopamine, GABA, etc. ECS interactions and mechanisms can be enhanced and prolonged with exogenous cannabinoid supplementation.

The compounds within this negative feedback mechanism ensure that the system remains balanced. A useful way to consider a negative feedback mechanism is looking at it as a mathematical ratio. Negative feedback systems are always striving to reach a zero balance. Concentrations of both endogenous and exogenous cannabinoids remain in a continual state of flux at the synapse to help ensure and maintain this balance.

Pharmacodynamically, stimulation of the ECS produces the widest therapeutic range and fewest adverse events when a true multi-compound plant product is administered (Gallily et al. 2015). Multi-compound products may be labeled several ways, such as “full spectrum”, “complete spectrum”, “broad spectrum”, or “whole plant” to name a few. Chapter 13 addresses how to assess products for therapeutic compounds and assess a certificate of analysis (COA). This consistent response is simple mathematics: our goal is to support and supplement the feedback mechanism with a product closely resembling the ligands within the ECS to maintain the intrinsic physiologic ratios. A true whole plant or full spectrum cannabis product should be composed of concentrations of multiple phytocannabinoids and terpenes in physiologically active concentrations, which are in specific proportions to one another.

The isolate (or single compound, single molecule) products narrow the therapeutic range of the cannabinoid and increase the risk of adverse events because higher doses of isolate products are required to obtain the same therapeutic effect seen with whole plant or full spectrum products. There is a growing body of research detailing the negative response characteristics of isolate products and highlighting the importance of administering a balanced product, which will more effectively support the intrinsic equilibrium within the ECS. This is in part due to the potentiating and mitigating factors which exist between all phytocannabinoids and other molecules within the cannabis plant, most notably terpenes. The term often used to describe this interplay is synergy or the “entourage effect” (Rosenberg et al. 2015).

Isolate products can have therapeutic value. They may be useful in specific acute conditions or as an additive therapy, such as in break-through pain, post-traumatic stress disorder (PTSD) trigger events, separation anxiety, and pre- or post-ictal phases of seizures. In addition, if a patient is already receiving a whole plant product

on a regular dosing schedule or regimen, the addition of an isolate product may help alleviate and control break-through or trigger-associated events.

The addition of a CBD-only product to a patient who is receiving a whole plant product may be an effective treatment regime for those conditions where a boost of exogenous cannabinoids is required to address an acute or break-through event. After the new event has subsided, the patient should be taken off the CBD-only product, or the dosing of that product should be reduced, while remaining on a broad or whole plant product as a maintenance therapy. This modified isolate pulse dosing, based upon need, can be utilized nearly indefinitely, while decreasing potential adverse events associated with single compound therapies.

Dose is as important as the product's composition. In CBD-dominant products, such as those from hemp, dosages at or above 2 mg/kg of CBD have demonstrated alkaline phosphatase (ALP) elevation (Gamble et al. 2018; McGrath 2018). Increased ALP elevations have not been described in the literature in patients receiving low to moderate dosages (0.1–1.5 mg/kg CBD) of multi-compound products but may still occur. However, this ALP elevation may still be observed in patient populations with hepatic pathology, patients receiving therapies which cause or exacerbate hepatic stress, or in patients on multiple pharmaceuticals or supplements metabolized by the liver (Ewing et al. 2019). Despite evidence indicating low dose CBD and THC administration may be hepatoprotective, any dosing of exogenous cannabinoid products warrants therapeutic monitoring of hepatic enzymes similar to long term NSAID administration (Goyal et al. 2018).

If a moderate to high dose (>2 mg/kg) CBD isolate product is utilized, the ECS will initially respond as with a whole plant product, but receptor saturation occurs within anywhere from 4 to 12 months (depending on the ECS tone of any specific patient) which may result in reduced clinical efficacy (Uliel-Sibony et al. 2018). The risk of negative response, or the need to increase dosages to compensate for diminished patient response, is significantly amplified with single compound therapy as compared with multi-compound product administration. More research is warranted to understand this process further, particularly as it pertains to the onset of receptor saturation.

That being said, the potential for diminished response to single compound administration is multifactorial: patient sensitivity, dosage (Gallily et al. 2015), and concentration of the compound (higher potency results in a faster onset of adverse events). Though there are multiple factors to consider, the primary underlying reason for these single compound reactions, in some patients, is the rate at which the negative feedback mechanism becomes saturated and overwhelmed, preventing it from maintaining or achieving the balance required to function appropriately. Once the inherent balance of the ECS is chemically altered it may stop responding, or the response may be significantly muted, even with significantly increased dosages. In fact, increasing dosages may accelerate the imbalance due to rapid and prolonged saturation. Additionally, a significant percentage of patients who experience this single compound over-saturation response demonstrate worsening clinical signs. This response is an antagonistic receptor response and is reversible, typically within 72–96 h (based on clearance rates of CBD) (Millar

et al. 2018), without any apparent lasting negative effects. Multi-compound product administration may reduce the time required to return to normal function as it offers the ECS compounds with which it can more readily return to its preferred 'zero.'

2.2 The Endocannabinoids

Endocannabinoids (ECs) are lipid metabolites defined as endogenous ligands of cannabinoid receptors. Endogenous cannabinoid ligands are classified as unsaturated fatty acid ethanolamides, glycerol ethers, or glycerols. They are biologically active lipid molecules which activate G-protein-coupled cannabinoid receptors (CBR), as well as forming heteromers with other G-protein-coupled receptor (GPCR) systems (Morales and Reggio 2017). Just as with the other components of the endocannabinoid system (ECS), ECs are found in all vertebrates and many invertebrate species tested to date, providing an interesting perspective on cannabis and evolution (Salzet et al. 2000).

The precursors of these ligands are present in lipid membranes, liberated in between one and three rapid enzymatic steps then released into the extracellular space (Lu and Mackie, 2016). This contrasts with classical neurotransmitters, which are synthesized without stimuli and stored in synaptic vesicles. ECs exert their effects via juxtacrine, paracrine, and autocrine routes. They function as neurotransmitters, neuromodulators, and immunomodulators.

There are five currently recognized endocannabinoids, of which anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are the most researched and thus the most well-known. Other endocannabinoids are suggested, yet researchers have not agreed upon an expanded list to date (Pertwee 2015) (Table 2.1).

Endocannabinoids are found in the central and peripheral nervous systems, plasma, and peripheral tissues. Endocannabinoids exhibit different binding properties and intrinsic activity at CB1 and CB2 receptors. Anandamide behaves as a partial agonist at both CB1 and CB2 receptors but has higher affinity for the CB1 receptor (Hillard et al. 1999; Howlett et al. 2002). The intrinsic activity of anandamide at CB1 receptors is 4- to 30-fold higher than at the CB2 receptor. However, 2-arachidonoylglycerol (2-AG) is a complete agonist at both CB1 and CB2 receptors and it exhibits less affinity than anandamide for both CB1 and CB2 receptors (Stella et al. 1997; Howlett et al. 2002) (Tables 2.2 and 2.3).

2.2.1 N-Arachidonoyl Ethanolamide Anandamides

Anandamide (AEA) is an N-(polyunsaturated fatty acyl) ethanolamine (NAPE), derived from and then hydrolyzed back into arachidonic acid (Lu and Mackie,

Table 2.1 Known endocannabinoids and endocannabinoid-like compounds that act on various cannabinoid receptors and other receptors not classically associated with the endocannabinoid system

n-6 eCBs derivatives			
AEA (Anandamide) (delete:—need chemical structure png)	CB1 [1]	NAT [11]	FAAH-1 [19]
	CB2 [1]	iNAT [12,13,14]	FAAH-2 [20]
	TRPV1 [8]	NAPE-PLD [15]	NAAH [21]
	PPARα [9]	ABHD4 [16,17,18]	LOXx [22]
	PPARγ [9]	Lyso-PLD [16,17,18]	COX-2 [23,24]
	GPR55 [10]	GDE1[16,17,18]	CytP450 [25]
		PTPN22 [16,17,18]	MAGL [30]
2-AG	CB1 [1]	PLCβ [27,28]	FAAH-1 [19]
	CB2 [1]	DAGLα [29]	ABHD6 [31,32]
	TRPV1 [26]	DAGLβ [29]	ABHD12 [31,32]
			LOXx [22]
			COX-2 [23,24]
NADA	CB1 [1]	Postulate condensation between the catecholamine with AA	Slow hydrolysis of the amide bond or the methylation of catecholamine
	TRPV1 [33]		
	PPARγ [34]		
Noladin ether	CB1 [35,36]	Unknown	Unknown
	CB2 [35]		
	GPR55 [37]		
	PPARα [9]		
Virodhamine	CB1 [35,36]	Unknown	Unknown
	CB2 [35]		
	GPR55 [37]		
	PPARα [9]		
n-3 eCBs derivatives			
DHEA	CB1 [39]	Postulate as other NAEs	Postulate as other NAEs
	CB2 [39]		
	PPARγ [40]		
EPEA	CB1 [39]	Postulate as other NAEs	Postulate as other NAEs
	CB2 [39]		
	PPARγ [40]		
Monounsaturated and saturated fatty acids derivatives			
PEA	PPARα [42,43,44,45,46,47,48,49,50,51]	NAT [11]	FAAH-1 [54]
	GPR55 [52]	iNAT [12,13,14]	FAAH-2 [21]
	GPR119 [53]	NAPE-PLD [15]	NAAH [55]
		Lyso-PLD [16,17,18]	
		GDE1[16,17,18]	
		PTPN22 [16,17,18]	

(continued)

Table 2.1 (continued)

OEA	PPARα [42,43,44,45,46,47,48,49,50,51]	NAT [11]	FAAH-1 [54]
	GPR119 [53]	iNAT [12,13,14]	FAAH-2 [21]
	GPR55[52]	NAPE-PLD [15]	NAAH [55]
		ABHD4 [16,17,18]	
		Lyso-PLD [16,17,18]	
		GDE1 [16,17,18]	
		PTPN22 [16,17,18]	
2-OG	GPR119 [53]	PLCβ [27,28]	MAGL [30]
		DAGLα [29]	FAAH-1 [54]
		DAGLβ [29]	

2016). It is the product of the formal condensation of the carboxyl group of arachidonic acid with an amino group of ethanolamine.

2.2.1.1 Biosynthetic Pathway

Anandamide is obtained from the one- to three-step enzymatic hydrolysis of a family of minor membrane phospholipids, N-arachidonoyl-phosphatidylethanolamines (NArPE). N-acyl-phosphatidylethanolamine specific phospholipase D (NAPE-PLD), an enzyme that has little in common with other phosphodiesterases, is Ca^{2+}-sensitive and catalyzes the hydrolysis of NArPE directly to anandamide (Okamoto et al. 2004).

NArPE biosynthesis is catalyzed by a membrane enzyme, N-acyltransferase (NAT), and is activated by Ca^{2+} and cyclic adenosine monophosphate (cAMP). Additionally, the sequential action of α,β-hydrolase-4 (ABHD4) and glycerophosphodiesterase-1 (GDE1) catalyzes the conversion of NArPE into 2-lyso-NArPE first, then to glycerophospho-anandamide and, finally, anandamide (Simon and Cravatt 2008). Formation of 2-lyso-NArPE can also occur through the action of a soluble phospholipase A2, followed by direct conversion into anandamide by a lyso-PLD (Sun et al. 2004). Finally, an as-yet-unidentified phospholipase C (PLC), followed by the action of various phosphatases (such as protein tyrosine phosphatase N22 or SH2 domain-containing inositol phosphatase), can convert NArPE first into phospho-anandamide and then anandamide (Di Marzo and De Petrocellis 2012).

Table 2.2 This table illustrates the known tissues in dogs and cats where endocannabinoid system receptors and other receptors that integrate heavily with the endocannabinoid system have been located

Species	Receptor	Organ/tissue/cell
Dog	CB1	CNS Hippocampus Claustrum Cerebral cortex Cornu Ammonis Midbrain Cerebellum Medulla oblongata Spinal cord Astrocytes PNS Dorsal root ganglia (neurons, satellite cells) Schwann cells Skin Keratinocytes Hair follicles Sebaceous glands Sweat glands Mast cells Fibroblasts Gastrointestinal tract Salivary glands Lamina propria cells Epithelial cells
	CB2	Lymph nodes B cells Skin Keratinocytes Hair follicles Sweat glands Sebaceous glands Mast cells Fibroblasts Endothelial cells CNS Astrocytes Spinal cord (glial cells) Gastrointestinal tract Mast cells Immunocytes Blood vessels Smooth muscle cells Submucosal plexus (neurons and glial cells) Spleen

(continued)

Table 2.2 (continued)

Species	Receptor	Organ/tissue/cell
	GPR55	Gastrointestinal tract Macrophages Smooth muscle cells
	PPARα	Gastrointestinal tract Blood vessels Smooth muscle cells Myenteric plexus (glial cells)
	TRPV1	Keratinocytes
Cat	CB1	CNS Hippocampus Cerebral arterial smooth muscle cells Skin Keratinocytes Sebocytes Hair bulb cells Macrophages Ovary Oviduct
	CB2	Skin Keratinocytes Sebocytes Hair bulb cells Sweat glands Lymph node macrophages
	PPARα	Skin Keratinocytes Hair follicle Dermal papillae

2.2.1.2 Catabolism

Anandamide is catabolized by two intracellular enzyme pathways. The enzymes responsible are fatty acid hydrolase-1 (FAAH-1, expressed in all mammals) and, to a much lesser extent, fatty acid hydrolase-2 (FAAH-2, not expressed in rodents) (Di Marzo and De Petrocellis 2012). FAAH is the subject of numerous research studies and clinical trials as a method to increase concentrations of anandamide to increase ECS tone. One experimental approach is designed to block FAAH production, similar to the action of serotonin reuptake inhibitors (SSRIs), in an attempt to slow and/or decrease the catabolism of anandamide, thus increasing its circulating concentration. This approach has had little success to date. Additionally, anandamide is also metabolized by lipoxygenases (LOXs) (Van der Stelt et al. 2002) and cyclooxygenase-2 (COX-2) (Rouzer and Marnett 2011; Funk 2001), and can be oxygenated by the CYP450 pathway (Snider et al. 2010) (Fig. 2.1).

Table 2.3 List of various cannabinoids, cannabinoid-like and synthetic analogs

Endocannabinoids	Endocannabinoid-like compounds	Phytocannabinoids	Synthetic cannabinoids
Anandamide	Palmitoylethanolamide	Delta-9-tetrahydrocannabinol	Dronabinol
2-Arachidonoylglycerol	N-docosatetraenoylethanolamine	Delta-8-tetrahydrocannabinol	Nabilone
Noladin ether	Di-homo-γ-linolenoyl-ethanolamide	Cannabidiol (CBD)	Rimonabant
Virodhamine	2-Oleoylglycerol	Cannabinol (CBN)	Methanandamide
Arachidonoyldopamine	N-oleoylethanolamine	Cannabigerol (CBG)	JWH-0133
N-arachidonoylserine	N-eicosapentaenoylethanolamine	Cannabichromen (CBC)	AM-251
Homo-γ-linolenoylethanolamide	N-docosahexaenoylethanolamine	Cannabivarin (CBV)	ACEA/ACPA
7,10,13,16-Docosatetraenoylethanolamide	Oleamide	Cannabielsoin (CBE)	WIN 55,212-2
	N-arachidonoylglycine	Cannabitriol (CBT)	CP 55,940
	1-Arachidonoylglycerol		HU-210
	N-arachidonoyltaurine		

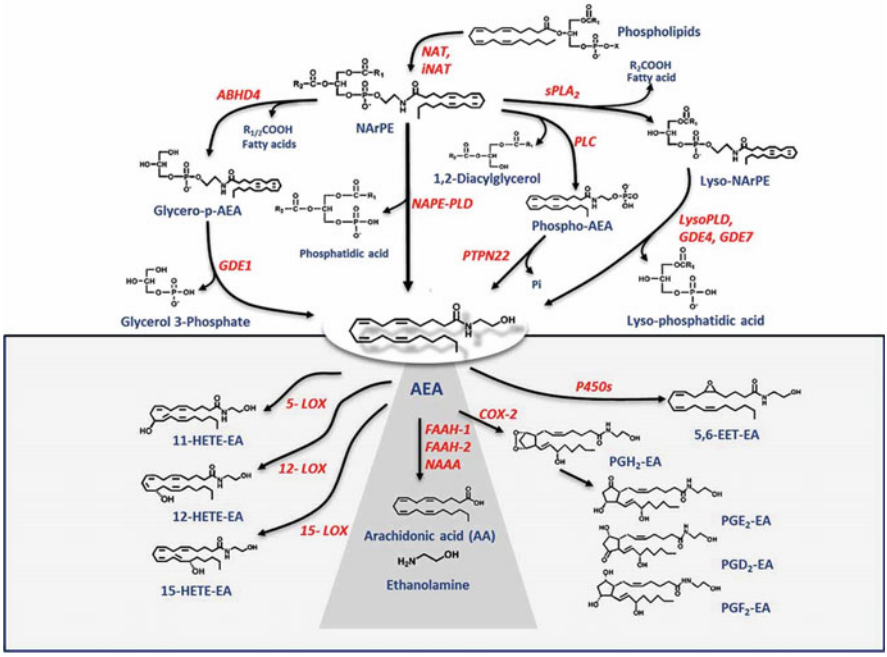

Fig. 2.1 Metabolic pathways of anandamide (AEA) (Permission to reproduce with full citation, Maccarrone, M. (2017). Metabolism of the Endocannabinoid Anandamide: Open Questions after 25 Years. *Frontiers in Molecular Neuroscience, 10*. doi:10.3389/fnmol.2017.00166)

2.2.1.3 Functions

Anandamide functions similarly to THC at the CB1 receptor. Anandamide exhibits high binding affinity for, and acts as a partial agonist of, the CB1 receptor. Although anandamide has more binding affinity for the CB1 receptor, it also binds with the CB2 receptor as a partial agonist. It acts as a vanilloid receptor (TRPV1) partial agonist and exhibits binding affinity for GPR55, GPR119, peroxisome proliferator-activated receptors (PPARα, β and γ), and possibly toll-like receptor 4 (TLR4) (O'Sullivan 2016; Cruz et al. 2018). Its secondary and tertiary metabolites impart physiologic effects via ECS receptors, as well as other receptors with which heteromers are formed (Ritter et al. 2016). Anandamide acts as a retrograde messenger at presynaptic cannabinoid receptors (CB1), where it regulates neurotransmitter release through its secondary transduction systems (mainly Ca^{2+} incorporated through voltage-gated calcium channels (VGCC) or glutamate NMDA (N-methyl-D-aspartate) receptors). Anandamide also acts as a neuromodulator of major transmitter systems, including dopamine, at postsynaptic cells, where it regulates excitability and synaptic plasticity through its modulation of potassium (K^+) channels and its regulation of a broad spectrum of protein kinases (PK), including protein kinase A and mitogen-activated protein kinases (MAPK) (De Fonseca et al. 2005).

Anandamide demonstrates important anti-proliferative, anti-inflammatory, and analgesic properties both in vivo and in vitro in animal models (Nagarkatti et al. 2009). Modulation of anandamide levels in the gut has demonstrated potential for treatment of inflammatory bowel disease and colon cancer (Alhouayek and Muccioli 2012). Also, anandamide derived from macrophages demonstrates anti-inflammatory effects both in the peripheral and central nervous systems (Nagarkatti et al. 2009). Anandamide significantly decreases blood pressure and heart rate (Hiley 2009). In addition, it is an anabolic regulator of metabolism. It increases the intake of food, promotes the storage of lipids, and decreases the expenditure of energy; it is also involved in the regulation of body temperature, locomotion, feeding behaviors, fear, and anxiety. In response to bacterial endotoxins, macrophages produce anandamide where it is involved in the pathology of septic shock and liver cirrhosis. This production of anandamide is suspected of causing significant hypotension in these patients (Liu et al. 2006). Anandamide is present in the reproductive fluids of both males and females and is believed to be important in reproduction (Sun and Sudhansu 2012; Meccariello et al. 2014). Apoptosis in several cell types is controlled directly and indirectly by anandamide (Maccarrone et al. 2000). Anandamide (and 2-AG and perhaps other long-chain ethanolamides) can modulate the activity of several antioxidant enzymes and control redox homeostasis by targeting CB and other receptors. It is considered an endovanilloid ligand due to its EC and TRPV interactions.

2.2.2 2-Arachidonoylglycerol (2-AG)

2-Arachidonoylglycerol (2-AG) is a monoglyceride that was first described in 1995 after isolation from the canine gastrointestinal tract and whose formation is closely associated with the metabolism of triacylglycerol, mainly by the receptor-dependent activation of phosphatidylinositol-specific phospholipase C (PLC). The standard model proposes that activation of metabotropic receptors coupled to the PLC and diacylglycerol (DG) lipase pathway will systematically lead to increases in 2-AG production (Stella et al. 1997). 2-AG formation is dependent upon Ca^{2+} and is regulated independently of anandamide synthesis and release.

2.2.2.1 Biosynthetic Pathway

Three major pathways have been proposed for 2-AG synthesis. The first is the production of 2-AG via a two-step process, starting with phosphatidylinositol 4,5-bisphosphate (PIP2), proceeding to the intermediate diacylglycerol (DAG), and finally, 2-AG (Farooqui et al. 1989). The first step is catalyzed by a phospholipase C-β (PLCβ) (Farooqui et al. 1989), whereas the second step is catalyzed by one of two diacylglycerol lipases (DAGLs) (Bisogno et al. 2003; Tanimura et al. 2010). This pathway appears to dominate in the CNS (Kano et al. 2009). The second

pathway involves the conversion of phosphatidyl lipid (PI) to 2-arachidonoyl-lyso PI by the action of a PLA1, and then to 2-AG by the action of lyso-PLC (Higgs and Glomset 1994). The third pathway involves LPA hydrolysis by an LPA phosphatase (Nakane et al. 2002). The involvement of these latter two pathways in the production of 2-AG in the CNS has not been evaluated in detail but may account for some reports of endocannabinoid-mediated synaptic plasticity that is insensitive to DAGL inhibitors (Zhang et al. 2011).

2.2.2.2 Catabolism

Monoacylglycerol lipase (MAGL) is the main catabolic enzyme responsible for 2-AG degradation. Two additional serine hydrolases, α/β-hydrolases domain 6 (ABHD6, with a postulated catalytic triad S148-D278-H306) and 12 (ABHD12, with a postulated catalytic triad S246-D333-H372), are involved in 2-AG hydrolysis (Blankman et al. 2007; Marrs et al. 2010). Of note, MAGL, ABHD6, and ABHD12 show distinct distribution within the CNS (Marrs et al. 2010; Savinainen et al. 2012), suggestive of a different physiological function of these three enzymes in regulating 2-AG signaling (Marrs et al. 2010; Tchantchou and Zhang 2013). In support of this view, anti-inflammatory effects of ABHD6 inhibition, without the deleterious side effects typically associated with MAGL inhibition, have been recently reported (Alhouayek et al. 2013). As with anandamide, 2-AG is metabolized by lipoxygenases (LOXs) (Van der Stelt et al. 2002) and cyclooxygenase-2 (COX-2) (Rouzer and Marnett 2011; Funk 2001). There may be some degradation activity by FAAH, but this is still being investigated.

2.2.2.3 Functions

2-AG functions as a full agonist at both the CB1 and CB2 receptors. Many of the functions of 2-AG are integral in brain metabolism. It is believed to act as a messenger molecule, modulating transmission signaling across synapses. At excitatory synapses, 2-AG creates a 'signalosome' consisting of a supra-molecular complex in a single functional unit containing three key players: phospholipase Cβ, an activator protein designated mGluR5, and DAGLα. One action of this activated complex and 2-AG synthesis is the depression of excitatory synaptic signaling by preventing excessive activation of glutamate receptors. 2-AG enables neuronal remyelination by enhancing the differentiation of oligodendrocyte progenitor cells and increasing clearance of myelin debris by microglia. Modulating effects of 2-AG are observed in energy metabolism, food intake, anxiety, depressive behaviors, and addiction.

2-AG regulates several CNS, PNS, and immune inflammatory processes (Du et al. 2011). It demonstrates activity suppression and expression of COX-2, as well as being involved in pain pathways via the ECS and other receptors systems. In platelets and macrophages, 2-AG is produced in response to injury, and its

production and action decreases pro-inflammatory mediators. Finally, it exhibits antiproliferative and invasion modulation capabilities. The result is that 2-AG exerts anti-cancer activities by inhibiting the proliferation, migration, and/or invasion of cancer cells. It also has activity which inhibits tumor angiogenesis (Peruzzotti-Jametti et al. 2014).

2.2.3 N-Arachidonoyl Dopamine

N-Arachidonoyl Dopamine (NADA) is an arachidonic acid derivative with a dopamine moiety in its structure. To date, it has been found in multiple areas of the CNS, particularly where dopamine is either produced and or utilized. Dopamine levels seem to be a reaction limiting step in synthesis.

2.2.3.1 Biosynthetic Pathway

Research on the synthesis and catabolism of this endocannabinoid is in its infancy. The following are potential synthesis and degradation pathways of NADA, including its targets. The first proposed pathway involves N-arachidonoyl tyrosine, which can be metabolized by tyrosine hydroxylase (TH) to N-arachidonoyl DOPA and by l-amino acid decarboxylase (AADC) to NADA. The second pathway describes the formation of NADA from arachidonic acid and dopamine by FAAH (Grabiec and Dehghani, 2017).

2.2.3.2 Catabolism

Currently, there are three different postulated ways in which NADA inactivation occurs: Catechol-O-methyl-transferase (COMT) mediates its transformation to an O-methyl derivative; fatty acid amide hydrolase (FAAH) hydrolases NADA to arachidonic acid; dopamine and cytochrome P450 (CYP450) pathway metabolizes NADA to omega hydroxylated metabolites (HETE-dopamine) (Grabiec and Dehghani 2017).

2.2.3.3 Functions

NADA acts mainly through CB1 and TRPV1 receptors (Sagar et al. 2004). It is considered an endovanilloid ligand due to its ECS and TRPV interactions (Redmond 2016; Wilhelmsen et al. 2014). It participates in several physiological activities in the body. NADA is neuroprotective, acts on immune cells, mediates pain response, regulates vasorelaxation, and inhibits platelet aggregation. NADA seems to affect the proliferation/migration and actions of immune cells, especially microglia.

NADA potentially mediates both anti- and pro-nociceptive responses depending on the balance between CB1 receptor and TRPV1 channel activation and the type of stimulus. A better understanding of NADA-mediated actions may lead to development of novel therapies in acute neurological disorders and in neuroinflammatory pain. However, we need to first understand exactly how its synthesis and degradation occur and in which cell type these processes take place, as well as more about the function of endogenous NADA (Wilhelmson et al. 2014; Grabiec and Dehghani 2017).

2.2.4 2-Arachidonyl Glycerol Ether (Noladin Ether, 2-AGE)

2-AGE belongs to the group of glycerol-type endocannabinoids like 2-AG. It is an ether formed from the alcohol analog of arachidonic acid and glycerol. No biosynthetic or catabolic pathways have been identified. It acts as an agonist or partial agonist at CB1, CB2, TRPV1 and GPR55 receptors. It may play a role in inflammation, analgesia, appetite regulation, and neuroplasticity (Deshmukh and Sharma 2012; Páldyová et al. 2008).

2.2.5 O-Arachidonoyl Ethanolamine (Virodhamine)

Virodhamine is arachidonic acid and ethanolamine joined by an ester linkage. It is not yet clear how virodhamine is stored, produced, or degraded. Virodhamine demonstrates partial agonist/antagonist activity at the CB1 receptor and agonist activity at the CB2 receptor (Pertwee 2014). In the periphery, the concentration of virodhamine is higher than anandamide, especially in tissues expressing CB2 receptors. Therefore, the potential exists that virodhamine plays a significant role in the periphery as a full agonist at the CB2 receptor.

There are several other compounds which have ECS activity. These include the omega-3 fatty acid ethanolamides, docosahexaenoyl ethanolamide (DHEA), and eicosapentaenoyl ethanolamide (EPEA). Lastly, there are the monounsaturated and saturated fatty acid derivatives palmitoylethanolamide (PEA), oleoylethanolamine (OEA), and 2-oleoylglycerol (2-OG).

2.3 Phytocannabinoids

Phytocannabinoids are exogenous cannabinoids produced by all species of the *Cannabis* genus. They exhibit a typical C_{21} terpenophenolic skeleton and are produced by differentiation of several common originator compounds found in the plant. Additionally, this term encompasses their derivatives and biotransformation products (Gertsch et al. 2010). To date, 142 phytocannabinoids have been identified.

They are produced in two specific areas in the *Cannabis* plant: within the resin produced by surface trichomes and by Bast cells which are the structural cells found within the main and secondary plant stems and branches (Andre et al. 2016). In the fresh plant, these compounds exist in acidic form which is decarboxylated via removal of a carboxyl group (COOH) by exposure to heat, light, oxygen, and or alkaline and acidic conditions.

2.3.1 Individual Phytocannabinoids

Studies have recently begun on several phytocannabinoids including: cannabicyclol (CBL), cannabichromevarin (CBCV), cannabicyclolvarin (CBLV), cannabielsoin (CBE), cannabivarin (CBV), cannabidivarin (CBDV), cannabitriol (CBT), cannabitriolvarin (CBTV), cannabifuran (CBF), and cannabiripsol (CBR) (Turner et al. 2017). There is limited data on these compounds outside basic biosynthetic and degradative pathways but research on these and other phytocannabinoids will inevitably increase with the shifting legal climate.

The precursors of phytocannabinoids originate from two distinct biosynthetic pathways: the polyketide pathway, giving rise to olivetolic acid (OA) or divarinic acid (DA), and the methylerythritol phosphate pathway, leading to the synthesis of geranyldiphosphate (GPP). The biogenesis of phytocannabinoids containing a n-pentyl side chain starts with the condensation of OA and GPP into cannabigerolic acid (CBGA) which is catalyzed by geranyl pyrophosphate-olivetolate geranyl transferase (GOT). Isoprenylation is followed by the activity of three corresponding oxidative cyclases which generate tetrahydrocannabinol acid (THCA), cannabidiolic acid (CBDA), and cannabichromenic acid (CBCA) from CBGA, the key intermediate molecule. Phytocannabinoid acids are also non-enzymatically decarboxylated into cannabigerol (CBG), delta-9-tetrahydrocannabinol (delta-9-THC), cannabidiol (CBD), and cannabichromene (CBC) (Aizpurua-Olaizola et al. 2016; Andre et al. 2016) (Fig. 2.2).

2.4 Pharmacokinetics

All phytocannabinoid compounds found in *Cannabis* species are highly lipophilic. This lipophilic property translates into compounds which are easily absorbed into the body but difficult to eliminate without significant biotransformation. In some studies, more than one plasma peak was observed, resulting in a biphasic absorption curve (Law et al. 1984). This can be explained by the lipophilic nature of these compounds, the need for extensive biotransformation, and significant enterohepatic circulation (Musshoff and Madea 2006). Route of administration, vehicle, dose, and physiological factors, such as absorption and rates of metabolism and excretion, influence the concentration of drugs in circulation, and these same factors apply to concentrations of phytocannabinoids in circulation.

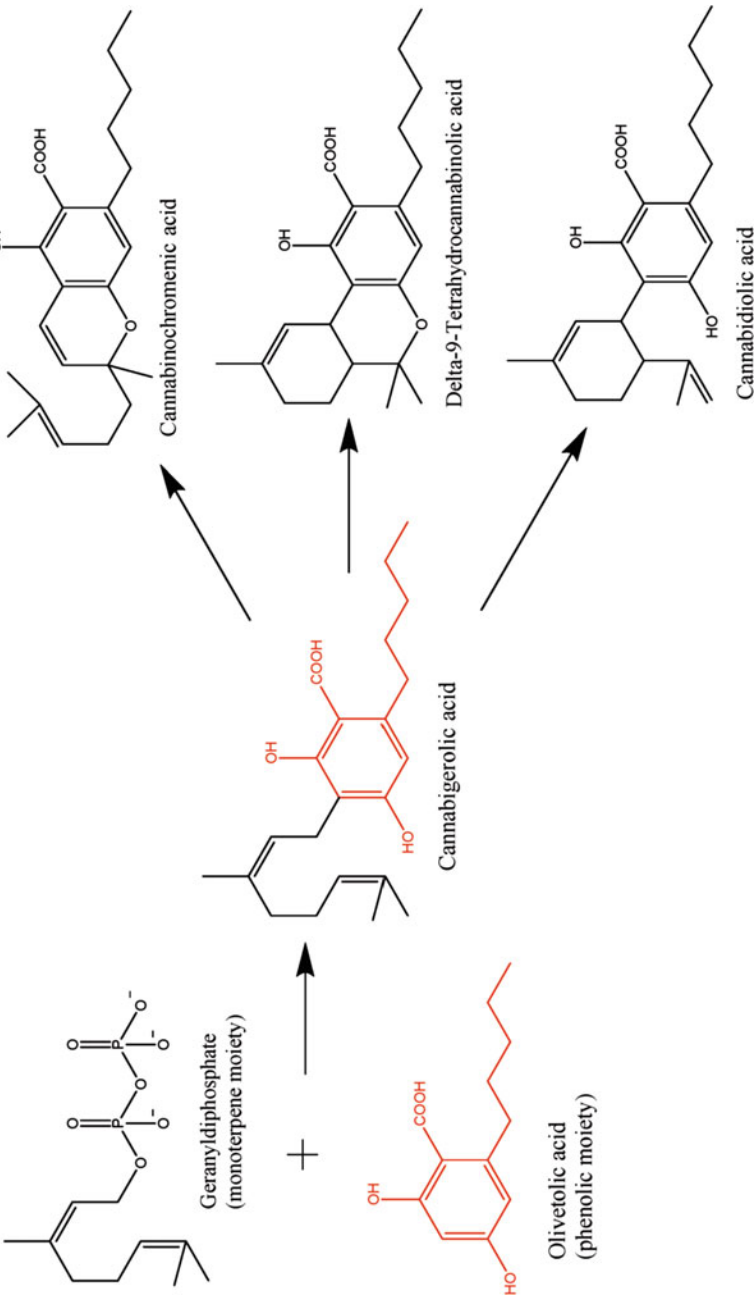

Fig. 2.2 Key steps in the biosynthesis of phytocannabinoids (Permission to reproduce with full citation, Kis, B., Ifrim, F. C., Buda, V., Avram, S., Pavel, I. Z., Antal, D., . . . Danciu, C. (2019). Cannabidiol—from Plant to Human Body: A Promising Bioactive Molecule with Multi-Target Effects in Cancer. *International Journal of Molecular Sciences, 20*(23), 5905. doi:10.3390/ijms20235905)

Systemic absorption after oral administration is slow and usually takes 1–2 h. Other routes of administration, which bypass biotransformation, result in quicker absorption and higher overall plasma concentrations (Pertwee 2014). However, quicker absorption, without biotransformation, can lower half-life. Bypassing biotransformation increases bioavailability but can lead to response inconsistencies which make alternative routes of administration difficult to formulate for maximum effectiveness and minimal adverse events. Oral bioavailability ranges between 6 and 20+% (Samara et al. 1988; Bartner et al. 2018). This range may be due to variable absorption, degradation of drug in the stomach, and/or significant first-pass metabolism resulting in active and inactive metabolites in the liver. When administered orally, as compared to inhalation, onset is delayed, peak concentrations are lower, and duration of pharmacodynamic effects generally are extended with a delayed return to baseline (Huestis 2006).

Most pharmacokinetic studies to date have focused on smoked or inhaled compounds in humans. However, an ever-increasing quantity of research is focusing on topical, oral, oromucosal, transcutaneous, rectal, intravaginal, intramuscular, and intravenous routes of administration in both humans and animals.

Peak plasma concentrations decrease rapidly, due to the efficient distribution into several compartment stores (central, peripheral, and special). Furthermore, hepatic metabolism causes a rapid decrease in plasma levels. These compounds equilibrate rapidly into adipose tissue, brain, heart, liver, lung, and spleen. Slower equilibration results in detectable concentrations in skeletal muscle. Although peak plasma levels decrease rapidly, the compartments into which phytocannabinoids are distributed act as reservoirs, which release these compounds back into circulation over an extended period. This reservoir archetype is one factor responsible for the extended clearance rates observed in PK studies. Tissues with lower perfusion accumulate these compounds more slowly and release them over an extended period. 90–95% of these compounds are protein bound.

In a THC porcine study (Brunet et al. 2006), the volume of distribution (V_d) was measured 0.5 h in various tissues after intrajugular administration of 200μg/kg. The results: blood 24μg/kg, kidney 272μg/kg, heart 178μg/kg, lung 1888μg/kg, muscle 55μg/kg, spleen 34μg/kg, fat 91μg/kg, liver 155μg/kg, brain 49μg/kg, bile 0.4μg/kg, and vitreous humor 1.2μg/kg. THC was eliminated most rapidly from the liver and was unmeasurable in liver tissue after 6 h (<5μg/kg). THC concentrations decreased more slowly in the brain as compared to blood but at the 6-h mark were only 9% of the levels seen at the 0.5 h mark. Fat had the highest THC retention, with detection beyond 24 h. 11-OH-THC (a primary THC metabolite) was only found in the liver, and THCCOOH was less than or equal to 5μg/kg in most tissues.

Hepatic metabolism, primarily microsomal hydroxylation and oxidation catalyzed by enzymes of the cytochrome P450 (CYP) complex, are the most common metabolic routes for these compounds. The brain, intestines, and lungs are the primary extrahepatic metabolic sites. More than 100 THC metabolites, including di- and trihydroxy compounds, ketones, aldehydes, and carboxylic acids, have been identified (Harvey 2001). Similarly, more than 100 CBD metabolites have been identified. The primary metabolic route for CBD is hydroxylation to 7-OH-CBD,

which is then metabolized further, resulting in the major CBD metabolites: 6α-OH-, 6β-OH-, 7-OH-, and 4″-OH-CBDs (Geneva, 4–7 June 2018, WHO conference committee). CBD is a potent inhibitor of CYP 450. The level of inhibition may be dose dependent. Extrahepatic metabolism of these compounds readily occurs and is mediated by the cytochrome families 1−4, which primarily metabolize xenobiotics. Cytochromes are found in decreasing concentrations within the liver, small intestine, peripheral blood, bone marrow, and mast cells, with the lowest concentrations in the brain, pancreas, gallbladder, kidney, skin, salivary glands, and testes. Within the brain, higher concentrations of CYP 450 enzymes are found in the brainstem and cerebellum (Krishna and Klotz 1994).

The secondary and tertiary metabolites are often more physiologically active than the administered primary compounds, due to the high level of cross reactivity with other G-protein coupled receptor systems. For example, carboxylic THC (11-COOH-THC), is a secondary biotransformation metabolite of THC and is water soluble (Ritter et al. 2016). It may be more inebriating than THC and imparts significant analgesic, anti-inflammatory, and antiproliferative properties within hydrophilic tissues.

The elimination timeline for these compounds is highly variable and nonlinear. It is dependent upon the individual compounds and from where and when test samples are obtained. The terminal half-life for THC in humans is approximately 4.1 days (Smith-Kielland 1999). Additionally, current testing methodologies are limited in their ability to detect and differentiate primary, secondary, and tertiary compounds, making compound identification, biochemical pathway involvement, cross-reactivity, and measurement difficult. Elimination ranges from 4 to 6 h in plasma and highly perfused tissues, to more than 12 days in higher dose administrations and less perfused tissues. 65% of *Cannabis* compounds are eliminated in the feces and 25% are eliminated via the renal system. Unlike THC, a significant portion of CBD is excreted unchanged in feces (Pertwee 2014). Numerous acidic metabolites are found in the urine, many of which are conjugated with glucuronic acid to increase their water solubility. The primary urinary metabolite is the acid-linked THC-COOH glucuronide conjugate (Williams and Moffat 1980), while 11-OH-THC predominates in the feces.

A common problem encountered when studying the pharmacokinetics of cannabinoids is the need for highly sensitive procedures to measure low cannabinoid concentrations in the terminal phase of excretion, and the requirement for monitoring serum concentrations over an extended period to adequately determine cannabinoid half-lives. Many studies utilize short sampling intervals of 24−72 h that underestimate terminal THC and THC-COOH half-lives. The slow release of THC from lipid-storage compartments and significant enterohepatic circulation contribute to a long terminal half-life of THC and other compounds in plasma (Marilyn A. Huestis 2007).

In a canine study conducted by Wakshlag et al., 24-h pharmacokinetics for CBD, CBDA, THC and THCA were similar regardless of the delivery form (oil vs chew). CBDA and THCA concentrations were two- to three-fold higher than CBD and THC concentrations, respectively. The 1- and 2-week steady state concentrations of

phytocannabinoids were not statistically different between the two different oil carriers or the soft chew forms, except for CBDA and THCA. Oil containing lecithin showed superior steady state absorption/retention of CBDA and THCA, while a non-lecithin-based oil demonstrated less THCA retention. 11-OH-THC was below the quantitation limit of the assay for nearly all samples. Overall, these findings suggest CBDA and THCA are absorbed more efficiently than decarboxylated CBD or THC, and that a partial lecithin base may provide superior absorption of CBDA and THCA. It is interesting to note that no ALP elevations were detected in the canine subjects despite administering 2–2.5 mg/kg CBD twice daily for up to 2 weeks as seen in a few clinical studies with dogs. Based on the current understanding of the entourage effect, it is likely that using multiple compounds offers hepatoprotective properties. Further study is warranted, particularly as testing capabilities allow for lower limits of quantitation and different mediums are assessed for absorption and retention of acid vs decarboxylated cannabinoids (Wakshlag et al. 2020).

In a feline study using 20 healthy subjects that were divided into five groups received either a placebo MCT oil, CBD in MCT oil (up to 30.5 mg/kg), THC in MCT oil (up to 41.5 mg/kg), sunflower oil placebo or CBD/THC in sunflower oil (up to 13 mg/kg CBD and 8.4 mg/kg THC). The phytocannabinoids, CBD and THC, were present in addition to their common metabolites 7-COOH-CBD and 11-OH-THC in the plasma. Interestingly, this study showed possible pharmacokinetic potentiation between CBD and THC at the tested ratio. Plasma levels of both CBD and THC after treatment with the CBD/THC combination product revealed CBD levels were approximately 2-fold, and 1.3–2.9-fold higher for THC, respectively. The plasma levels of phytocannabinoid metabolites were also elevated by approximately 1.7–2.0-fold (7-COOH-CBD) and 2.3–4.2-fold (11-OHTHC) with the combination product. Blood chemistry analysis in this study focused on liver markers (ALP, ALT, AST, GGTP) which remained within the normal reference ranges for all doses and cats with the exception of one cat in the placebo MCT oil group who had an elevation in ALT that normalized after one week of the final dose (Kulpa et al. 2021). This is similar to a transient ALT elevation seen by Deabold et al. (2019) where one out of eight cats showed a transient elevation in ALT after a 2 mg/kg dose, twice daily dosing of a CBD/CBDA full spectrum product in fish oil at week four. Overall, absorption, metabolite production and safety of relatively high doses of CBD and THC appear to be well tolerated with adverse events occurring as doses escalate in the domestic cat.

2.5 Pharmacodynamics

The distribution of CB1 and CB2 receptors is well documented and only summarized here (Mackie 2008). The CB1 receptor is primarily found in the central nervous system, peripheral nervous system, and autonomic nervous system. The CB1 receptor is one of the most abundant and widely expressed G protein-coupled receptors in the mammalian brain. CB1 receptors are expressed in the olfactory bulb, neocortex,

entorhinal cortex, piriform cortex, hippocampus (extensive concentration), amygdala (extensive concentration), several parts of basal ganglia, thalamic and hypothalamic nuclei, cerebellar cortex, neocortex, medial prefrontal cortex (vMPFC), cornu ammonis (CA), nucleus accumbens (NAcc), dentate gyrus, medulla oblongata, brainstem nuclei, and grey matter of the spinal cord (Freundt-Revilla et al. 2017).

In subcortical regions, CB1 receptors are present at relatively high levels in the septal region (lateral and medial septum, and vertical and horizontal nuclei of the diagonal band). Dense expression is found in the fibers of the globus pallidus and substantia nigra surrounding immunonegative neurons. Some expression is observed in the medial and lateral preoptic hypothalamic nucleus, magnocellular preoptic nucleus, and paraventricular nucleus (PVN). In the caudal hypothalamus, CB1 receptor expression is demonstrated in the premammillary nucleus. In the lateral hypothalamus, CB1 receptors are present in scattered cells. In the thalamus, CB1 receptors are present in the lateral habenula, reticular thalamic nucleus, and zona incerta. There are some CB1 receptors present in tyrosine hydroxylase-expressing neurons in the ventral tegmental area (VTA) and in dopaminergic terminals in the striatum. CB1 receptors are also found in the anterior and intermediate lobes of the pituitary gland. Astrocytes, satellite cells of the dorsal root ganglia, and myelinated Schwann cells express moderate to dense concentrations of CB1 receptors as well. Finally, thyroid, adrenal, male and female reproductive system, hepatic, adipocytes, pulmonary, renal, myocardium, vascular, gastrointestinal, and neuronal tissues all contain CB1 receptors (Walker et al. 2019).

The distribution of CB2 receptors is primarily found within the immune system, spleen, tonsils, thymus, gastrointestinal tract, osteocytes, monocytes, macrophages, microglia, B-cells, and T-cells. However, CB2 receptors are also found in varying concentrations in the following areas: cerebral cortex, hippocampal pyramidal cells, globus pallidus, cerebellar Purkinje cells, cerebellar granule cells, cerebellar nuclei, vestibular nuclei, dorsal motor nucleus of the vagus nerve, nucleus ambiguus, spinal trigeminal nucleus, substantia nigra, and spinal sensory neurons (Magid et al. 2019). Immunohistochemical analysis has elucidated the distribution of cannabinoid receptors CB1, CB2, G protein-coupled receptor 55 (GPR55), and peroxisome proliferation activation receptor alpha (PPARα) in the canine gastrointestinal tract (Stanzani et al. 2020).

Within the immune system and the inflammatory cascade, CB2 receptors exhibit both cellular and humoral modulation. The following cellular hierarchy represents immune cells which either produce endocannabinoids or present cannabinoid receptors on their cell surface or both: macrophages, monocytes, natural killer cells (NKC), lymphocytes, mast cells, CD8+, and CD4+. Other cells which interrelate with the ECS are: leukocytes, B cells, dendritic cells, platelets, microglia, and Kupffer cells. Humoral ECS interaction is observed with the following interleukins: IL1-α/β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-14, IFNγ, and TNFα (Basu and Dittel 2011). The ECS is also involved in the protein kinase C (PKC) pathway and, as currently understood, to a lesser degree in protein kinase A and B pathways.

Two receptors were recently discovered and the extent of their distribution and effects is currently being researched. CB4 receptors appear to be predominantly

located in endothelium and the function of this receptor is not fully understood; it may be involved in vascular tone and endothelial cytokine interaction. The CB6 receptor and its distribution is unclear, but it appears to act in an anti-inflammatory capacity, though in a different manner than either the CB1 or CB2 receptors.

2.6 ECS Receptor Response

Endocannabinoid (eCB) receptors have variable responses depending upon ligand concentration. Nearly all ligands which interact with eCB receptors can produce either a partially agonistic, inversely agonistic, agonistic, or antagonistic response. Usually, the higher the dose administered, and thus higher ligand concentration at the receptor, will result in an inverse or antagonistic response.

This receptor response variation and effects from oversaturation are two causes of adverse events which whole plant products at low to moderate dosages do not seem to impart. It is the author's hypothesis that high doses of a whole plant product may have similar effects, but the timeline will be different than with administration of an isolate product. As previously stated, isolate products increase the risk of negative response while decreasing the therapeutic range.

The synergistic effect imparted by a whole plant product helps reduce and/or eliminate this ligand concentration associated receptor response and decreases the likelihood of receptor or pathway over-saturation. The complex interplay between dose, type of product, ligand concentration, PK, receptor response, ECS tone, receptor cross-reactivity, and metabolite cross-reactivity are among the reasons why these compounds are difficult to accurately dose in a generalized manner. These compounds work best when administered together in specific ratioed concentrations. Moreover, they should be started in a 'low and slow approach' and tailored for each patient. Adhering to these simple guidelines provide the best response and fewest side effects.

An example of moderate to high dose isolate administration and feedback loop oversaturation is potentially the emesis syndrome phenomenon, observed recently in human emergency facilities, termed Cannabis Hyperemesis Syndrome (CHS) (Galli et al. 2011; Richards 2018). In CHS, patients present with severe to extreme nausea, vomiting, and gastrointestinal distress. These patients, prior to presentation, were using high concentration THC products over the course of several hours to days. The adverse response is multifactorial due to an oversaturation of the negative feedback mechanism and an inverse agonistic or antagonistic response to high THC concentrations at eCB receptors. THC, at low to moderate doses, normally produces antiemetic, anti-nausea, and smooth muscle relaxant effects. However, if the THC dose, or any other compound found in the *Cannabis* genus, is increased, users begin to see opposite and idiosyncratic pharmacodynamic effects, due to receptor oversaturation and increased ligand concentration at the receptor. The adverse effects caused by these two factors can be dramatic. These individuals do not appear

to experience these adverse reactions if utilizing a low to moderate dose of a true whole plant product.

A final example of dose-related response is sedation. CBD is a potent sedative and when administered as an isolate; the sedation threshold is much lower than if CBD is administered in conjunction with THC and several other *Cannabis* compounds, which greatly mitigate the sedative effects of CBD (Niesink 2013). The inverse is true with THC, where CBD blocks the dysphoric effects of THC and potentiates positive effects. Based on current research, it appears all *Cannabis* compounds work in a similar manner.

Some studies have shown that CBD may reduce or antagonize some of the effects of THC. The mechanism for this is unclear, with some suggesting that CBD may be a weak CB1 receptor antagonist. Recent evidence suggests that CBD may be a negative allosteric modulator of the CB1 receptor, thereby acting as a noncompetitive antagonist of the actions of THC and other CB1 agonists (McPartland et al. 2015; Laprairie et al. 2015). One study suggests CBD may act as an allosteric modulator at the CB2 receptor as well (Martínez-Pinilla et al. 2015). CBD may also interact with the endocannabinoid system through indirect mechanisms such as enhanced action of the endogenous cannabinoid ligand anandamide, resulting in a blockade of anandamide reuptake and the inhibition of its enzymatic degradation by fatty acid amide hydrolase (FAAH1).

2.7 Cross-Reactivity with Other Receptor Systems

In the brain, the role of the ECS is to modulate neurotransmitter release which requires an exquisite degree of endocannabinoid control to affect necessary changes in other neuronal signaling systems. The existence of an endogenous antagonist at the CB1 receptor adds a new form of regulation to cannabinoid synapses in the central nervous system (Porter et al. 2002).

2.8 Orphan Receptors (GPCR)

An ever-increasing segment of the research community hypothesizes several of the orphan receptors are, in actuality, mislabeled cannabinoid receptors (Ryberg et al. 2007; Morales and Reggio 2017). The following receptors either interact directly with eCB, exogenous cannabinoids, their biosynthetic and catabolic enzymes, or the significant secondary and tertiary cannabinoid metabolites, of which there are currently 115 identified for Δ9-THC and 108 for CBD. The orphan receptors which demonstrate ECS interaction are:

- GPR3, found in oocytes
- GPR6, up-regulates cyclic adenosine monophosphate (cAMP) levels and promotes neurite outgrowth

- GPR12, found in the limbic system and promotes brain growth
- GPR18, roles in microglial migration/inflammation and peripheral vasodilation, expressed in the rostral ventromedial medulla (RVM)
- GPR55, osteoclast function and neuroimmunological regulation
- GPR119, expressed in the pancreas and gastrointestinal tract and may have a role in glucose homeostasis. (Pertwee 2014)

There are several others, but this research is in the nascent stages.

2.9 Transient Receptor Potential Vanilloid (TRPV) and Transient Receptor Potential Melastatin (TRPM)

Additionally, there is interaction between the ECS and vanilloid receptors: TRPV1, TRPV2, TRPV3, TRPV4. The vanilloid receptors (TRPV) are ligand-gated, non-selective cation channels expressed predominantly by sensory neurons. Research concerning TRPV1 is increasing and current results indicate significant interaction with a normally functioning inflammatory cascade. There is also ECS interaction with the melastatin non-selective cation channel receptor TRPM8 (Muller et al. 2018).

2.10 Peroxisome Proliferator-Activated Receptors

ECS interaction is observed between peroxisome proliferator-activated receptors (PPAR) (O'Sullivan 2007). PPAR receptors are ligand-activated transcription factors of a nuclear hormone receptor superfamily composed of the following three subtypes: PPARα, PPARγ, and PPARβ or δ. Activation of PPAR-α reduces triglyceride levels and is involved in regulation of energy homeostasis (Tyagi et al. 2011). Activation of PPAR-γ causes insulin sensitization, enhances glucose metabolism, and may inhibit inflammatory cytokines; whereas activation of PPAR-β/δ enhances fatty acid metabolism (Naughton et al. 2013). Thus, the PPAR family of nuclear receptors play a major regulatory role in energy homeostasis, metabolic function, immune regulation, and inflammatory cytokine production and regulation (O'Sullivan 2016).

2.11 Gamma-Aminobutyric Acid and Glutamate

The neurotransmitter group including gamma-aminobutyric acid (GABA), GABAergic interneurons, glutamate, glutamatergic neurons, and cholecystokinin (CCK), is interrelated with the endocannabinoid system. The ECS inhibits hippocampal GABA release, GABAergic transmission, and network oscillations (Lee

et al. 2010). Additionally, the ECS has also been demonstrated to increase glutamatergic transmission in the hippocampus. Furthermore, it modulates glutamate and decreases GABAergic synaptic transmission in the amygdala (Scarante et al. 2017). CB1 receptors are found overwhelmingly on the nerve terminals of a distinct group of GABAergic interneurons, which, besides GABA, also contain the neuropeptide cholecystokinin (CCK). Similarly, CB1 receptors are found in the cerebellum and striatum in high concentrations on the terminals of the excitatory glutamatergic fiber systems (Hoffman and Lupica 2013; Freundt-Revilla et al. 2017).

Endocannabinoids, depending on the brain region, can regulate the release of GABA, cholecystokinin, or glutamate with precision. In part, this regulation modulates the different phases of memory processes: acquisition, consolidation, retrieval, reconsolidation, and extinction. Moreover, the regulation plays a role in plasticity and neuronal depolarization control.

2.12 Opioid Receptors (OPM1 and OPD1)

There is extensively detailed research revealing a significant correlation and interaction between the ECS and OPM1 (mu) and OPD1 (delta) receptors (Befort 2015). This is confirmed and observed in a bidirectional upregulation between CB1 receptors and the OP1 receptors in the periaqueductal gray (PAG), rostral ventromedial medulla (RVM), locus coeruleus (LC), dorsal horn, peripheral unmyelinated C-fibers, and some distribution in myelinated A fibers. It is important to understand that the ECS is interwoven into all phases of the pain cascade: transduction, transmission, perception, and modulation (Yam et al. 2018). The ECS modulates C-fibers and 'wind up' pain by decreasing sensitivity to substance P, reducing sprouting, and altering N-methyl-D-aspartate (NMDA) and glutamate concentrations (Zieglgänsberger 2019; Alfulaij et al. 2018). As with OPM1 and OPD1, there exists cross reactivity between the ECS and OPK1 (kappa) receptors, but this research is limited in scope (Bushlin 2011).

2.13 N-Methyl-D-Aspartate Receptors

Cross reactivity has been observed between N-methyl-D-aspartate Receptors (NMDARs) and the ECS, which is involved in the production and binding of N-methyl-D-aspartate (NMDA) (Sánchez-Blázquez et al. 2013; Rodríguez-Muñoz et al. 2016). NMDARs are distributed throughout most structures in the brain, as well as in the cervical spinal cord, dorsal root and vestibular ganglia, and in pineal and pituitary glands. Moderate to dense concentration is observed in the olfactory bulb, neocortex, striatum, some thalamic and hypothalamic nuclei, the colliculi, and many reticular, sensory and motor neurons of the brainstem and spinal cord. The densest concentration appears in the pyramidal and hilar neurons of the CA3 region

of the hippocampus, Purkinje cells of the cerebellum, supraoptic and magnocellular paraventricular neurons of the hypothalamus, inferior olive, red nucleus, lateral reticular nucleus, peripheral dorsal cochlear nucleus, and motor nuclei of the lower brainstem and spinal cord (Hansen et al. 2018).

These receptors are glutamate-gated cation channels with high calcium permeability. They are critical for the development of the central nervous system (CNS), generation of rhythms for breathing and locomotion, and the processes underlying learning, memory, and neuroplasticity (Gonda 2012).

2.14 Adenosine Receptors

Another receptor group which exhibits ECS interaction is the adenosine receptor (AR) A3 (Koscsó 2011; Ferré et al. 2010). It is found in high concentrations in the testes and mast cells, in moderate concentrations in the cerebellum and hippocampus, and in low concentrations in the thyroid, most of the brain, adrenal gland, spleen, liver, kidney, and heart (Sheth et al. 2014). Activation of this receptor imparts cardioprotection during cardiac ischemia. A3 helps inhibit neutrophil degranulation in neutrophil-mediated tissue injury, has both neuroprotective and neurodegenerative effects, and may also mediate both cell proliferation and cell death (Maroon and Bost 2018; Bih et al. 2015).

2.15 Muscarinic Acetylcholine Receptors (mAChR)

There is interaction between muscarinic receptors M1 and M4 and the ECS (Thomas 2017; Christopoulos and Wilson 2001). M1 receptors are found in the hippocampal and cortical regions of the brain as well as in the parasympathetic ganglia. This receptor type is involved in the initiation of neuronal excitation, learning and memory, and regulation of the force and rate of heart contractions. The M4 receptor is abundant in the neostriatum, the cortex, and hippocampus (Tzavara et al. 2004). Its function is thought to mediate an inhibitory effect on striatal dopamine-mediated locomotor activity and appears to have smooth muscle effects as well (Merrer 2013).

2.16 Serotonin Receptors (5HT)

The 5HT-1A, 5HT-2A, and 5HT-3A serotonin receptors exhibit substantial interaction with the ECS (Haj-Dahmane and Shen 2011). 5HT-1A receptors inhibit adenylyl cyclase and open K^+ channels. This receptor type is widely distributed throughout the CNS and is present in both pre- and postsynaptic sites. Presynaptically, 5HT-1A receptors are exclusively located on cell bodies and dendrites of 5HT

(serotonin) neurons in the dorsal and median raphe nuclei, and tightly regulate 5HT neuronal activity (Altieri et al. 2013).

Postsynaptically, the highest number of 5HT-1A receptors is found in the limbic system, specifically in the entorhinal cortices, cingulate and lateral septum, with particularly high expression in the hippocampus. At the cellular level, the postsynaptic 5HT-1A receptors are expressed in cortical pyramidal neurons as well as pyramidal, GABAergic, and granular cells of the hippocampus. In the hippocampus, the 5HT-1A receptors are located on somata and dendrites of pyramidal and granular neurons, as well as on the dendritic spines of pyramidal neurons. Neuronal hyperpolarization occurs with activation of 5HT-1A receptors and leads to an effect mediated by pertussis-toxin-sensitive $G\alpha_{i/o}$ proteins. $G\alpha_{i/o}$ proteins are negatively coupled with the signaling pathway of adenylyl cyclase and thereby decrease the cAMP formation (Altieri et al. 2013).

5HT-2A receptors stimulate phosphoinositide-specific phospholipase C and close K^+ channels. Their location is in postsynaptic membranes of 5HT target cells and they are widely distributed in many brain areas, including limbic regions such as the hypothalamus, amygdala, nucleus accumbens (NAcc), striatum, hypothalamus, and prefrontal cortex (PFC). Additionally, these receptors are found in the claustrum, cerebral cortex, and olfactory tubercle (Raote 2007). This receptor type has been shown to play a role in appetite, temperature and blood pressure regulation, neuroendocrine function, and behavior.

5HT-3A receptors are ligand-gated ion channels (LGIC) and therefore differ from all other 5HT receptors whose actions are mediated via G proteins. They are primarily expressed on GABAergic interneurons in neocortex and limbic structures, derived from the caudal ganglionic eminence (Thompson and Lummis 2006). However, these receptors are also found in the following areas: hippocampus, entorhinal cortex, amygdala, nucleus accumbens (NAcc), solitary tract nerve, trigeminal nerve, motor nucleus of the dorsal vagal nerve, area postrema, and the dorsal horn of the spinal cord (Morton et al. 2015).

2.17 Dopamine Receptors (D1 and D2)

The ECS correlates with the dopamine receptors, D1 and D2 (Covey et al. 2017). D1 receptors are widely expressed in the brain, with the highest levels found in the caudate-putamen, nucleus accumbens (NAcc), substantia nigra, pars reticulata, and olfactory bulb. They are associated with learning, memory, locomotor activity, and reward mechanisms (Bhatia and Saadabadi 2019). D2 receptors are highly expressed in the caudate, putamen (basal ganglia), nucleus accumbens (NAcc), ventral tegmental area, and the substantia nigra, and in lower concentrations in the septal region, amygdala, hippocampus, thalamus, pituitary gland, cerebellum, and cerebral cortex. Specifically, in the cerebellum, the highest concentration of D2 receptors is found in lobules IX and X (Bhatia and Saadabadi 2019). D2 receptors can be located both presynaptically, where they regulate the release of dopamine and other

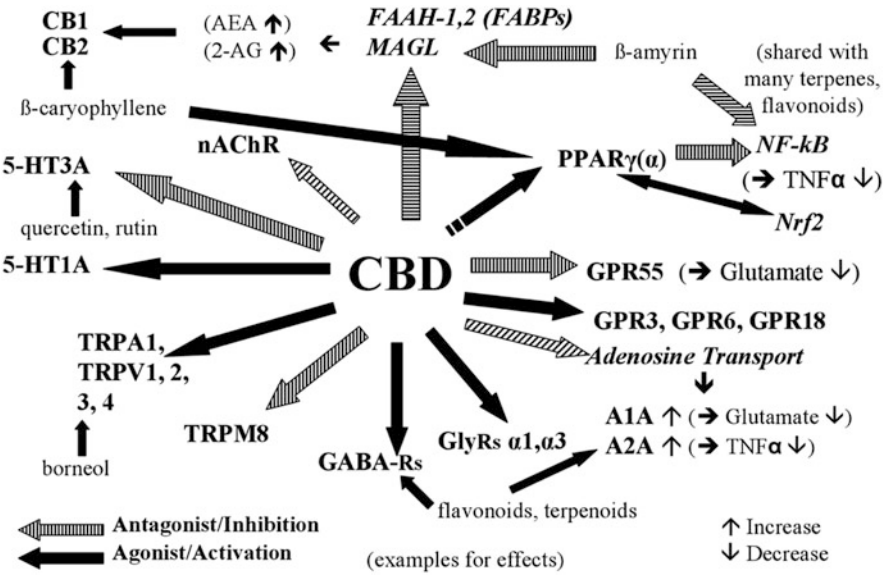

Fig. 2.3 Illustration of the dynamic interaction of cannabidiol on various receptors and with other endogenous and exogenous molecules (Permission to reproduce with full citation Nahler, G. (2018). Pure Cannabidiol versus Cannabidiol-Containing Extracts: Distinctly Different Multi-Target Modulators. *Alternative, Complementary & Integrative Medicine, 4*(1), 1–11. doi:10.24966/acim-7562/100048)

neurotransmitters, and postsynaptically, where they can exert a variety of functions, ranging from inhibition of long-term depression at midbrain excitatory synapses, to inhibition of calcium channels, to control of pacemaker activity and resting potential through activation of G protein-coupled inwardly-rectifying potassium channels (GIRK) channels (Fig. 2.3).

2.18 MDR1 Expression and Modulation

Multi-drug Resistance 1 (MDR1) is a genetic mutation found in many of the herding breeds, some sighthound breeds, and many mixed dogs. This mutation can have a significant impact on drug sensitivity. The MDR1 gene is responsible for production of a protein called P-glycoprotein. The P-glycoprotein molecule (P-gp) is a drug transport pump that plays an important role in limiting drug absorption and distribution (particularly to the brain) and enhancing the excretion/elimination of many drugs used in dogs. As a result, dogs with the MDR1-mutation may have severe adverse reactions to some common drugs (Arnold et al. 2012).

CBD and Δ9-THC at 10μM transiently induced the MDR1 transcript in P-gp overexpressing cells at 4 h but not 8 h or 48 h incubation durations. CBD and THC

also concomitantly increased P-gp activity as measured by reduced accumulation of the P-gp substrate Rhodamine 123 in these cells with a maximal inhibitory effect observed at 4 h that slowly diminished by 48 h. CEM/VLB100 cell lines were shown to express CB2 and TRPV1 receptors. Δ9-THC effects on MDR1 expression were mediated by CB2 receptors. The effects of CBD were not mediated by either CB2 or TRPV1 receptors alone but required activation of both receptors to modulate MDR1 mRNA expression. It appears that both CB2 and TRPV1 receptors cooperate to modulate MDR1 expression (Jonathon 2012).

In patients whom practitioners suspect MDR1 expression, confirmation testing is optimal, and the accumulation of testing results will help future patients. However, testing may not be an option. Pharmaceutical and supplement administration in this patient population should start at the lowest dosage and increase based upon patient response. Moreover, a patient may not have any adverse effects when only one or two pharmaceuticals or supplements are administered but the addition of another drug or supplement may either alter the response to the new drug or supplement or alter the patient response to an existing pharmaceutical or supplement regimen.

Cannabis compounds should be administered cautiously, as any pharmaceutical or supplement, in this patient population. Starting doses may need to be lower than the low end of recommended dose range and monitoring and adjustment based upon patient response is expected. Research into this mutation and cannabis is in the nascent stages. Testing and data accumulation on the precise way in which these compounds modulate and interact in this patient population is vital in understanding and establishing efficacious treatment protocols and perhaps mitigating this mutation in future generations.

2.19 Phytocannabinoid Potentiation and Interactions with Other Pharmacologic Agents

2.19.1 Opioids

A synergistic interaction exists between cannabinoids and opioid receptors; specifically OPM1, OPD1, and OPK1 receptors. Moreover, significant crosstalk exists between the ECS and all opioid receptors with a bidirectional upregulation of both ECS and opioid receptors after administration of opioids and/or phytocannabinoids (Bushlin 2011).

This upregulation occurs primarily in the periaqueductal gray (PAG), rostral ventromedial medulla (RVM), locus coeruleus (LC), and dorsal horn. For example, when cannabinoids are administered, OPM1 and OPK1 (to a lesser degree) receptors upregulate in those areas. Post opioid administration, there is an upregulation of CB1 and, to a lesser degree, CB2 receptors. Additionally, activation of CB2 receptors stimulates the release of endogenous opioids (Ibrahim et al. 2005; Machado et al. 2013). These interactions may necessitate the need to decrease opioid dosages for patients receiving both phytocannabinoids and opioids.

2.19.2 Benzodiazepines

This class of pharmaceuticals' mechanism of action is direct action at the gamma amino butyric acid ($GABA_A$) receptor. As the ECS exerts direct and indirect control over the production of, binding affinity for, and degradation of GABA, there exists the potential for phytocannabinoid and benzodiazepine potentiation. An alteration in benzodiazepine dosage may be necessary (Purcell 2019). Clinical experience has shown the addition of phytocannabinoids to a regimen of benzodiazepines can induce THC intoxication-like symptoms.

2.19.3 Gabapentin

The precise mechanisms by which gabapentin produces its analgesic and antiepileptic actions are unknown. Gabapentin is structurally related to the neurotransmitter gamma-aminobutyric acid (GABA) but has no effect on GABA binding, uptake, or degradation. There is a definite potentiation between phytocannabinoids and gabapentin (Guerrero-Alba et al. 2019). The potentiation interaction is beneficial and is a highly effective analgesic combination. However, the dose of gabapentin must be lowered to or taken below the low-end dose range to avoid any adverse events associated with potentiation.

2.19.4 Serotonin Reuptake Inhibitors

The ECS interacts with all 5-HT receptors. This interaction can theoretically lead to serotonin syndrome if a patient is on a high SSRI dose or is sensitive to SSRI therapy. The syndrome can be potentially life-threatening, although no deaths have been reported with concurrent phytocannabinoid administration. This syndrome is precipitated by the use of serotonergic drugs and overactivation of both the peripheral and central postsynaptic 5HT-1A and, most notably, 5HT-2A receptors. This interaction is rare but should be considered when starting a patient on any phytocannabinoid regimen (Kaminer 2010).

2.19.5 Phenobarbital and Other Antiepileptic Drugs (AED)

It is no surprise the ECS intimately interacts with neuronal receptors, resulting in improved seizure regulation. This effect occurs within the CNS at the receptor level, and mitigates secondary effects of seizure events such as, cerebral hyperemia, cognition changes (anxiety, aggression), transient systemic hypertension,

hyperthermia, muscle fatigue and soreness, etc. Ongoing research is expected to produce details regarding ECS interaction with receptors involved for commonly used antiepileptic drugs (AEDs) as well as determine ideal dosages for concurrent CB1 agonist and AED administration.

Phenobarbital warrants separate mention due to its CYP interaction and associated known potential drug interactions (Sasaki and Shimoda 2015; Perucca 2006). CBD is a potent CYP enzyme inhibitor and there exists the potential for negative interaction resulting in elevated phenobarbital blood levels. To the author's knowledge, no studies exist on this topic in veterinary species yet. Theoretically, this is only likely to occur when CBD isolate products, at moderate to high doses, are administered alongside moderate to high doses of phenobarbital and/or in cases of known hepatic dysfunction.

The author's experience using AEDs concurrently with CB1 receptor agonists, specifically low dose (<1.5 mg/kg CBD:THC 2:1-10:1) whole plant products, or moderate to high doses (>2 mg/kg CBD) isolate products as pulse therapy, supports CBD modulation of CYP enzymes and the benefits of more dynamic profiles of cannabinoids. As is typical in clinical medicine, using the lowest effective dose for symptom relief is the best course of action, but there is currently no need to avoid concurrent AED and CB1 receptor agonist administration. Signs of interaction will often resemble any initial adverse clinical signs observed when a patient is first started on an AED regime.

For example, if a patient is on phenobarbital and potassium bromide (KBr) and a cannabinoid product is added, adverse clinical signs will resemble those first observed when the patient started receiving phenobarbital. The patient may appear ataxic, lethargic, and have an increased appetite. Dosages should be reevaluated and readjusted, based upon serial testing and patient response.

2.20 Cytochrome P450

Erik Amazonas

One of the largest and most important classes of drug-metabolizing enzymes is the cytochrome P450 (CYP) family, which are responsible for oxidation of small molecules in order to be removed from the body. They are highly non-specific and able to metabolize many different chemical substrates, accounting for an estimated 60–70% of all drug metabolization (Sevrioukova and Poulos 2013). CYPs' expression and activity occurs mainly in the liver, but significant activity can also be found in the lungs and intestinal tract.

It is well established that any drug-based therapy requires attention to both the metabolism of the drug as well as the interactions between two or more drugs. As CYPs are the most important drug-clearance mechanism, one may easily conclude that altering CYP activity will result in differences in drug metabolization. Reduced CYP activity could lead to increased potency and/or duration of effects, and

may result in unwanted side-effects or even toxicity. At the other extreme, increasing CYP activity may result in loss of therapeutic efficacy of medications due to excess metabolization (Zendulka et al. 2016).

When practicing cannabinoid therapy, one must have in mind that cannabinoids directly change CYP activity and can lead to undesirable side effects. Hexobarbital clearance by CYP2C9 was found to be reduced by cannabidiol (CBD) (Benowitz et al. 1980) as was cyclosporine oxidation by CYP3A4 (Jaeger et al. 1996). Thus, CBD can promote greater or longer activity of those chemicals and this must be taken into account in therapeutic methodologies. Several drugs may have delayed metabolization secondary to inhibited CYP activity and therefore may either result in increased activity, toxicity, or lack of function due to little to no conversion into the bioactive compounds, as some drugs are administered as prodrugs and need metabolization from CYPs to become active. A comprehensive guide to drug interactions on CYPs can be found at the Indiana University's *Cytochrome P450 Drug Interaction table* (Flockhart 2007).

Cannabinoid interaction with CYPs is very complicated. From all studied cannabinoids, CBD is the most potent inhibitor of the CYP family. By inhibiting CYP3A4, CBD can delay drug metabolization and clearance and thereby raise the plasma concentration of several other drugs increasing their duration and/or effects (Pertwee 2014).

The inhibition of CYPs inhibition by cannabinoids varies based on their molecular structure. CBD has two free hydroxyl on the resorcinol moiety, whilst Δ-9-THC possesses only one. THC demonstrates only 20% of CBD's inhibition power upon CYP1A1, CYP2B6, CYP2D6, CYP3A4, and CYP3A5 (Yamaori et al. 2011). Other CYP family members are also inhibited by means of the other structures of cannabinoid molecules, such as CYP2C19, which is inhibited by the pentyl side chain (five carbon tail) of the CBD molecule (Jiang et al. 2013).

2.20.1 Cannabinoid Metabolism

Cannabinoid metabolism occurs mainly by hydrolytic pathways by FAAH and MAGL. Anandamide (AEA) is hydrolyzed by FAAH into arachidonic acid, ethanolamine, and into inflammatory prostaglandin ethanolamides by COX-2, an important inflammatory enzyme (Zendulka et al. 2016). MAGL, and to some extent also FAAH, hydrolyse 2-Arachidonoylglycerol (2-AG) rendering the same metabolic path as AEA (Zendulka et al. 2016).

Cannabinoids, both endo- and phyto-, are also metabolized by the oxidative pathways of CYPs, in addition to the hydrolytic pathways described previously and mediated by FAAH and MAGL. The enzymes CYP2C9, CYP2C19, and CYP3A4 catalyze most of their hydroxylations (Pertwee 2014). These pathways render metabolites with both lower and higher affinity to receptors. Anandamide can undergo epoxygenation by several CYPs and some of these oxidation routes lead to a more bioactive compound, 5,6-EET-EA. In the same manner, 2-AG can also be

converted to a more bioactive compound, EET-G. CYP2C9 catalyzes the conversion of the phytocannabinoid Δ-9-THC into the more bioactive 11-OH-THC, which is then converted to 11-COOH-THC by CYP3A4. Thus, whenever an animal is undergoing treatment with any drug that alters CYP activity, attention must be paid while cannabinoid therapy takes place. Ketoconazole, a largely used antifungal drug, has been known for strongly inhibiting CYP3A4 and other isoforms of CYP450 (Sevrioukova and Poulos 2013). It directly affects the way cannabinoids are converted to their active or inactive forms, resulting in greater activity of THC and CBD (Stout and Cimino 2014). Dexamethasone, a commonly used glucocorticoid, increases CYP family activity, leading to a large increase in the active metabolites of cannabinoids (Zendulka et al. 2016), such as 11-OH-THC, which might in turn result in the increased occurrence of undesired effects.

It is by now well understood that cannabinoid-based therapy benefits greatly from the synergistic effects of different cannabinoids, terpenes, and flavonoids acting together to create the "*entourage effect*" (Ben-Shabat et al. 1998; Mechoulam and Ben-Shabat 1999). CBD is well-known to reduce THC's negative side effects (Russo and Guy 2006) and, in addition to the direct action on cannabinoid receptors throughout the body, a large part of that mitigation is accomplished via alteration of CYP2C9 and CYP3A4 activity (Devitt-Lee 2018). Strong inhibition of CYPs activity can occur, and severe side effects may result, when using CBD isolates, which possess no entourage effect to counteract over-inhibition (Devitt-Lee 2018).

For the clinical practitioner, there is an important statement made by Stout and Cimino (2014) concerning the inhibition or induction of CYPs activity by cannabinoids:

> "(. . .) given the lack of inhibition data at several CYP-450 isoforms, the **wide variability** in cannabinoid product **content** and **dosing**, and the inherent **imprecision** of using **concentration/inhibition potency** ratios to predict in vivo drug interaction potential, **clinically significant inhibitory effects cannot be ruled out entirely**."

There is great interest in studying possible side effects and drug-drug interactions concerning cannabinoids. Although there is a non-negligible list of side effects from cannabinoid therapy (e.g., disorientation, ataxia, incoordination and sedation), most of the severe side effects generally arise from the actions of concurrent medications in use. Oncologists often use the maximum non-lethal dosage of chemotherapeutics in order to eliminate cancer cells. As many first-line chemotherapy drugs are metabolized by CYPs, the dosage may have to be lowered when combined with cannabinoids, since delayed clearance of these chemicals could result in systemic toxicity (Devitt-Lee 2018). As the actual effect of several drugs can be augmented by cannabinoid therapies, it is common for the clinical practitioner to consider reducing the doses of the drug(s) with the most severe collateral side effects. This is particularly true in clinical practice for opioid-based therapies. Many opioids such as oxycodone and tramadol undergo CYP3A4 metabolization which also produce other bioactive metabolites and, thus, cannabinoids can greatly influence the potency of opioids (Holmquist 2009). Dose adjustment should be considered for chemotherapeutics, neuroleptic medications, glucocorticoids and NSAIDs therapies (Table 2.4).

Table 2.4 CYP interactions with and regulation by THC and CBD

Phytocannabinoid	CYP3A4	CYP3A5	CYP2C9	CYP2C19	CYP1A2	CYP2D6
THC	THC is metabolized by Inhibitors slightly increase THC levels Inducers slightly decrease THC levels		THC is metabolized by		THC induces Theoretically, THC can decrease serum concentrations of clozapine, duloxetine, naproxen, cyclobenzaprine, olanzapine, haloperidol, and chlorpromazine	
CBD	CBD is metabolized by Inducers slightly decrease CBD levels CBD is a potent inhibitor Metabolizes about a quarter of all drugs, CBD may increase serum concentrations of macrolides, calcium channel blockers, benzodiazepines, cyclosporine, sildenafil (and other PDE5 inhibitors), antihistamines, haloperidol, antiretrovirals, and some statins (atorvastatin and simvastatin, but not pravastatin or rosuvastatin)	CBD metabolized by	CBD is metabolized by	CBD is metabolized by		CBD is a potent inhibitor Metabolizes many antidepressants, so CBD may increase serum concentrations of SSRIs, tricyclic antidepressants, antipsychotics, beta blockers and opioids

Original table, Reference: Yamaori (2012), Stout and Cimino (2014), Flockhart (2007)

2.21 Future Thoughts

The authors are curious to learn, out of over 500 known compounds in the *Cannabis* genus, what novel therapeutics will emerge: antiepileptic, anti-inflammatory, analgesic, anti-nausea, antidepressant, and others? Certainly, more research is needed to add to the already significant quantity currently available. These research efforts run the gamut of scientific endeavors including botany, genetics, biochemistry, pharmacology, and clinical and behavioral sciences. It is clear the ECS is one of the most important biochemical systems within the body. It is designed to function optimally as a homeostatic system when it and all associated compounds are in balance. The ECS, and the molecules and compounds that interact with it, contain vast potential to change the veterinary medical field, improving the lives of our patients.

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Chapter 3
Toxicology

Ahna Brutlag

3.1 Introduction

Along with the increased popularity and accessibility to cannabis, the incidence of accidental exposure and intoxication in pets, as reported to Pet Poison Helpline, a 24/7 animal poison control center serving the United States (US) and Canada, has increased dramatically. With accidental pet intoxication on the rise, it is imperative for veterinary professionals to understand how the common types of cannabis products can impact their patients following intentional or inadvertent exposure. This chapter will review cannabinoid containing products along with synthetic marijuana products (synthetic cannabinoids or SCBs) which are illegal substances with a much greater affinity for cannabinoid receptors.

As therapeutic benefits of cannabinoids come to light and societal perceptions change, veterinary professionals are expected to see a continued increase in legalization and decriminalization of marijuana and individual cannabinoids, and thus likely to see an increase in inadvertent companion animal exposure and intoxication.

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3.2 A Brief History of Cannabis

For many centuries, cannabis plants have been used to treat various ailments, for their psychotropic or intoxicating properties/recreational use, and in religious ceremonies. The use of cannabis for medical purposes is recorded back to 2700–2600 BCE for treatment of various maladies including constipation, rheumatic pain, malaria, menstrual health, venereal disease, headaches, fever reduction, appetite stimulation, and as a sleep aid. Use for these ailments continued well into the nineteenth century, particularly in 1839 when Irish physician W.B. O'Shaughnessy began investigating its usefulness in the treatment of seizures, tetanus, rabies, and rheumatism in animal studies. He recognized its benefits as an antispasmodic agent with anxiolytic and antiemetic properties, although he also noted side effects such as catalepsy (Adams and Martin 1996).

Over the last 60 years, scientists have gained considerable knowledge on the topic of the cannabis plant and its cannabinoid compounds. Delta-9-tetrahydrocannabinol, or THC, was identified as the major psychoactive cannabinoid in 1964 (Marzo et al. 2004). The structure of cannabidiol or CBD, the primary non-intoxicating cannabinoid, was discovered in 1963 (Long et al. 2005). The design of enantiomerically pure analogues or synthetic varieties of THC began in the 1960s in both the pursuit of analgesics and of endogenous receptors presumed present in mammals at which THC, CBD, and other cannabinoids act (Marzo et al. 2004; Obafemi et al. 2015). Cannabinoid receptors were discovered in 1988 and specific receptors CB1 and CB2 were cloned in 1990 and 1993 respectively, at which time they were identified as G-protein coupled receptors affected by endogenous cannabinoids, termed *endocannabinoids*, during the early 1990s (Marzo et al. 2004).

Marijuana and synthetic cannabinoids have progressed to be the most widely used illicit drugs in the world, and, over the past 60 years, most countries categorized them as drugs of abuse. However, the more recent legal fluidity of plant-based cannabinoids production and processing for sale is rapidly evolving. Synthetic cannabinoid varieties developed to mimic THC have also gained popularity for their intoxicating properties, most notably since 2009 (Obafemi et al. 2015).

Although considered a drug of abuse, the therapeutic benefits of THC specifically have not been ignored. Since the 1980s, synthetic THC-based medications dronabinol (Marinol®) and analogue nabilone (Cesamet®) have been used in treatment of inappetence and nausea in chemotherapy and AIDS patients (Janczyk et al. 2004; Marzo et al. 2004). In 2010, Sativex®, a 1:1 ratio of THC:CBD, was approved in many European countries as an oromucosal spray for treatment of spasticity due to multiple sclerosis. As of 2020, the drug was approved by more than 25 countries around the globe, including Canada, Israel, Australia, New Zealand, and in Latin America, but not in the US. In 2018, Epidiolex® (cannabidiol) was approved in the US by FDA for the treatment of seizures associated with two rare forms of human epilepsy (Greenwich Biosciences 2018).

3.3 The Endocannabinoid System Review

The endocannabinoid system is akin to the endogenous opioid system in which opioid receptors (i.e., delta, kappa, mu) are activated by endogenous opioid peptides (e.g., endorphins). Mammals have cannabinoid receptors in plasma membranes that are activated by endogenous ligands called endocannabinoids. The endocannabinoid system encompasses complex intracellular signaling including enzymes for ligand biosynthesis and inactivation. It plays a physiological role in several systems, namely neurological, inflammatory, and immune.

Anandamide (AEA) and 2-Arachidonoylglycerol (2-AG) are the two most commonly recognized and studied endocannabinoids. Both are produced "on demand" in response to stress. These endocannabinoids bind to G-protein coupled receptors and perform several neurotransmission functions, including inhibition (mostly) of adenylate cyclase, inhibition of voltage-gated calcium channels, stimulation of protein kinases, and stimulation of potassium channels. Receptor binding and subsequent effects ultimately translate into biological responses within a complex system designed for modulating neurotransmitter release (Marzo et al. 2004).

The primary targets for endocannabinoids and THC are cannabinoid receptors 1 and 2 (CB1 and CB2). These receptors are present on the postsynaptic neuron and act via retrograde synaptic signaling mechanisms to inhibit neurotransmitter release from presynaptic neurons (Pirone et al. 2015). Endocannabinoids are synthesized, as needed, from membrane phospholipids to act in an autocrine (upon the same cell) or paracrine (upon nearby cells) fashion and are quickly inactivated via hydrolysis after internalization into the cell (Marzo et al. 2004; Murray et al. 2007).

Endocannabinoids are 4-20 × less potent than THC and have a significantly shorter duration of action (Adams and Martin 1996). Administration of exogenous cannabinoids such as THC or synthetic analogues will disrupt the subtle endocannabinoid signaling process and may result in the common THC tetrad of delusions, hallucinations, paranoia, and sedation (Adams and Martin 1996; Murray et al. 2007).

- **CB1 receptors**
  - Primarily located in the central nervous system (CNS) with lower concentrations in the peripheral nervous system (PNS).
  - In the CNS, CB1 is involved in cognitive function, emotion, motion/movement, hunger, and neuroprotection in both post-traumatic events and degenerative diseases.
  - Sensory and autonomic CB1 receptors are involved in pain perception, cardiovascular, gastrointestinal, and respiratory effects.
  - CB1 is responsible for the psychotropic effects of THC.
  - Activation inhibits retrograde release of acetylcholine, dopamine, GABA, serotonin, histamine, glutamate, and/or noradrenaline, among others.

- **CB2 receptors**
  - Primarily located in the PNS and do not result in intoxicating effects.
  - Involved in reducing inflammation and chronic pain relief (Marzo et al. 2004).
  - Activation inhibits pro-inflammatory cytokine production and subsequent release of anti-inflammatory cytokines (Landa et al. 2016).

Cannabinoid receptors are found in abundance within the epithelial tissues of the developing embryo with highest concentrations in the nervous system, sensory organs, and thyroid tissue. CB1 is important in the normal neuronal differentiation and axonal growth during neuronal development. The developed animal has highest CB1 concentration in the basal ganglia and cerebellum (Pirone et al. 2015). Endocannabinoid signaling is required for motor learning in the cerebellum, extinction of aversive memories in the amygdala, and as an aid in memory encoding.

For an in-depth understanding of the endocannabinoid system and receptor physiology, please see Chaps. 1 and 2 of this book.

3.4 Toxicity of Δ9-THC

THC, the primary intoxicating cannabinoid in the cannabis plant, is found in a range of products and formulations including edibles, oils, tinctures, topical salves/balms/etc., capsules, vaping liquids, hashish, and "concentrates". It is also the compound upon which the potency of cannabis products, especially plants used for recreational purposes, is traditionally measured (Obafemi et al. 2015).

Other forms of THC can be found in the cannabis plant including THCA (tetrahydrocannabinolic acid) and THCV (tetrahydrocannabivarin), which are not intoxicating. Whereas new forms of the THC molecule, including THCB (Δ9-tetrahydrocannabutol) and THCP (tetrahydrocannabiphorol), in more recently discovered cultivars are dramatically more potent than the popular Δ9-THC. While not readily available on the legal or black market, these newly discovered cannabinoids may be a potential intoxicating substance to consider in the near future.

The concentration of THC in cultivated cannabis plants has increased in the US, mostly as a result of cross-breeding and hydroponic year-round growing operations (Adams and Martin 1996). In the 1960s, the average concentration of THC in cannabis plants was 1.5%, rising to 3.5% by the mid 1980s (ElSohly et al. 2016). As of 1995 and 2014, concentrations in plants used for recreational purposes increased from 4% to 12%, respectively, while the concentration of CBD simultaneously declined from 0.28% to <0.15% (ElSohly et al. 2016). This increase in potency correlates with a shift in production from cannabis to sinsemilla which are unpollinated, sterile flowering tops from the cultivated female cannabis plant. Sinsemilla is more potent than traditional marijuana and is gaining popularity in the US, presumably due to demand for plants with greater psychoactive effects (Adams and Martin 1996; ElSohly et al. 2016). In cannabis plants, THC is most

concentrated in the flowering buds, followed by the leaves, stems and roots. The seeds do not contain notable levels of THC (Adams and Martin 1996).

The dose of THC found in cannabis products varies greatly with formulation. Marijuana joints (cigarettes) typically contain 0.5–1 g of plant material with THC concentrations varying from 0.4 to 20%. The average 1 g joint contains 150 mg of THC (Raber et al. 2015).

Commercial edible products may also contain widely varying amounts of THC, although typical doses for adults are ~10 mg per serving. The most common commercial edible products are chocolates and gummies. Brownies or cookies made using "marijuana butter" or various cooking oils that have been used to extract the lipid soluble THC from plant matter, are both commercially available and readily made at home. Marijuana butter/oil can contain very high concentrations of THC and pose a greater risk for poisoning than ingestion of plant material alone. Additionally, chocolate present in food may also lead to intoxication in pets. Human foods that also pose risk for poisoning in dogs are xylitol, grapes/raisins, and macadamia nuts. Other commonly sold THC containing food products are truffles, caramels, lollipops/hard candies, ice cream, savory baked goods, beverages, etc. Sachets of powdered THC intended to be added to foods and beverages are also available.

Processed cannabis products, referred to as "concentrates," can contain >80–90% THC. These may be used recreationally or medically and require smaller doses to achieve stronger and more long-lasting effects compared to plant material. Concentrates come in varying formulations and compositions, sometimes divided into "hash", concentrates made from dry or water based extractions, and techniques using solvents and CO_2 (Raber et al. 2015). Hashish is a sticky resin collected from the flowering buds that may be shaped or formed into a cake, ball, sticks or slabs and typically contains about 10% THC. Hash oil is a liquid or semi-solid with a higher THC concentration than hashish, typically 20–50%.

Solvent-based concentrates are increasingly common products, often recreational, and made by soaking plant material in various solvents which are then boiled off. Such products are typically called RSO (Rick Simpson Oil) as an homage to the person who popularized the technique. Other names for solvent-based concentrates include "butane hash oil" (BHO), "honey oil," "honeycomb," "wax," "shatter," "budder", "errl", and "CO_2 oil". Together, these may be collectively referred to as "dabs", the act of which inhaling them is called "dabbing" or "doing a dab" (Raber et al. 2015). In addition to containing THC, concentrates may be contaminated with high concentrations of solvents, pesticides, and other chemicals that can also complicate the clinical picture when ingested by pets.

3.4.1 Exposure Scenarios in Pets

The most common route of accidental exposure to cannabis products containing THC in companion animal patients is via ingestion, although some are exposed via inhalation from secondhand smoke or smoke intentionally blown in their face. Approximately 66% of the cannabis exposures reported to Pet Poison Helpline

involve pets ingesting homemade or commercial edible goods (Pet Poison Helpline and SafetyCall International 2019—Database last accessed: 2019). The second most common source of cannabis exposures involve ingestion of plant material (~19%), followed by OTC medical cannabis preparations or prescription medications such as dronabinol and nabilone (~9%) (Pet Poison Helpline and SafetyCall International 2019—Database last accessed: 2019).

Legalization of both recreational and medical cannabis has also increased both the prevalence of and pet exposure to formulations such as vaping liquids, topical balms/salves/oils, and oral preparations such as oils, capsules, sublingual sprays, tinctures, etc. Doses and concentrations of such products can vary widely. Any of these products can also be mixed with other chemicals; a common example is the addition of peppermint essential oil to topical products.

3.4.2 Pharmacokinetics and Toxic Doses

For an in-depth discussion on pharmacokinetics of cannabis in dogs and cats, please see Chap. 2.

THC is readily absorbed when inhaled leading to a rapid onset of clinical signs. Absorption is slower and less predictable following ingestion. Consuming THC products with a fatty meal will increase absorption due to its lipophilic nature. The majority of THC is metabolized in the liver and undergoes enterohepatic recirculation, with a small amount excreted as metabolites in the urine (Fitzgerald et al. 2013). Due to its lipophilicity, THC is rapidly distributed into the tissues and crosses the blood-brain barrier (Sharma et al. 2012). This accounts for a short plasma but long biological half-life (Donaldson 2002). When large doses are ingested, it is not uncommon for animals, especially dogs, to remain symptomatic for 1–2 days. In rare cases, clinical signs may last 3–4 days as observed by Pet Poison Helpline.

The pharmacokinetic and toxic dose information listed below pertains to dogs unless otherwise indicated.

- Suggested lethal dose >3–9 g plant material (THC dominant)/kg, but a real LD_{50} has not been established (Donaldson 2002) (https://www.tandfonline.com/doi/pdf/10.1080/24734306.2018.1434470, https://www.sciencedirect.com/science/article/pii/0041008X73903104?via%3Dihub)
- Clinical signs in dogs (oral ingestion) starting at 0.3–0.4 mg/kg THC, possibly lower have been observed in Pet Poison Helpline cases; however, the reported doses in these cases is based on the package label which, due to lack of regulatory oversight, may not be accurate (Pet Poison Helpline and SafetyCall International 2019). (See *Toxicity of CBD*—Sect. 3.5 for more information.)
- T_{max} 1–2 h (oral)
- Onset of signs: minutes (inhaled); 1–2 h (oral) (Pet Poison Helpline and SafetyCall International 2019)

  - Experimentally, in fasted dogs dosed orally with THC, the onset of neurological signs such as ataxia, tremors, hyperesthesia, hypothermia, and hypertonia were ~4 h post-dosing (Vaughn et al. 2020)

- Excretion: 85% in feces via biliary excretion; 15% renally excreted
- Half-life (biological): 30 h; 80% of THC is excreted within 5 days (Fitzgerald et al. 2013)
- Clinical recovery after ingestion occurs within 24 h in most cases, potentially up to 72 h (Donaldson 2002; Pet Poison Helpline and SafetyCall International 2019)

Interactions between THC and CBD have been documented, with CBD often touted as an antagonist of THC; however, this is not always the case. Data suggest CBD can potentiate the psychoactive and physiological effects of THC as well. The exact mechanism(s) for interaction is still being investigated and depends on whether CBD is given before THC or the two are dosed simultaneously. The ratio of CBD to THC also plays a considerable role in expected signs, with higher dose ratios (e.g., 8.1) resulting in antagonistic effects of CBD on THC, whereas ratios of 1.5–1.8 have resulted in potentiation of effects from THC. Therefore, when THC is given simultaneously in combination with CBD, the dose at which adverse effects occur may be less than had an equivalent dose of THC been administered alone. This phenomenon was observed in a canine escalating dose study by Vaughn et al. which compared adverse effects in dogs divided into three groups: dogs dosed with CBD (~2–62 mg/kg), THC (~2–49 mg/kg), and CBD/THC (ratio 1.5:1, ~1.5 mg/kg CBD + 1 mg/kg THC to ~12 mg/kg CBD + 8 mg/kg THC) (Vaughn et al. 2020). Dogs in the CBD/THC arm experienced more severe adverse effects (severe ataxia and/or lethargy), resulting in cessation of dosing halfway through the trial, whereas dogs dosed with CBD alone or THC alone were allowed finish.

3.4.3 Clinical Signs of Poisoning

Exposure to THC is associated with a high morbidity but low mortality rate meaning that clinical signs are common, but death is very rare. Common signs of exposure in dogs include lethargy, CNS depression, ataxia, vomiting (especially if plant material was ingested), urinary incontinence/dribbling, increased sensitivity to motion or sound, mydriasis, hyperesthesia, ptyalism, and bradycardia (Donaldson 2002; Janczyk et al. 2004; Pet Poison Helpline and SafetyCall International 2019). See Fig. 3.1 for signs reported to Pet Poison Helpline and Fig. 3.2 for the percentage of cases in which veterinary intervention was recommended. Acute onset urinary incontinence, especially in younger animals, is not commonly observed with other toxin exposures and serves as a helpful indicator for veterinary staff to consider THC exposure. Less common signs include agitation, aggression, bradypnea, hypothermia, hypotension, tachycardia, and nystagmus (Janczyk et al. 2004; Pet Poison Helpline and SafetyCall International 2019; Vaughn et al. 2020). Rare signs include seizures or comatose conditions. Although seizures are uncommon, it has been

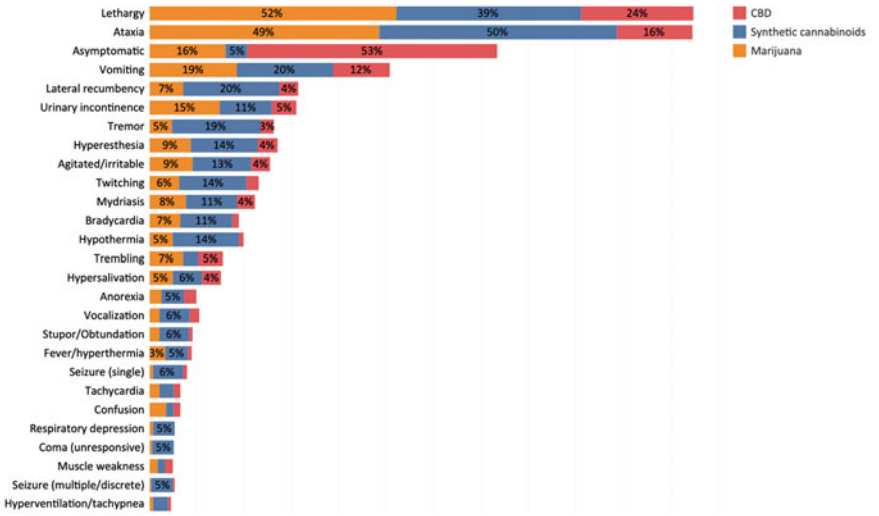

Fig. 3.1 Clinical signs associated with exposure to marijuana (i.e., THC containing products; ~3750 patients), synthetic cannabinoids (~70 patients), and CBD predominant cases (~350 patients) as reported to Pet Poison Helpline between 2010–2019. Canines represent ~87% of the displayed data, and cats ~10%. The remainder includes equines, livestock, and exotics. Confirmation of exposure was not obtained in all cases, nor could co-ingestants such as chocolate or other toxicants be ruled-out. Therefore, these data are meant to portray general trends only. Additional clinical signs were reported but excluded from the graphic due to space limitations

suggested that a higher expression of CB1 receptors on GABA releasing, rather than on glutamatergic terminals, in the neocortex could explain pro- or anti-convulsant effects in a dose dependent nature. Additionally, seizures may be caused by co-ingestants such as chocolate or other drugs.

Fatality in pets from THC intoxication is very rare and not well described in veterinary literature. Two canine euthanasias were reported in conjunction with the ingestion of baked goods made with marijuana butter although the cases became complicated and the exact cause of death was not determined (Meola et al. 2012). No confirmed fatalities or deaths thought to be a result from THC exposure have been reported to Pet Poison Helpline, despite consultation on many thousand cases (Pet Poison Helpline and SafetyCall International 2019).

3.5 Toxicity of CBD

Cannabidiol (CBD) is the most well-known and widely discussed non-intoxicating phytocannabinoid with concentrations ranging from 0.3 to 4.2% in cannabis plants—although this is ever changing with selective breeding of various cultivars (Long et al. 2005).

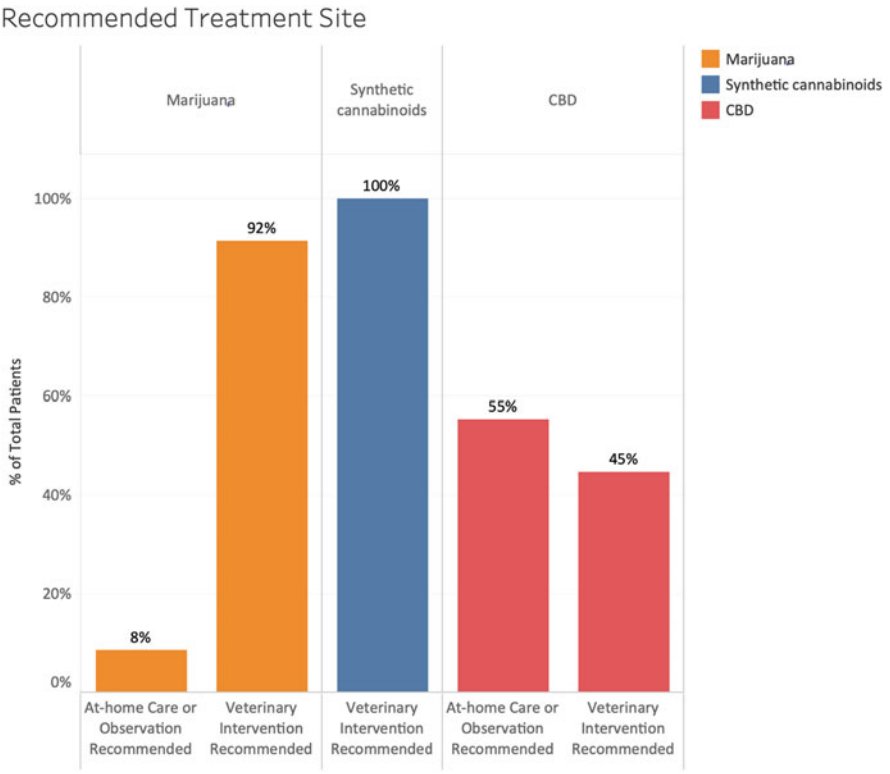

Fig. 3.2 Comparison of the ultimate recommended management site for dogs and cats exposed to marijuana (i.e., THC containing products), synthetic cannabinoids, and CBD-predominant containing products for Pet Poison Helpline cases. Referral for veterinary intervention is based on a thorough patient risk assessment which includes patient signalment, dose, expected or current clinical signs, current and prior medical history, and co-ingestants or concomitant medications

Despite their ubiquity on the consumer market, OTC products containing CBD are not FDA-approved, nor do they have *any* regulatory oversight. As of 2020, there was only one CBD product in the United States with regulatory approval, the human prescription pharmaceutical Epidiolex®. Approved by FDA in 2018, Epidiolex® is formulated as an oral solution (100 mg/mL) and labeled for the treatment of seizures associated with Lennox-Gastaut syndrome or Dravet syndrome in patients 2 years of age and older (Greenwich Biosciences 2018).

The lack of regulatory oversight for CBD containing products has resulted in a 'buyer beware' situation due to concerns over product quality. In 2015, the FDA tested various OTC "CBD containing products", including those marketed specifically for pets, and found many did not contain the amount of CBD stated on the label (if any) while others contained unlabeled THC (US Food and Drug Administration 2018). Since then, regulators and numerous independent laboratories have repeatedly tested OTC "CBD-predominant" products and found similar results. For

example, a product may contain only a small fraction of or several times the amount of labeled CBD. The same product may contain very large amounts of THC and/or synthetic cannabinoids, both of which can lead to severe clinical signs in pets or people. In a veterinary paper published by Wakshlag et al. (2020) the researchers found very similar results for veterinary labeled products as described above. (https://doi.org/10.2147/VMRR.S248712). The lack of regulation for pet products leaves consumers vulnerable to unscrupulous manufacturers and poor quality products.

3.5.1 Published Therapeutic and Adverse Effects

There is limited data regarding the safety and adverse effects of CBD in pets although this is an emerging area of research. Recent articles on the subject are discussed below.

A randomized, placebo-controlled, blinded, cross-over study published in 2018 by Gamble et al. demonstrated statistically significant reduction of pain and an increase in activity in a group of 16 pet dogs with concurrent osteoarthritis at Cornell University (Gamble et al. 2018). The dogs were given 2 mg/kg of CBD oil by mouth twice a day for 4 weeks. Dogs were also allowed to remain on NSAIDs, fish oil, and/or glucosamine/chondroitin supplements during the study. Laboratory work showed a statistically significant, progressive increase in alkaline phosphatase (ALP) from baseline through week 4 of CBD treatment in nine dogs (56%).

A study performed at Colorado State University examined the safety, toxicity, and pharmacokinetics of CBD in 30 purpose-bred beagle dogs (McGrath et al. 2018). Dogs received CBD via transdermal cream applied to the ear, oral capsules, or oral oil tinctures at ~10 mg/kg or ~20 mg/kg daily for 6 weeks (due to formulation inconsistencies, the dosages were less than the goal dosage of 10 and 20 mg/kg). Adverse effects included diarrhea in all dogs, with 20% (6/30) exhibiting single episodes of vomiting. The authors suspected the episodes of diarrhea were due to CBD administration but extenuating causes such as dietary variation and stress may have had an impact. Only dogs receiving oral CBD experienced vomiting although no correlation with respect to formulation or dose was identified. Erythematous pinnae were reported in all but one dog receiving the transdermal formulation. The only reported clinically significant laboratory change was a dose-dependent elevation in ALP in 11 dogs (36%) receiving oral CBD. Significant changes, defined as more than two times the high end of the normal range (140 IU/L), were first documented at week 2 and progressed through week 6. Most elevations ranged from 400–600 IU/L, with one dog peaking ~1000 IU/L. Both fasting and postprandial bile acids remained normal in all dogs, including those with elevated ALP. Other reported effects that were not necessarily attributed to CBD administration included nasal and ocular discharge, salivary staining of the feet or ventral abdomen,

transient and intermittent elevated nictitans and body temperature, weight-bearing lameness, and transient isosthenuria, hyposthenuria, and/or proteinuria.

In 2019, Deabold et al. published a study on the pharmacokinetics and safety of whole-plant CBD dominant products in dogs and cats. Eight healthy, purpose-bred beagle dogs received 2 mg/kg of a 50% mix of CBD and CBDA in a soft chew formulation twice daily, while fasted, for 12 weeks. Serum chemistry values, including ALP and ALT, remained within the normal reference range for all dogs during the 12 weeks. Diarrhea was the most commonly recorded adverse event and occurred intermittently in all dogs. A small number of vomiting episodes were also recorded. Eight healthy, purpose-bred domestic shorthair cats received 2 mg/kg of a 50% mix of CBD and CBDA in an infused fish formulation twice daily, while fasted, for 12 weeks. Although changes in CBC parameters occurred, none were considered clinically significant. Serum chemistry values remained within reference ranges with the exception of one cat. The affected cat had an elevated aminotransferase (ALT) noted at week 4 (first measurement since week 0) of ~300 U/L. ALT decreased but remained elevated at the 8- and 12-week measurements, measuring ~150 U/L each time. The researcher noted after publication that the ALT in this one cat did return to normal. No clinical signs related to the cat's elevated ALT were observed. Other adverse effects included licking and head shaking during oil administration, pacing, chomping/chewing, gagging, vomiting, hypersalivation, being uncooperative, and grimacing. No diarrhea was observed in any cat. Food consumption and body weight remained consistent and ongoing physical exams revealed no abnormalities or changes in behavior over the 12 weeks. As with any study, it should be noted that recorded adverse events are not necessarily *directly caused* by the product and should not be interpreted as such. Of interest, the Cmax of CBD in cats was approximately 1/5 that of dogs, suggesting larger doses may be needed for therapeutic effect. For more detail on the pharmacokinetics reported in this study, see Chap. 2.

In 2020, Vaughn et al. published a randomized, placebo-controlled, blinded, parallel study in dogs comparing effects from escalating doses of three cannabis oil formulations containing predominantly CBD, THC, or a CBC/THC combination (1/5:1). Twenty healthy, purpose bread Beagle dogs were divided into groups of four with the intent of administering 10 escalating doses to fasted dogs, by oral gavage, with at least 3 days between administrations. In addition to laboratory diagnostics and measurement of plasma cannabinoids, all adverse events were recorded and categorized as mild, moderate, or severe/medically significant. The dogs in the CBD group were initially dosed ~2 mg/kg, with the 10th and final dose at ~62 mg/kg. Although adverse events were documented in the CBD arm, they were all classified as mild, defined as "activities of daily life were not impacted, and no intervention was indicated". The vast majority of these effects were GI related (nausea, emesis, diarrhea), although constitutional effects (lethargy, hyperesthesia), neurological effects (muscle tremor, ataxia), and those classified as ocular, dermatological, and respiratory were also noted. Unlike some of the other studies, minimal impact on hepatic enzyme elevation was noted. In the CBD group, one dog developed a 2.9-fold increase in ALP (127 U/L) compared to baseline (44 U/L) 24 h following its

10th dose (~62 mg/kg). Seven days after the 10th dose, the concentration had dropped to 93 U/L. At no point, did this animal's ALP exceed the normal reference range of 5–131 U/L. Overall, the authors concluded that the escalating doses of CBD were well tolerated by all dogs. The dogs in the THC and CBD/THC group developed more clinically significant adverse effects as compared to those in the CBD group. See the *Toxicity of THC*—Sect. 3.4 in this chapter for more detail on these two groups.

In people, dose-related elevation of alanine transaminase (ALT) and aspartate aminotransferase (AST) have also been reported. During clinical trials for Epidiolex®, enzyme elevation typically occurred in the first 2 months. In approximately two-thirds of cases, resolution occurred following discontinuation or dose reduction of the drug (or concurrent agents resulting in drug interactions); in one-third of cases, enzyme elevation resolved without dose reduction (Greenwich Biosciences 2018).

The reason for increased hepatic enzyme elevation, in all species, is thought to be due to induction of various cytochrome p450 enzymes in the liver, although more research and data is needed for a full understanding. Drug interactions can also play a role in elevated hepatic enzymes. In people, ALT elevation was more common in patients concurrently taking valproate (Greenwich Biosciences 2018).

CBD can also inhibit certain cytochrome P450 enzymes. Both inhibition and induction can lead to drug-drug interactions. This is an area of considerable concern and research, especially in human medicine. For example, in people, CBD is metabolized by CYP3A4 and CYP2C19. Concurrent administration of other drugs which also inhibit these enzymes can result in inhibition or slowed metabolism of these medications (Narimatsu et al. 1990). Dogs and cats also express CYP450 2C19 although data regarding interaction with CBD is lacking as enzyme nomenclature does not imply a one to one functional comparison (Greb and Puschner 2018). Regardless, monitoring of hepatic enzymes in animals chronically exposed to CBD is strongly recommended.

Lab animal reproductive toxicity studies with CBD revealed evidence of toxicity. Pregnant rabbits dosed orally at 0, 50, 80, or 125 mg/kg/day throughout organogenesis displayed decreased fetal body weights and increased fetal structural variations at the highest dose tested; this dose was also associated with maternal toxicity (Greenwich Biosciences 2018). Pregnant rats dosed at 0, 75, 150, or 250 mg/kg/day throughout the period of organogenesis displayed embryo-fetal mortality at the highest dose tested (Greenwich Biosciences 2018). When administered orally to rats, at doses of 75, 150, or 250 mg/kg/day throughout pregnancy and lactation, the following effects were observed: decreased growth, delayed sexual maturation, and neurobehavioral changes (decreased activity). Adverse effects on male reproductive organ development (small testes in adult offspring) and fertility were observed in the offspring at the mid and high dose. All effects occurred in the absence of maternal toxicity (Greenwich Biosciences 2018). It is important to note the dosages used in toxicity studies are considerably higher than thereputic dosges.

3.5.2 Clinical Signs of Poisoning

Due to the lack of intoxicating properties and wide margin of safety, less severe effects are expected in cases of pet exposure/overdose of CBD compared to those of THC containing products. However, exposure can be complicated by poor quality CBD products that may contain unlabeled THC or other agents such as synthetic cannabinoids.

Of the CBD exposures reported to Pet Poison Helpline from 2009 to 2019, the majority of cases (53%) remained asymptomatic. The most commonly reported clinical signs were lethargy/CNS depression, ataxia, vomiting, urinary incontinence, trembling, hyperesthesia, agitation, mydriasis, hypersalivation, and lateral recumbency (see Fig. 3.1). Many of these signs are consistent with pets exposed to THC/marijuana. As such signs are not expected with pets exposed to CBD alone, even in fairly large acute doses, the possibility that these signs may have been caused by adulterated products must be considered. Regardless, the overall lesson to the treating veterinarian is that patients exposed to CBD-containing products may become symptomatic. This reminds us that the adage, "treat the patient, not the poison" should be heeded. See Fig. 3.2 for the percentage of CBD exposure cases in which Pet Poison Helpline recommended veterinary intervention.

Other risks of accidental CBD product ingestion include exposure to carriers such as oils (aspiration), alcohols, toxic human foods, or a massive number of treats or novel food sources that could result in GI upset or other issues. Although significant systemic health effects are unlikely when ingested, IV dosing of 150–200 mg/kg in rhesus macaques did lead to tremors, hypopnea, respiratory arrest, and cardiac arrest in a dose dependent nature (Rosenkrantz et al. 1981). Such severe signs would not be anticipated following oral exposure in small animals.

3.5.3 Retrospective Study on CBD Cases Reported to Animal Poison Control

To further explore the development and spectrum of clinical signs following CBD exposures in pets, Pet Poison Helpline performed a retrospective study focused on 2 years of cases originating in the US or Canada. The study included any case originating between January 1, 2018 and January 12, 2020 that was documented in Pet Poison Helpline's database. All cases were searched for any exposure to CBD-containing products which resulted in 346 patients. A "CBD-predominant product" was defined as any commercial, OTC, prescription, or homemade product which was labeled as containing CBD. Commonly reported products included pet treats; oral oils/capsules/tinctures marketed for use in either people or pets; topical balms, salves, or lotions marketed for use on people; human foods; vaping liquids; etc. Many of the products were labeled as "full spectrum". To avoid clinical complication due to coingestants or THC, any patient with documented evidence

Table 3.1 Patients exposed to CBD-containing products over the 2-year retrospective study period. Age and weight are reported as the mean with corresponding full ranges as was reported by the pet owner or treating veterinary professional

	Dog	Cat	Parrot
Number of patients	232 (90%)	23 (9%)	1 (0.4%)
Age (years) (mean & range)	4.8 (0.25–16)	6.8 (0.58–17)	15
Weight (kgs) (mean & range)	19.8 (2.2–74.6)	4.92 (2.3–10.0)	0.68

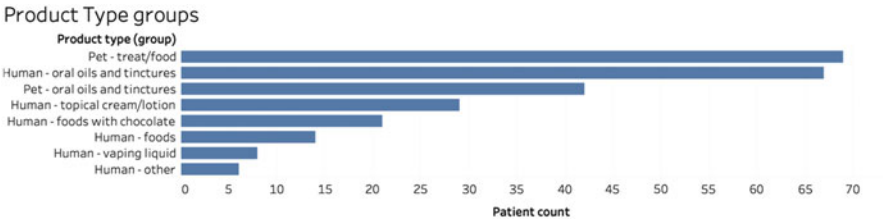

Fig. 3.3 Number of patients exposed to CBD-containing products, categorized by product type, over a 2-year period as reported to Pet Poison Helpline. Whenever possible and reasonable, the dose of CBD that the patient was exposed to was recorded. In some instances, the dose or concentration of CBD in the product was not listed on the product label or the package was too destroyed by the pet to read the labeled dose. As has been discussed elsewhere in this chapter, at the time this data was collected there was no regulatory oversight of OTC CBD containing products for pets or people, meaning that there were greater concerns for product quality and less confidence that the dose listed on the label matched the actual dose present in the product. See Fig. 3.4 for the reported clinical signs associated with specified dose ranges of CBD from this study

of exposure to products other than CBD were excluded. This includes products with labeled THC. CBD containing foods, even those containing chocolate, were allowed to remain in the study group. This reduced the total number of included patients to 256. Species, age, and weight ranges are described in Table 3.1.

Patients were categorized by the general type of CBD containing products to which the pet was exposed. The three most common, in descending order, were: pet treats 27% (n = 69); oral oils/capsules/tinctures marketed for use in people 26% (n = 67); and oral oils/capsules/tinctures marketed for use in pets 16% (n = 42). See Fig. 3.3 for full product category lists and associated patients counts.

3.6 Synthetic Cannabinoid Exposure

While this chapter focuses on plant derived cannabinoids, synthetic cannabinoids (SCBs), initially created many decades ago, became popular for recreational use in the US around 2000 and were marketed as a “legal high”. These compounds are dissolved in solvents and applied to dried plant material, intended to be smoked as an alternative to marijuana (Williams et al. 2015). They are often sold under a multitude of street names such as K2, Spice, Skunk, Wild Greens, Purple Haze, etc. They are

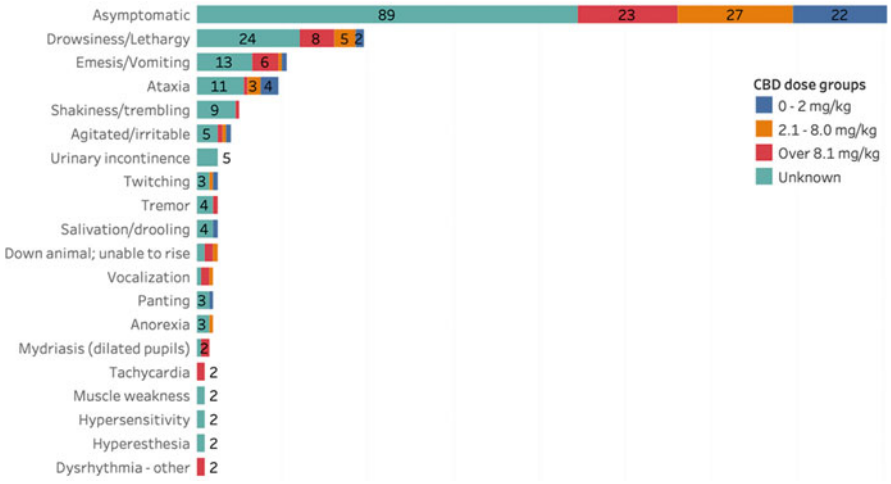

Fig. 3.4 Clinical signs associated with the specified CBD dose range as reported to Pet Poison Helpline in 256 patients. Individual patients may have exhibited more than one clinical sign. The total number of patients that developed clinical signs in this study group was 95 (37%). Signs which occurred in only one patient were excluded from this graphic. As these cases involve reports spontaneously made to an animal poison control center by a pet owner or veterinary professional, confirmation of exposure could not be obtained in all cases and the product dose was recorded at face value based on the label. As is the nature of spontaneously reported adverse event data, not all patients underwent medical evaluation, nor were these cases evaluated for causality. Therefore, these data are meant to portray general trends only. Based on the spectrum of clinical signs reported and significant concerns with OTC product quality in the US at the time this data was collected, there is a strong suspicion that some of these supposedly "CBD only" containing products were adulterated with THC, synthetic cannabinoids, or other substances. The product exposure categories were also individually reviewed with respect to the percentage of symptomatic patients. The only product in which 100% (n = 8) of exposed patients were symptomatic was vaping liquid. The category with the second highest percentage of symptomatic patients was pets exposed to oral oils and tinctures marketed for use in people, in which 50% (n = 34) of patients were symptomatic. This was followed by human foods *with no chocolate* (43%, n = 6), human topical formulations (41%, n = 12), oral oils and tinctures marketed for use in pets (33%, n = 14), human foods *with* chocolate (33%, n = 7), and pet treats (20%, n = 14)

typically packed in small sachets/envelopes and sometimes labeled as "incense" or "potpourri" and carry a "warning" stating they are "not for human consumption" to avoid detection by an unwitting public or law enforcement. In addition to containing SCBs, these products may have added chemicals/drugs such as caffeine or other stimulants. Although initially legally available in gas stations, head shops, tattoo parlors, etc., many of these products were banned by the 2011 Synthetic Drug Control Act.

3.6.1 Clinical Effects

Synthetic cannabinoids are engineered to cause potent psychotropic effects. They have a higher affinity for cannabinoid receptors than traditional marijuana, which results in more severe clinical effects (Gugelmann et al. 2014; Williams et al. 2015). In people, synthetic cannabinoids are 2–3 times more likely to cause sympathomimetic effects including tachycardia and hypertension, five times more likely to cause hallucinations, and have a higher incidence of seizures in comparison to marijuana (Murphy et al. 2012). Other signs may include cyclical agitation, aggression and incontinence in people (Gugelmann et al. 2014). Rare but significant cases of acute kidney injury have been reported in humans as well (Murphy et al. 2012).

Clinical effect data in animals exposed to SCBs is limited, although similar signs to those seen in humans have been reported. Exposure typically occurs via ingestion or, less often, inhalation. A case report involving both a pet owner and a dog affected by SCBs reported hyperesthesia, tremors, miosis, hyper-responsiveness to stimuli, ataxia, seizure-like activity, aggression and mild respiratory acidosis in the dog (Gugelmann et al. 2014). Similar signs were observed in the pet owner. Another case report involving presumptive SCB intoxication detailed signs of progressive ataxia, inappropriate mentation, hypothermia, stupor, and intermittent aggression with rapid progression to comatose condition, apnea, tremors, and opisthotonos (Williams et al. 2015). Cases reported to Pet Poison Helpline are more likely to involve severe signs such as tremors and seizures, when compared to marijuana, and all cases were deemed serious enough to warrant veterinary evaluation (see Figs. 3.1 and 3.2). These data seem to mirror the increase in severity of signs described in human medicine.

3.6.2 Pharmacokinetics

The pharmacokinetics of SCBs may be similar to other cannabinoids, although limited information is available. The oral bioavailability of various SCB products is likely low as case reports in people have indicated milder signs of shorter duration post-inadvertent ingestion of baked goods laced with SCBs (Obafemi et al. 2015).

- Half-life: 72–96 h in dogs and people (Williams et al. 2015)
- Recovery typically occurs within 24 h in people, perhaps shorter in duration if ingested (4–10 h) (Obafemi et al. 2015; Williams et al. 2015)
- LD_{50} and minimum toxic dose have not been established (McGrath et al. 2018)

3.7 Diagnosis of Poisoned Pets

There is no reliable point-of-care test available to detect the presence of cannabis or synthetic cannabinoids in veterinary patients. The OTC human urine drug screen assays designed to detect marijuana exposure are ineffective for SCB detection in

both people and animals and often yield false negatives in veterinary patients exposed to marijuana/THC (Teitler 2009). Liquid chromatography mass spectrometry (LC/MS) remains the gold standard for drug screening in both humans and animals. Due to the wide variability of SCBs, clinicians wishing to submit samples for testing are advised to consult with the diagnostic lab prior to sending.

The reason(s) for which most human urine drug screens often yield false negative results in veterinary species is unknown and likely multifactorial. Possibilities include the differences in urinary THC metabolites produced in dogs or cats, as compared to people; impacts from laboratory handling (THC binds to glass and rubber stoppers); and increased patient water consumption resulting in dilute urine (Donaldson 2002; Fitzgerald et al. 2013).

False positives when human urine screens are used may also occur. In people, NSAIDs such as ibuprofen, naproxen, and niflumic acid, and efavirenz (antiviral drug) may cause false positives depending on the brand of test. Whether or not these agents could affect testing with dog or cat urine is not known (Saitman et al. 2014).

3.7.1 Non-specific Diagnostics

Routine diagnostics can alert clinicians to secondary intoxications or unrelated medical problems, as well as help guide supportive care treatments.

- Radiographs: Monitor for evidence of ingested foil, other packaging materials, or batteries in the instance of a vaporizer pen ingestion, and the rare risk for foreign body obstruction if baggies, pipes, or vape pens are consumed.
- CBC/chemistry/pre-fluid urinalysis: Following acute exposure to most cannabinoids, no immediate lab abnormalities are expected. Therefore, use these tests to rule out underlying issues, other causes, and/or establish normal baselines.
- Initial diagnostics: PCV/TP to monitor hydration status; electrolytes (monitor sodium if multi-dose activated charcoal is given and monitor potassium if severely symptomatic); blood glucose (monitor intermittently in severely affected patients); renal profile (in the event hypotension leads to hypoperfusion); liver enzymes, especially in patients with chronic exposure.

3.7.2 Differential Diagnoses

The clinical signs for THC/marijuana and SCBs are non-specific and differential diagnoses must be considered if exposure cannot be confirmed. Differentials may include but are not limited to alcohols (ethanol, methanol, ethylene glycol, diethylene glycol, propylene glycol), opiates, benzodiazepines, muscle relaxants, tranquilizers, bromethalin (neurotoxic rodenticide), macrocyclic lactones (ivermectin, milbemycin), and illicit drugs (LSD, PCP, hallucinogenic mushrooms).

3.8 Treatment for Patients Exposed to Toxic Doses of Cannabinoids

3.8.1 Decontamination

1. Emesis
   (a) Emesis should be considered if all the following conditions are met:
      (i) A toxic dose was ingested.
      (ii) Exposure was within the last 30–60 min or a significant amount of material remains in the stomach.
      (iii) The patient is asymptomatic and at low risk for aspiration.
      (iv) Spontaneous vomiting has not occurred. Spontaneous vomiting is usually secondary to irritation of GI tract from plant material.
   (b) Emetics
      (i) **Dogs**: Apomorphine (0.03 mg/kg IV) or hydrogen peroxide, 3% (1–2 mL/kg PO, food in the stomach increases chance of success).
      (ii) **Cats**: Dexmedetomidine (7–10 mcg/kg IM), hydromorphone (0.1 mg/kg SQ), or xylazine (0.44 mg/kg IM). Reverse as needed with atipamezole or naloxone. Do not use hydrogen peroxide or apomorphine in cats.
2. Activated charcoal
   (a) Multi-dose activated charcoal may be considered in asymptomatic patients who are well hydrated and at low risk for aspiration.
      (i) Administer one dose of activated charcoal (1–2 g/kg PO) with sorbitol to start the series.
      (ii) Administer a half-dose of activated charcoal (or 0.5–1 g/kg PO) without sorbitol every 6–8 h × 1–2 additional doses.
      (iii) Have patients on IV fluids when receiving multi-dose charcoal and monitor serum sodium q 12 h. If acute CNS signs occur, check serum sodium STAT.
      (iv) Do not administer if the patient is at increased risk for aspiration, hypernatremia, dehydrated, or not passing stool prior to re-dosing.
3. Gastric lavage
   (a) Massive ingestions may benefit from gastric lavage with patients under anesthesia and airway secured. A dose of activated charcoal with sorbitol may be placed through the stomach tube.
4. Enemas
   (a) If suspect material is noted upon rectal exam, enemas may expedite clearance.

3.8.2 Supportive Care

1. In general, most animals recover with appropriate supportive care.
2. Antiemetics as needed. Do not use maropitant or antiemetics with a prokinetic effect if the patient is at risk for a foreign body obstruction.
3. IV fluids 1–1.5× maintenance; adjust as needed for perfusion changes. IV fluids are not expected to expedite or enhance excretion of cannabinoids to a large degree.
4. Thermoregulation
   (a) Warming or cooling therapy as needed. Hypothermia is more common with marijuana/THC exposure while hyperthermia is more commonly associated with SCB exposure. Do not cool below 103.5 °F.
5. Oxygen therapy if respiratory depression.
6. Nursing care
   (a) Generalized nursing care should be provided to obtunded or profoundly sedate patients including body rotation, ocular lubrication q 4–6 h, etc.
   (b) Keep the patient clean and dry. Although rarely necessary, an incontinent patient may benefit from a temporary urinary catheter.

3.8.3 Monitoring

1. Mildly affected patients may be monitored at home if kept in a safe environment with no fall risk.
2. Monitor vitals and blood pressure q 1–6 h depending on patient status.
3. Monitor laboratory values as needed:
   (a) PCV/TP to monitor hydration status, especially if giving activated charcoal.
   (b) Electrolytes: Monitor sodium if administering multi-dose activated charcoal and potassium if severely symptomatic.
   (c) Blood glucose, intermittently, in severely affected patients.
   (d) BUN/creatinine if hypotension leads to hypoperfusion.
   (e) Liver enzymes, especially in patients with chronic exposure or if hypotension leads to hypoperfusion.

3.8.4 Medications

1. Agitation: butorphanol (0.1–0.4 mg/kg IM or IV), diazepam (0.5–1.0 mg/kg IV

PRN), or ± acepromazine (0.01–0.2 mg/kg slow IV, IM or SQ, titrate dose for effect). Avoid acepromazine in hypotensive patients.
2. Tremors: methocarbamol (44–220 mg/kg slow IV to effect, select dose based on severity of signs). Re-dose PRN.
3. Seizures: diazepam, phenobarbital, propofol, levetiracetam
4. Bradycardia: atropine

3.8.5 *Intravenous Lipid Emulsion and Extracorporeal Therapy*

ILE has been suggested for marijuana and SCB intoxications in pets and used with varied success (Fitzgerald et al. 2013; Meola et al. 2012; Pet Poison Helpline and SafetyCall International 2019; Williams et al. 2015). The potential therapeutic benefit of ILE is based upon the knowledge that THC and cannabinoids are extremely lipophilic. All pharmacologically active cannabinoid compounds have a LogP > 4.5 (Thomas et al. 1990).

- THC (CSID:15266 n.d.):
  - LogP = 7.68
  - LogD at pH 7.4 = 7.25
- CBD (CSID:559095 n.d.):
  - LogP = 7.03
  - LogD at pH 7.4 = 6.43

The veterinary toxicologists at Pet Poison Helpline do not routinely recommend the use of ILE in cases of marijuana or SCB exposure, in part because of scant supportive data, both in the literature and from the Pet Poison Helpline database, but also because ILE may negatively impact the effect of therapies such as sedatives or anticonvulsants. If a patient's clinical signs are severe enough to consider ILE, consultation with an animal poison control center is highly recommended prior to starting.

The use of extracorporeal therapy (ECT) has been described in one case report (Culler and Vigani 2019). In this case the dog ingested synthetic THC oil and presented with extreme anxious behavior, dysphoria, severe/frantic vocalization, severe hyperesthesia, and seizures refractory to anticonvulsants, necessitating general anesthesia and mechanical ventilation. The dog was concurrently hyperlipidemic, so ILE was not attempted. ECT was accomplished using both a hemoperfusion cartridge and hemodialysis filter in series. Within 1 h of initiating ECT, the dog's mechanical ventilation was discontinued. Throughout ECT, the patient's neurological status progressively improved. At the end of the 3h session, the dog was described as alert, responsive, and ambulatory with no signs of seizure activity. Six hours after ECT, the dog was quiet, alert, slightly ataxic but eating

readily. The patient had radiographic evidence of aspiration pneumonia prior to ECT and, as such, remained in ICU for 36 h for IV antibiotic and nebulizer treatment. One-week post discharge, the dog was reportedly normal.

3.9 Prognosis

Most companion animals recover from acute, accidental cannabis exposures within 24–36 h. Severe cases may be affected for up to 72 h, barring secondary complications. Prognosis is generally good with supportive care.

3.10 Conclusion

Cannabis, THC, CBD, and SCB exposures and intoxications have been increasing in frequency in both human and veterinary medicine. Veterinary professionals are expected to see an increase in inadvertent companion animal exposure and intoxications as the additional therapeutic benefits of cannabinoids come to light, societal perceptions change, legislators and regulators move toward legalization and decriminalization, and access to cannabinoid products increases. Vigilant and careful physical exams with attentive and empathetic history taking skills devoid of judgment are imperative to help in diagnosing and treating companion animal patients.

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Chapter 4
Terpenes and Flavonoids: Cannabis Essential Oil

Liz Hughston and Melissa Conarton

4.1 Introduction

An essential oil is an extraction of the volatile plant molecules that confer distinctive colors, flavors, and aromas to different plant species (aka the "volatiome" of a plant). This diverse group of molecules are produced by all plants and are considered secondary metabolites. While primary metabolites are those substances created to meet the basic survival needs of the plant, secondary metabolites provide adaptive and protective mechanisms for plants, while also regulating the plant's development and metabolism, and are classified by nitrogen content. The non-nitrogen containing secondary metabolites include cannabinoids, terpenes, flavonoids, lignans, saponins, and fatty acids (Wink 2010). The volatile molecules of particular interest in cannabis for clinical and therapeutic use are terpenes and flavonoids. These molecules provide each variety of cannabis its distinctive color, aroma, and flavor profile, while potentially conferring therapeutic benefits in their own rights.

4.2 Terpenes

Terpenes are volatile, lipophilic hydrocarbons with high vapor pressure, meaning they evaporate at a lower temperature than non-volatile compounds and can be easily perceived by olfaction. Terpenes create the distinct, unique smell and flavor of each

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and every plant and fruit, while also providing protection against predators and attracting pollinators. Terpenes are the largest category of plant chemicals, with 15–20,000 molecules completely characterized (Langenheim 1994). Cannabis terpenes are synthesized and stored in trichomes, which are primarily found on both the inflorescence (flowers) and leaves of primarily the female plant. Terpenes are categorized according to their number of repeating 5-carbon building blocks, known as "isoprene units". Monoterpenes have 10 carbons, sesquiterpenes have 15 carbons, and triterpenes contain 30 carbons. Terpenes comprise between 0.3 and 5% of the dry weight of cannabis flowers, depending on variety (Andre et al. 2016; Nuutinen 2018). There are at least 120 terpenes identified in cannabis (Flores-Sanchez and Verpoorte 2008), with some researchers reporting higher numbers. While the terms "terpenoid" and "terpene" are often used interchangeably, terpenoids are oxygen-containing compounds derived from isoprene units (aka oxidized terpenes) (IUPAC 2006). For the purposes of this chapter, we will use the term terpene to refer to all compounds with isoprene unit skeletons.

Terpenes—as a class of chemicals—have been granted Generally Regarded As Safe (GRAS) status by the FDA as an active odor and flavor ingredient in many different products; acute oral LD_{50} in rats is greater than 5 g/kg (Tisserand et al. 2014). They are currently being investigated for use in many different applications, both cosmetic and medicinal. Two patent reviews (Guimarães et al. 2014; Silva et al. 2018) revealed many patents already granted for terpene-based medicines for analgesia and cardiovascular diseases, respectively. Given the safety profile of these molecules, their use as medicines is a focus of ongoing research.

While the cannabinoids THC and CBD are the primary drivers of the effects of cannabis, terpenes can fine-tune and modulate those effects. As many others have said, if cannabinoids are the "engine" or "motor" of cannabis, then terpenes are the "steering", enabling users to adjust both the medical and intoxicating effects of cannabis to pinpoint the exact therapeutic effect desired. Cannabinoids, terpenes, flavonoids, and other molecules in different combinations deliver different synergistic effects; this is commonly known as the "entourage effect". The complex mechanisms of these interactions are not well understood but research is ongoing to help clarify why combinations of these molecules are reportedly more effective than any single molecule in isolation (see "Entourage Effect", below).

4.3 Biosynthesis

Thus far, the biosynthesis of only 30 cannabinoids and terpenes has been elucidated (Booth and Bohlmann 2019). Based on current research, terpenes appear to be synthesized by two isoprenoid pathways. Isoprenoids, the precursors to all terpenes, are integral to plant cell membrane maintenance and hormone synthesis. The mevalonic acid (MVA) pathway occurs in the cytosol of the plant cell, and the 2C-methyl-d-erythritol-4-phosphate (MEP) pathway occurs in plastids (plant organelles). Both pathways result in the formation of d-isopentenyl pyrophosphate (IPP) and its isomer dimethylallyl pyrophosphate (DMAPP); these molecules are then

synthesized to geranyl pyrophosphate (GPP) (Pichersky 2006). GPP is the precursor for the monoterpenes myrcene, linalool, limonene, terpinolene, and pinene which are formed in plastids in cannabis trichomes. GPP is further synthesized in the cytosol of the cells to farnesyl pyrophosphate (FPP) which is the precursor for beta-caryophyllene, as well as other sesquiterpenes, triterpenes, and steroids (Thomas and ElSohly 2016). GPP is also the precursor to cannabinoid synthesis.

(Also see Fig. 4.1 later in the chapter).

4.4 Metabolism

Because terpenes are volatile and aromatic, two areas of metabolism should be considered: metabolism secondary to inhalation/olfaction and metabolism secondary to ingestion. When essential oils are inhaled (via diffusion into the environment, or from skin or surfaces to which oil has been applied, or during the process of ingestion) the volatile molecules, such as terpenes, bind to olfactory receptors (ORs) found in the cilia of olfactory sensory neurons, triggering the perception of smell. ORs are G-protein coupled receptors (GPCRs) and represent one of the largest families of GPCRs (Kang and Koo 2012; Lee et al. 2018). ORs have been discovered in non-olfactory tissues throughout the body including the testes, kidneys, liver, heart, and enterochromaffin cells in the GI tract (Lee et al. 2018). In the GI tract, when odorants such as terpenes bind to ORs on enterochromaffin cells, they trigger the release of serotonin, a key modulator of gut motility (Braun et al. 2007). Only 10% of known ORs have been categorized by function; future research on therapeutic targets for ORs is certain to include terpenes.

Inhaled terpenes are often excreted unchanged during exhalation, but they can be metabolized by odorant-metabolizing enzymes (OMEs) co-located with ORs in the epithelium of the respiratory and olfactory tracts. These OMEs are cytochrome P450 (CYP-450) enzymes, glutathione transferases, and others found in both the epithelium of the olfactory system as well as the mucus produced therein. In some cases, metabolites are excreted into the mucus, whereas others are exhaled and still others will be absorbed. The metabolism of odorants is rapid, occurring within milliseconds of OR stimulation (Heydel et al. 2019). Previous studies of odorant metabolism focused on toxicological properties of odorants and their metabolites; future physiological studies may help further elucidate the systemic effects of inhaled terpenes.

In addition to being volatile and absorbed via inhalation/olfaction, terpenes are lipophilic compounds rapidly absorbed from the gastrointestinal tract. They undergo extensive first pass metabolism in the liver by the CYP-450 pathway. Terpenes are oxidized to create hydrophilic compounds that can be eliminated in the urine (phase 1 metabolism). Following the initial metabolism, the terpene metabolites undergo conjugation (phase 2 metabolism) to further increase hydrophilicity and enhance the ability for the metabolites to be excreted. Some terpene molecules, and their volatile metabolites, reach the lungs and are exhaled but the majority of metabolites are

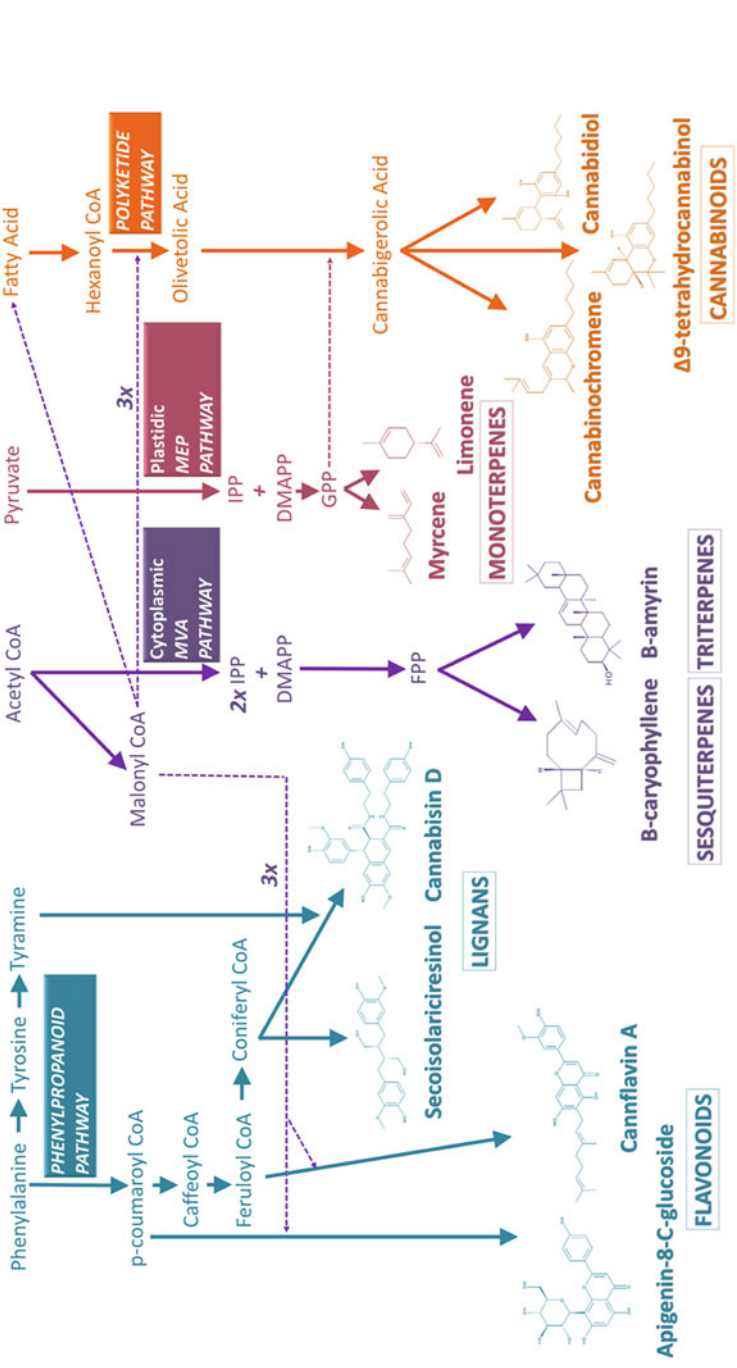

Fig. 4.1 Schematic of the biosynthetic pathways leading to the Cannabis secondary metabolites. Transport of precursors are represented by dashed arrows. Direct catalytic reactions are depicted by bold arrows. Abbreviations used: Isopentenyl diphosphate (IPP), geranyl diphosphate (GPP), dimethylallyl diphosphate (DMAPP), farnesyl diphosphate (FPP), mevalonate diphosphate (MVA), methylerythritol (MEP). (Reprint permission with full citation from Andre, C. M., Hausman, J., & Guerriero, G. (2016). Cannabis sativa: The Plant of the Thousand and One Molecules. *Frontiers in Plant Science*, *7*. doi:10.3389/fpls.2016.00019)

excreted by the kidneys into the urine, with a small amount excreted in the feces. Metabolism is rapid with metabolites identified in the blood and urine simultaneously. Excretion is also rapid; terpene levels in the blood demonstrate an elimination half-life of approximately 5 h, with almost no evidence of the terpene or its metabolites within 24 h of administration (Schmidt and Göen 2016).

4.5 Entourage Effect

In order to understand the idea of the entourage effect (also discussed in Chap. 2), it may be useful for the veterinary practitioner to consider the interplay of molecules within cannabis as a form of polypharmacy. We are adept (or becoming so) at using multiple pharmacological agents in different combinations to achieve clinical results that are synergistic, where the combination provides more profound effects than might be expected with each agent given on its own. For example, multi-modal anesthesia and analgesia—where a combination of sedatives, analgesics, anxiolytics, dissociative agents, etc.—is now considered the standard of care in veterinary medicine. Similarly, combinations of different chemotherapeutics often are more effective than using single agents alone, targeting different mechanisms of metastasis and malignancy. The synergy of the molecules within cannabis is no less powerful or wide-ranging in its clinical effects.

The term "entourage effect" was coined by Ben-Shabat et al. in 1998 to describe the interaction of endogenous cannabinoids and esters that increased agonism of CB1 and CB2 receptors by 2-AG without binding directly to those receptors. Support for the idea of synergy among the molecules in the cannabis plant has been demonstrated in many studies showing increased effectiveness of whole-plant, full spectrum extracts and products when compared to individual molecules (Russo 2011). In these studies, the synergy between phytocannabinoids, especially THC and CBD, has been explored, but terpenes may also play a role in the increased effectiveness of full spectrum cannabis products. In fact, further investigation into the interactions of terpenes with different receptors in the body may be the key to further understanding the entourage effect. One interesting area of research involves the transient receptor potential (TRP) channels such as TRPV1, which has been identified as important in the realm of analgesia. TRPV1 channels are activated not only by terpenes and other volatile compounds but are also activated by inflammatory signaling pathways (Maffei et al. 2011), pointing to a potential for synergy with cannabinoids like CBD, which are known to impact inflammatory processes.

Proponents of the medical applications of cannabis have long touted the benefits of whole plant or full-spectrum products, citing the entourage effect as the principle behind the efficacy expected of these products. However, skepticism justifiably remains as few high quality, placebo-controlled, blinded studies have been performed evaluating biochemical or metabolic markers that definitively prove this effect and expose its underlying mechanisms of action. One study (Blasco-Benito et al. 2018) showed increased effectiveness of a "Cannabis Drug Preparation"

(composed of THC, CBD, and the terpenes beta-caryophyllene, humulene, nerolidol, linalool, and beta-pinene) over pure THC against several human breast cancer cell lines, both in vitro and in vivo. However, the study ruled out the possibility that the increased cancer-killing properties came from the terpenes found in the preparation. Santiago et al. in 2019 found no evidence that six of the most common terpenes found in cannabis modulated agonism of either the CB1 or CB2 receptors and postulated that the entourage effect may be due to actions at different sites, or alterations to cannabinoid metabolism and distribution, or may arise from distinct actions outside of the ECS that affect ECS tone. These findings were further confirmed by Finlay et al. in 2020, where researchers pointed out the possibility that the medicinal effects of terpenes could be related to other receptor interactions, or via olfaction. More research is definitely needed on the terpene and flavonoid components of cannabis to determine their role in the entourage effect.

4.6 Cannabis "Strains"

Historically, in both the recreational and medicinal cannabis marketplace, flowers with different effects have been characterized as "strains" with many fanciful and descriptive names. A more accurate description of this variation in effects, appearance, and aroma of different cannabis flowers would be to refer to "chemovars", "varietals", or "varieties" of cannabis plants. These differences can be attributed to differing terpene and flavonoid profiles contributing to different aromas, flavors, and colors of each chemovar. Through the years there have been different attempts to classify the chemovars or varieties of cannabis and the effects—both medicinal and recreational—they impart to users. This led to the vernacular of "Indica" and "Sativa" employed by purveyors of cannabis to inform the end user about the expected effects of the plant. "Indica" cannabis is traditionally linked to those varieties with a calming or sedative effect (aka "couch lock" or "body high"), while "Sativa" varieties are described as uplifting or energizing ("head" or "cerebral high"). These variations in effect were once thought to be due to the differences in THC potency in the plant, but recent research has shown that these effects are more likely explained by variations in the terpene profiles of different varieties (Jin et al. 2020; Elzinga et al. 2015). When counseling pet owners about the use of cannabis for their companion animals, it is important that medical professionals avoid these vernacular classifications in favor of focusing on the molecular composition and the expected beneficial therapeutic effects of any particular product.

In a 2019 study, Booth et al. evaluated the terpene profile of a hemp variety of *Cannabis sativa* known as "Finola" in an effort to identify terpene synthases present in the plant and to explore the possibility of creating varieties of cannabis with specific terpene profiles based on plant genomics. The researchers found the most prevalent terpenes, on a dry weight basis, to be the monoterpenes alpha-pinene, beta-pinene, myrcene, limonene, ocimene, and terpinolene; and the sesquiterpenes beta-caryophyllene and humulene. The monoterpenes were measured at a

concentration of 389μg/dry weight gram of inflorescence, while the sesquiterpenes were 34μg/g. In their conclusion, Booth et al. commented, "The fact that terpenes have persisted throughout domestication as a substantial and diverse component of cannabis resin highlights their significance for human preferences."

In a 2020 study, Jin et al. measured terpene, flavonoid, and cannabinoid content in all parts (inflorescence, leaves, stems, and roots) of three different cannabis varieties. This study also found the most prevalent terpenes in the trichomes of the flowers of the different varieties to be myrcene, and both alpha- and beta-pinene. The researchers also found limonene and linalool at lower concentrations. The most prevalent sesquiterpenes were beta-caryophyllene and humulene, which matched the prevalence found in the Booth and Bohlmann (2019) study of "Finola". A review of several Certificates of Analysis (CoAs) for pet hemp products revealed that the prevalence and distribution of terpenes in these products was very similar to the aforementioned studies.

A discussion of the six most common cannabis terpenes (see Table 4.1) follows. The potential therapeutic value is reviewed along with the purported method of action (if known).

4.7 Common Terpenes Found in Cannabis

4.7.1 beta-Caryophyllene

Beta-caryophyllene is found in high concentrations in many varieties of cannabis and is generally one of the most prevalent terpenes in hemp varieties used for veterinary products. It demonstrates in vitro cytoxocity against human breast cancer cells and cervical cancer cells (Kubo and Morimitsu 1995), and human and mouse melanoma cells (Kubo et al. 1996). It demonstrates both in vitro and in vivo cytotoxic effects against Ehrlich ascites tumors and tumor cells in mice (Silva et al. 2007). Beta-caryophyllene also demonstrated inhibition of cell growth in vitro of human leukemia cells (Lampronti et al. 2006). The oxidized form of beta-caryophyllene, caryophyllene oxide, also demonstrated moderate cytotoxic effects in vitro against human prostate cancer cells, human breast cancer cells, human bladder carcinoma, and human melanoma cells (Sibanda et al. 2004; Loizzo et al. 2007). Caryophyllene oxide also enhanced the cytotoxicity of doxorubicin against three different human cancer cell lines in vitro (Di Giacomo et al. 2017).

Perhaps the most well-known properties of beta-caryophyllene are as both an anti-inflammatory and anti-oxidant agent. Essential oils containing beta-caryophyllene in high amounts are as effective at reducing inflammation as etodolac and indomethacin (Russo 2011). This anti-inflammatory effect can be attributed, at least in part, to beta-caryophyllene's full agonism of CB_2 receptors, making it both a terpene and a cannabinoid (Gertsch et al. 2008; Maffei et al. 2011; Alberti et al. 2017; Nuutinen 2018); it is the only molecule found in plants other than *Cannabis*

Table 4.1 The most common cannabis terpenes with descriptions

Terpene	Type	Aroma	Also found in	Medicinal effects	Reference(s)
beta-Caryophyllene	Sesquiterpene and cannabinoid (CB_2 agonist)	Peppery and spicy	Black pepper, clove, cinnamon, rosemary, hops, basil, oregano, ylang-ylang, green tea	• Analgesic	• Katsuyama et al. (2012b); Klauke et al. (2014); Paula-Freire et al. (2014); Fidyt et al. (2016)
				• Anti-inflammatory	• Fernandes et al. (2007); Russo (2011); Horváth et al. (2012); Cheng et al. (2014); Alberti et al. (2017); Ames-Sibin et al. (2018)
				• Antioxidant	• Kubo et al. (1996); Horváth et al. (2012); Ames-Sibin et al. (2018)
				• Anti-tumoral	• Kubo and Morimitsu (1995); Lampronti et al. (2006); Legault and Pichette (2007); Loizzo et al. (2007); Silva et al. (2007)
				• Bone growth	• Yamaguchi and Levy (2016)
				• Gastro-protectant	• Tambe et al. (1996)
Humulene (aka alpha-caryophyllene)	Sesquiterpene	Hoppy	Hops, cloves, coriander, ginseng, tobacco, sunflowers, sage	• Anti-inflammatory	• Fernandes et al. (2007); Passos et al. (2007); Rogerio et al. (2009)
				• Anti-tumoral	• Legault et al. (2003); Legault and Pichette (2007)
Limonene	Monoterpene	Citrus	Citrus fruits (especially concentrated in rinds), rosemary, juniper, peppermint, dill, fennel, celery, caraway seeds, hops	• Antidepressant	• Komori et al. (1995)
				• Anti-inflammatory	• Rufino et al. (2015); d'Alessio et al. (2013); Hirota et al. (2010, 2012); Chi et al. (2012)
				• Antioxidant	• Rehman et al. (2014)

				• Anti-tumoral	• Maltzman et al. (1989); Haag and Gould (1994); Murthy et al. (2012); Russo et al. (2014)
				• Anxiolytic	• Carvalho-Freitas and Costa (2002); Komiya et al. (2006); Pultrini et al. (2006); Lima et al. (2013)
Linalool	Monoterpene	Lavender	Lavender, cinnamon, rosewood, birch, coriander, sage, thyme	• Analgesic	• Ghelardini et al. (1999); Batista et al. (2008); Katsuyama et al. (2012b)
				• Anti-convulsant	• Elisabetsky et al. (1995, 1999)
				• Anti-inflammatory	• Batista et al. (2008); Li et al. (2016a) (*topical application*)
				• Anti-tumoral	• Jana et al. (2014); Chang and Shen (2014); Cheng et al. (2017)
				• Anxiolytic	• Linck et al. (2010); Guzmán-Gutiérrez et al. (2015); Harada et al. (2018)
				• Hepatoprotective	• Jana et al. (2014)
				• Neuroprotective	• Sabogal-Guáqueta et al. (2018)
				• Sedative	• Buchbauer et al. (1991); Linck et al. (2009, 2010)
Myrcene	Monoterpene	Herbal, earthy, fruity, clove-like	Mango, hops, lemongrass, basil, bay leaves, cardamom	• Analgesic	• Rao et al. (1990); Lorenzetti et al. (1991) Paula-Freire et al. (2015)
				• Anti-inflammatory	• Lorenzetti et al. (1991); Rufino et al. (2015)
				• Antioxidant	• Ciftci et al. (2011); Bonamin et al. (2014); Ciftci et al. (2014); Burcu et al. (2016)

(continued)

Table 4.1 (continued)

Terpene	Type	Aroma	Also found in	Medicinal effects	Reference(s)
alpha- and beta-Pinene	Monoterpene	Coniferous (alpha) and herbal (beta)	Alpha: pine needles, rosemary Beta: basil, dill, parsley, hops	• Analgesic (alpha)	• Li et al. (2016a) (*topical application*)
				• Antidepressant (beta)	• Guzmán-Gutiérrez et al. (2015)
				• Anti-inflammatory (alpha)	• Gil et al. (1989); Bae et al. (2012); Kim et al. (2015); Li et al. (2016b)
				• Antioxidant (alpha)	• Porres-Martínez et al. (2015, 2016)
				• Anxiolytic (alpha)	• Kasuya et al. (2014)
				• Bronchodilator (alpha)	• Falk et al. (1990)
				• Sedation/sleep (alpha)	• Yang et al. (2016)
				• Short-term memory enhancer (alpha)	• Perry et al. (2000, 2002); Miyazawa and Yamafuji (2005)

that directly interacts with cannabinoid receptors in the ECS. Beta-caryophyllene counteracts neuroinflammation in mouse models of multiple sclerosis and the neuropathic pain associated with inflammation (Klauke et al. 2014; Alberti et al. 2017). It also protects brain tissue from damage via inhibition of several inflammatory cytokines (Alberti et al. 2017).

Beta-caryophyllene also acts an analgesic, especially in neuropathic pain conditions, directly via CB2 receptor agonism (Fidyt et al. 2016), and indirectly by virtue of its anti-inflammatory properties. In addition, beta-caryophyllene was found to protect gastrointestinal cells in the face of anti-inflammatory medication administration (Tambe et al. 1996).

4.7.2 Humulene (aka alpha-Caryophyllene)

Humulene is found most prevalently in cannabis and hops (*Humulus lupulus*), and at lower concentrations in many other plants. It has anti-inflammatory, anti-tumoral, and wound healing properties. It demonstrates synergy with beta-caryophyllene in many essential oils, particularly in its anti-tumoral effects.

Fernandes et al. (2007) reported the anti-inflammatory effect of humulene to be equal to that of dexamethasone in in vivo (rat) models of inflammation. Humulene was also found to be equal to dexamethasone in reducing airway inflammation in vivo in rats (Rogerio et al. 2009), without inducing the side effects seen in study subjects treated with steroids alone. It was effective as both a preventative and a therapeutic agent of allergic airway inflammation.

Humulene inhibits the release of both tumor necrosis factor alpha (TNFα) and interleukin-1β in addition to blocking COX-2 expression and prostaglandin production (Fernandes et al. 2007). However, it increased the secretion of the pro-inflammatory cytokine interleukin-8 (IL-8) from human gastrointestinal cells in vitro; IL-8 influences migration of inflammatory cells and promotes angiogenesis, which could be helpful in enhancing wound healing, but may contribute to tumor growth (Satsu et al. 2004).

As an anti-tumoral agent, humulene encourages creation of reactive oxygen species (ROS) which damage cancer cells (Legault et al. 2003). Legault and Pichette (2007) found that humulene was cytotoxic in vitro to human breast adenocarcinoma and its cytotoxic effects were potentiated when it was combined with beta-caryophyllene. Derivatives of humulene found in the *Asteriscuc vogelii* plant were found to be cytotoxic to human lymphoma, lung carcinoma, colon carcinoma, and melanoma cells in vitro (Rauter et al. 2001). It is not yet known if these same derivatives exist in cannabis.

There is in vitro evidence of humulene's effectiveness as an anti-bacterial agent against *Staphylococcus aureus* (Pichette et al. 2006) and various pathogenic oral bacteria (Azizan et al. 2017). Also in vitro, humulene showed effectiveness against *Bacteroides fragilis* (a causative agent of Inflammatory Bowel Disease in humans), including the ability to break down the biofilm created by these bacteria (Jang et al.

2020). In vivo studies have not been performed. Anecdotally, humulene has been attributed with sedative and appetite-suppression properties, but these effects have not yet been formally studied.

4.7.3 Limonene

Limonene has a well-documented safety profile and is used in many consumer products such as: a solvent in cleaning products; a flavoring and odorant in food products; in perfumes; and as an insecticide (Nuutinen 2018). It is well known for its anti-inflammatory and anti-cancer properties and its effect on mood and anxiety.

In an in vitro model of osteoarthritis, limonene inhibited interleukin-induced nitric oxide production, thereby reducing inflammation (Rufino et al. 2015). In an in vivo rat model of colitis, limonene reduced blood levels of TNFα to a greater extent than that seen in rats treated with ibuprofen or subjects in the control group. The limonene-treated rats also had less weight loss and lower inflammation scores than the control group or ibuprofen-treated rats (D'Alessio et al. 2013). The same study conducted in vitro examination of colonic epithelial cells and found that limonene preserved gut barrier function. In the human arm of the study, elderly people taking limonene as an oral supplement had lower levels of inflammatory markers in their blood (D'Alessio et al. 2013).

In models of asthma, limonene inhibits cytokine and ROS production, and inactivates the migration of eosinophils in vitro, making it a potential therapeutic agent in this disease (Hirota et al. 2010). Hirota et al. (2012) went on to demonstrate this effect in vivo in a mouse model of bronchial asthma, wherein airway modeling was also reduced when limonene was inhaled. Further in vivo experimentation showed that limonene also reduced lipopolysaccharide-induced inflammation in a mouse model of acute lung injury (Chi et al. 2012).

Limonene has been shown to prevent induced mammary carcinomas in rats when administered in the early stages of the disease (Maltzman et al. 1989) and to induce regression of these tumors via its primary metabolite, perillic acid (Haag and Gould 1994). Apoptosis of human colon cancer cells was induced and angiogenesis to these cells was reduced by limonene (Murthy et al. 2012), showing it to be a potent anti-cancer agent. In an in vitro study of neuroblastoma, limonene was found to modulate autophagy of cells in a dose-dependent manner (as a constituent of bergamot essential oil) (Russo et al. 2014). Along with its direct anti-cancer properties, limonene was shown to protect kidney cells from oxidative and inflammatory damage secondary to doxorubicin administration in rats (Rehman et al. 2014).

In a 1995 study by Komori et al., inhaled limonene improved depression scores in a small group of human subjects. Interestingly, the inhaled citrus fragrance also normalized levels of immune cells, whether they were elevated or depressed pre-treatment, potentially demonstrating the ability of limonene to restore immune system homeostasis, though the sample size in this study was very small by human research standards. Inhaled orange oil, comprised of over 95% limonene, decreased

anxiety and elevated mood in patients in a dental office (Lehrner et al. 2005). Limonene was found to reduce anxiety by boosting serotonin and dopamine levels in the central nervous system (Carvalho-Freitas and Costa 2002; Komiya et al. 2006; Pultrini et al. 2006). These anti-anxiety effects were not blocked by flumazenil, suggesting a non-benzodiazepine method of action (Lima et al. 2013).

4.7.4 Linalool

Linalool is present in high levels in lavender essential oil and is a relatively minor terpene constituent in most cannabis varieties. It is known to have sedative, anxiolytic, anti-depressant, anticonvulsant, analgesic, anti-inflammatory, anti-tumor, antioxidative, neuroprotective, hepatoprotective, and anti-microbial properties (Nuutinen 2018).

Research has shown that inhaled linalool (as lavender essential oil) increases sedation and decreases normal motor activity without a loss of motor function in mice (Buchbauer et al. 1991; Linck et al. 2009, 2010). In the 2010 study, Linck et al. found that inhaled linalool increased social interaction and decreased anxiety in mice. Further studies showed linalool exerts its sedative and anxiolytic effects via both the monoaminergic and GABAergic pathways (Guzmán-Gutiérrez et al. 2015; Harada et al. 2018). Linalool exerts further central nervous system effects by modulating stimulation of glutamate receptors, thereby exerting ant-convulsant properties (Elisabetsky et al. 1995, 1999). Its antagonism of glutamatergic receptors contributes to linalool's central analgesic properties (Batista et al. 2008), and interferes with both short- and long-term memory functions in healthy rats (Coelho et al. 2011). Interestingly, in a rat model of cerebral ischemia, researchers found that linalool protected astrocytes and neurons and rats treated with oral linalool recovered more quickly from the ischemic event than those in the control group (Sabogal-Guáqueta et al. 2018).

In addition to its central effects, linalool also demonstrates local anesthetic properties equal to the effects of procaine and menthol (Ghelardini et al. 1999). It also produced anti-nociception in a rat model of chronic inflammatory and neuropathic pain (Batista et al. 2008). A 2012 study demonstrated that linalool's local anti-nociceptive effects may be attributed to action on peripheral opioid receptors (Katsuyama et al. 2012a). It is also effective (as a constituent of frankincense essential oil) as a topical anti-inflammatory and anti-nociceptive agent via modulation of COX-2 expression (Li et al. 2016a).

Linalool administered orally to mice in a sarcoma model decreased tumor volume in vivo, while demonstrating apoptosis of cancer cells in vitro. While linalool was found to be a pro-oxidant in cancer cells, it protected liver cells from oxidative damage, making it a potential adjunctive or even primary treatment for sarcomas and other solid-tumor cancers (Jana et al. 2014). Linalool's pro-oxidant effect was also seen in an in vitro study of glioma, where it was found to induce apoptosis via the formation of reactive oxygen species within glioma cells (Cheng et al. 2017). In

another in vitro study, linalool induced apoptosis in several lines of cancer cells, while also preventing migration of those cells, making it a potential anti-metastatic agent (Chang and Shen 2014).

4.7.5 Myrcene

Myrcene is one of the most abundant terpenes found in commonly available cannabis varieties; it is the terpene most often cited as contributing to the sedative or "couch-lock" effect of some varieties of cannabis. Anecdotally, cannabis users and "budtenders" claim that myrcene enhances the intoxicating effects of THC by aiding penetration of the blood brain barrier by the cannabinoid THC. There is no scientific evidence for the claim that myrcene lowers the blood brain barrier, and only weak evidence for myrcene's sedative effects. In fact, in the one study of the sedative properties of myrcene in mice, sedation was only seen at very high doses and those same doses also induced anxious behavior in the study subjects (Do Vale et al. 2002).

Myrcene does have a number of therapeutic applications including anti-inflammatory and antioxidant effects in neuronal, cardiac, and skin tissues, as well as analgesic properties (Nuutinen 2018). Rao et al. (1990) demonstrated that myrcene's analgesic effects occur via stimulation of alpha-2 adrenergic receptors both centrally and peripherally. Lorenzetti et al. confirmed the peripheral analgesic properties of myrcene and elucidated the mechanism of action was via inhibition of inflammatory prostaglandins. Myrcene (as a constituent of wild basil essential oil) was effective against neuropathic pain in mice, also via alpha-2 adrenergic receptor agonism (Paula-Freire et al. 2015). Myrcene also demonstrated anti-inflammatory effects in an in vitro model of osteoarthritis (Rufino et al. 2015), with evidence of protection of chondrocytes.

Further to its cytoprotective properties, myrcene protected rat livers from persistent exposure to an environmental pollutant by exerting antioxidant effects (Ciftci et al. 2011) with those effects increasing over time. Myrcene also protected gastric and duodenal cells in vivo from peptic ulcer formation induced by ethanol and indomethacin administration, as well as those induced by ischemia-reperfusion (stress) injury (Bonamin et al. 2014). This protective action was attributed to myrcene's antioxidant properties, as well as direct antimicrobial action against *Helicobacter pylori*, a known causative agent of GI ulcers. Myrcene reversed neurodegeneration in a mouse model of global cerebral ischemia, demonstrating a potential therapeutic use in stroke or other ischemic brain injuries (Ciftci et al. 2014). In addition to neuroprotective effects, myrcene demonstrates protection of cardiac cells after reperfusion injury, making it a potential treatment for this complex condition (Burcu et al. 2016).

4.7.6 Pinene

Pinene has two primary isomers that are found in cannabis—alpha-pinene and beta-pinene—each with their own therapeutic effects. Alpha-pinene has anti-tumoral, anti-inflammatory, antioxidant, anxiolytic, and bronchodilation effects. It also serves as a molecular basis for the development of some CB2 receptor ligands (Nuutinen 2018). beta-Pinene may have a role in the entourage effect and increasing the effectiveness of other medications.

alpha-Pinene has been shown to inhibit acetylcholinesterase (AchE) both in vitro and in vivo as a constituent of sage essential oil (Perry et al. 2000, 2002; Miyazawa and Yamafuji 2005). This inhibition allows for persistence of acetylcholine in the neuronal junction, giving it more time to interact with receptors which results in different actions, depending on the location of the neurons. In the musculoskeletal system, for example, AchE inhibition can encourage muscular contraction. In the central nervous system, acetylcholine is implicated in the formation of memories (Hasselmo 2006) and inhibition of AchE may improve short-term memory. In human medicine, this property may be useful in the treatment of Alzheimer's Disease (Miyazawa and Yamafuji 2005).

Oral administration of alpha-pinene improves the quality and duration of sleep in mice via GABA benzodiazepine receptors (Yang et al. 2016). Inhalation of alpha-pinene is anxiolytic, demonstrated by increases in tyrosine hydroxylase, the rate-limiting enzyme in the production of dopamine, in the mid-brains of mice (Kasuya et al. 2014). beta-Pinene has demonstrated both anxiolytic effects and anti-depressant effects via interactions with the monoaminergic pathway (particularly in combination with linalool (Guzmán-Gutiérrez et al. 2015).

When inhaled, alpha-pinene expands airway diameter, making it a potential treatment option for restrictive airway conditions (Falk et al. 1990). Intraperitoneal administration of alpha-pinene to mice with induced acute pancreatitis inhibited inflammatory cytokine production (Bae et al. 2012), which may attenuate damage to distant organs often seen in acute pancreatitis. alpha-Pinene also inhibited inflammatory cytokine production in vitro in macrophages in a mouse model of lipopolysaccharide-induced peritonitis (Kim et al. 2015). When applied topically, alpha-pinene exhibits local anti-inflammatory effects via the down-regulation of local COX-2 enzymes (Li et al. 2016a). In vitro, alpha-pinene demonstrated antioxidant effects by scavenging reactive oxygen species created by hydrogen peroxide in rat pheochromocytoma cells and in astrocytes (Porres-Martínez et al. 2015, 2016) (Table 4.2).

Table 4.2 Summary of terpene biological activity

Terpenoid	Known properties
β-Myrcene	Analgesic, anti-inflammatory, antibiotic, antimutagenic
β-Caryophyllene	Anti-inflammatory, cytoprotective, antimalarial, CB2 agonist
d-Limonene	Immune potentiator, antidepressant, antimutagenic
Linalool	Sedative, antidepressant, anxiolytic, immune potentiator
Pulegone	Acetylcholinesterase (AChE) inhibitor, sedative, antipyretic
1,8-Cineol	AChE inhibitor, stimulant, antibiotic, antiviral, anti-inflammatory, antinociceptive
α-Pinene	Anti-inflammatory, bronchodilator, stimulant, antibiotic, antineoplastic, AChE inhibitor
α-Terpineol	Sedative, antibiotic, AChE inhibitor, antioxidant, antimalarial
Terpineol-4-ol	AChE inhibitor, antibiotic
p-Cymene	Antibiotic, anticandidal, AChE inhibitor

4.8 Cannabis Flavonoids

Flavonoids make up about 10% of the compounds found in cannabis. The current consensus is that there are 21–26 flavonoids found in cannabis and most of these flavonoids are also present in other plants including fruits, vegetables, and herbs which both humans and animals consume. Flavonoids not only protect the plant and provide diverse characteristics of odor and flavor depending on variety, they also have known medicinal properties including anti-inflammatory, anti-oxidative, anti-carcinogenic, cardioprotective, antiviral, and anti-mutagenic effects with the additional capability of regulating enzymatic function at a cellular level (Ibrahima et al. 2010; Panche et al. 2016).

Although research on cannabinoid flavonoids previously has been somewhat stymied due to federal regulations on cannabis as a whole, a few of these flavonoids unique to cannabis (cannflavins) warrant further investigation due to their anti-inflammatory and anti-cancer properties. Cannflavins work in concert with cannabinoids and terpenes to contribute to the entourage effect.

4.8.1 Flavonoids

Flavonoids are secondary metabolites that aid in biological activities in plants, animals, and bacteria. They are responsible for color and aroma of flowers and attractiveness to insects which serve as pollinators to plants. Flavonoids have several functions within plants: they provide protection from UV rays by filtering the radiation at the cellular level; they protect against both biotic and abiotic stressors; they function as signal molecules within the plant; and act as detoxifying agents and antimicrobial defense compounds (Panche et al. 2016).

Flavonoid compounds are found in several parts of the plant and belong to a class of low-molecular weight phenolic compounds (Panche et al. 2016). The flavonoid structure consists of two aromatic rings (A and B) with an oxygenated heterocycle (ring C). The heterocycle is formed when the two aromatic rings become bound together by three carbon atoms. The subclasses of flavonoids are determined by the molecule's activity and type of heterocycle. Flavonoids are divided into 12 major subclasses based on their chemical structure. The six dietary flavonoids include: anthocyanins, flavanones, flavones, flavonols, isoflavones, and flavanols (catechins and proanthocyanidins) (Manach et al. 2004).

Dietary flavonoids are found naturally in fruits, vegetables, tea, wine, cocoa, medicinal plants, and herbs. The biologic effects of flavonoids are associated with their ability to modulate cellular activity and antioxidant activity, however bioavailability in vivo for the latter has been discovered to be low. Despite the diminished antioxidant activity within plasma concentrations in humans, research demonstrates the ability of flavonoids to modulate cell signaling pathways (Spencer et al. 2001, 2003). Due to the effect on cell signaling and enzyme inhibition (e.g. xanthine oxidase (XO), lipoxygenase, phosphoinositide 3-kinase and cyclo-oxygenase) flavonoids have been studied for use in various pharmaceutical applications. Several randomized, controlled trials have been published on the consumption of specific flavonoids (flavan-3-ols and anthocyanins), showing beneficial effects on cardiovascular health and vascular endothelial function (https://lpi.oregonstate.edu 2019; Cassidy et al. 2015).

4.9 Flavonoid Classification

Flavonoids can be divided into different subgroups based on the position of carbon on the C ring and the linkage of the B ring. This chapter will focus on the subgroups that are derived through dietary means. The subclasses within the flavonoid subgroups are found in specific areas of the plant or fruit and promote specific functions both within the plant and when consumed by mammals.

4.10 Subgroups

4.10.1 Flavones

Flavones are present in leaves, flowers, and fruits of plants as glucosides. This subgroup happens to be one of the most important among all of the flavonoids. Flavones are present in foods and plants such as chamomile, mint, ginkgo biloba, parsley, citrus fruits, and celery. A few of the subclasses of flavones include apigenin, luteolin, baicalein, rhoifolin, chrysin, genistein, and tageritin. Flavones can affect cytochrome P45 (CYP450) metabolic activity. Dietary intake of plant flavones

has demonstrated antioxidant, anticancer, and neuroprotective properties (Rea et al. 2019).

4.10.2 Flavanones

Flavanones are typically present in citrus fruits and grapes. This subclass includes: hesperetin, eriodictyol and naringenin. Flavanones provide free radical scavenging properties. Studies have demonstrated cytoxic properties of prenylated flavanones and these molecules have been shown to induce apoptosis in cell line studies (Chen et al. 2012).

4.10.3 Flavanols (Flavan-3-ols or Catechin)

Flavanols are found in fruits and vegetables. This subgroup is the building block of proanthocyanidins (PACs). The most studied subclasses of flavanols consist of monomers (known as catechins) dimers, and polymers. These compounds occur abundantly in a variety of vegetables and fruits including tea, cocoa, grapes, and wine. Research on flavanols suggests that these molecules may reduce cardiovascular disease (Hertog et al. 1993a).

4.10.4 Isoflavonoids

Isoflavonoids are a distinctive subgroup of flavonoids with limited distribution within the plant kingdom. Isoflavonoids are predominantly found in soybeans and legumes. Subclasses of isoflavonoids include daidzein, genistein, glycitein, and homoisoflavonoids.

The majority of dietary isoflavones are present as glycosides. The isoflavones found in soybeans are known to have anti-inflammatory properties (Yu et al. 2016). Isoflavonoids are known to act as phytoestrogens in mammals. Although additional high-quality clinical research is needed, isoflavones appear to provide health benefits in certain conditions. Wei et al. (2012) research proved soybean isoflavones' (specifically genistein and daidzein) ability to suppress immune sensitization by suppressing dendritic cell (DC) maturation. This action allowed the isoflavones to regulate mucosal immune responses in mice. These positive findings warrant additional studies on this compound as an immunoregulatory molecule that may be useful in the treatment of allergies and respiratory hypersensitivities.

4.10.5 Flavonols

Flavonols are 3-hydroxyl derivatives of flavanones. Flavonols contain the 3-hydroxyflavone backbone due to the position of the hydroxyl group on the C ring (bound to position 3). Flavonols are found in blueberries, cranberries, apples, bananas, onions, red wine, and tea.

Flavonols are the natural antioxidants of food. They protect ascorbic acid from autoxidation in fruit juices and a few studies have mentioned their possible role in the prevention of chronic diseases (D'Andrea 2015; Böhm et al. 1998; Shi et al. 2013). Myricetin, quercetin and kaempferol are subclasses of flavonols. Studies for health benefits include lowered risk of vascular disease and their role as antioxidants.

Flavonols in cranberries are often utilized in veterinary patients with recurrent urinary tract infections (UTIs) to prevent bacterial adhesion. A research study demonstrated flavonols (along with proanthocyanidins (PACs)) were more efficient at lowering the force adhesion of *E. coli* to host surfaces (Gupta et al. 2016). These findings may warrant further research into the potential of adjunctive or alternative antibacterial treatments.

4.10.6 Anthocyanins

Anthocyanins are the pigments that provide the richness in color of purple, red, and blue plants. They are found in high levels in vegetables, grains, roots, and red to purplish-blue colored, edible leafy vegetables. They are consumed as a source of antioxidants. Examples are berries, black currants, and other blue-colored fruits. Anthocyanin-rich black carrot, purple potato, and red cabbage are foods that have been consumed for disease prevention (Khoo et al. 2017).

Anthocyanins have been used in herbal medicine as treatments for high blood pressure, urinary tract infections, and to shorten the duration of symptoms of the common cold. Subclasses of anthocyanins include cyanidin, delphinidin, malvidin, pelargonidin, and peonidin.

4.11 Cannabis and Flavonoids

Flavonoids are components of cannabis that provide a variety of functions within the plant including filtration of UV light, providing color to attract pollinating insects, and both antifungal and antibacterial properties.

Within the cannabis plant, flavonoids occur as glycosides, aglycones, and methylated derivatives with the basic flavonoid structure (Kumar and Pandey 2013). Flavonoids are important for both the survival of cannabis and its medicinal properties. The total content of flavonoids in cannabis leaves and flowers may be about

1–2.5% of its dry weight. This percentage depends on environmental factors and the specific variety of cannabis. The main flavonoid subgroups identified in cannabis include flavones and flavonols. Several important flavonoid glycosides (C-/O-) include subclasses apigenin, kaempferol, luteolin, quercetin, orientin, vitexin and the unique-to-cannabis flavonoids cannflavin A, B, and C.

4.11.1 Apigenin

Apigenin is a color flavonoid often used to dye fabrics. Current in vivo research in mice produced evidence of antioxidant, anti-inflammatory and anti-carcinogenic properties (https://patents.justia.com/patent/20180098961). According to one review study: "Apigenin was reported to suppress various human cancers in vitro and in vivo by multiple biological effects, such as triggering cell apoptosis and autophagy, inducing cell cycle arrest, suppressing cell migration and invasion, and stimulating an immune response" (Yan et al. 2017).

4.11.2 Kaempferol

This polyphenol antioxidant has been studied for the reduction of risk of cancer and chronic disease. It is thought to augment the body's antioxidant defense against free radicals which could mean a decreased potential of cancer development in mammals. The review study by Chen and Chen (2013) concluded that kaempferol reduces the risk of cancer by modulating apoptosis, angiogenesis, inflammation, and metastasis.

4.11.3 Luteolin

A review study published by Lin et al. (2008) mentions luteolin, from the flavone subgroup, as having multiple biological effects. This study is also notable for elucidating the mechanism of action for luteolin's selective cytotoxicity in cancerous cells while preserving normal, healthy cells. Luteolin can function as either an antioxidant or pro-oxidant biochemically. Luteolin may have a place in hormone replacement therapy due to estrogenic activity at low concentrations.

4.11.4 Quercetin

This color flavonoid possesses anti-inflammatory potential in both human and animal models. In a review study by Li et al. (2016b) titled "Quercetin, Inflammation

and Immunity," the researchers found quercetin possesses both mast cell stabilizing and cytoprotective activity, has immunosuppressive effects on dendritic cell function that can be expressed on different cell types in both animal and human models, and exerts inflammatory- and immune-modulating activity in several murine models of autoimmunity.

In vivo experiments on animals demonstrate the anti-inflammatory effects of quercetin. A study by Mamani-Matsuda et al. (2006) produced data indicating that quercetin has potential as both an anti-inflammatory therapeutic and inflammation prevention agent. The administration of quercetin led to reduced clinical signs of chronic adjuvant-induced arthritis in rats, compared to untreated controls. The possible mechanism was quercetin's ability to target the inflammatory response of macrophages. Although quercetin is an often-used dietary supplement, the levels of effective bioavailability in vivo have not been substantiated for immunity enhancement.

4.11.5 Orientin

Orientin is a water-soluble flavonoid and a known, potent radioprotectant. The study by Satyamitra et al. (2014) found that the antioxidants orientin and vicentin (another cannabis flavonoid) significantly reduced 4 Gy-induced DNA damage and hastened repair of ex vivo irradiated mouse splenocytes. The study mentions the ability of both orientin and vicentin to facilitate repair of radiation-induced injury. However, further studies are indicated (Satyamitra et al. 2014).

4.11.6 Vitexin

Vitexin is a flavonoid which is commonly used to prevent heart disease. Vitexin is cardioprotective and, according to a review conducted by Aslam et al. (2015), has exhibited potent hypotensive, anti-inflammatory, and anti-metastatic potential along with antispasmodic properties. In this review, vitexin is credited with a hypotensive effect due to its ganglion-blocking properties and anti-inflammatory effects through its antihistamine, anti-bradykinin, anti-serotonin, and anti-oxidative properties.

4.12 Cannflavins

Although extensively studied in many other plants, the biosynthetic pathways of flavonoids unique to cannabis have not been studied (Andre et al. 2016). Of the 21–26 flavonoids isolated in cannabis, the cannflavins A, B, and C are exclusive to

this plant. These specific flavonoids have been found to provide anti-inflammatory effects and are currently being isolated and studied for this purpose.

Cannflavins are part of the flavone subgroup. They were first identified in the 1980s by Dr. Marilyn Barrett who discovered two diprenylated flavonoids in cannabis by isolating them through extraction into a "pure compound" then utilizing mass spectrometry to view the structure. Ultraviolet spectroscopy was used for confirmation. The flavonoid compounds were referred to as cannflavin A and cannflavin B. Cannflavins were of interest due to the recognition of their ability to inhibit the release of inflammatory prostaglandins from cells in culture. A study conducted in 1986 by Barrett et al. confirmed cannflavin A and B inhibited prostaglandin production by human rheumatoid synovial cells in culture. Cannflavin A and B are the first aglycone flavonoids discovered to be unique to the cannabis plant. It was not until 2013 that Mahmoud Elsohly et al. identified the third cannabis-unique cannflavin: cannflavin C.

4.13 Cannflavin Research

One of the first studies of cannabis-unique flavonoids found these compounds to be 30 times more potent than aspirin when they were assayed for their ability to inhibit prostaglandin E2 (PGE2) release from human rheumatoid cells in culture (Barrett et al. 1985). Another study performed in 2014 by Oliver Werz et al. discovered sprouting hemp seeds induced the production of cannflavins A and B, using a cannabinoid-free variety of *Cannabis sativa* L. In this study, the researchers demonstrated the ability of cannflavins A and B to inhibit, in vivo, production of prostaglandin E2 and leukotrienes, two pro-inflammatory mediators (Wertz et al. 2014). A study published in 2019 by Rea et al., found that luteolin is converted to chrysoeriol by O-methyltransferase and then is catalyzed from prenyltransferase so that chrysoeriol produces cannflavins A and B. This study presents a sequence for the biosynthesis of cannflavins A and B and suggests a pathway to develop strategic metabolic engineering of these relevant cannabis compounds. This study's interest focused on the approach to gene discovery through individualizing enzymatic steps that were identified throughout the search of sequences and analysis of prenyltransferases in cannabis. Rea et al. (2019) are pursuing their interest in phylogenomics-driven gene discovery in cannabis research and have already produced patent applications.

4.13.1 Cannflavins A, B, and C

Cannflavin A has been described as the most potent of the three discovered cannflavins. Cannflavin A has moderate antioxidant action and has shown good antiprotozoal activity. Antimicrobial and antiprotozoal activity have also been

demonstrated by cannflavin B and both cannflavins A and B are known to exert anti-inflammatory action targeting microsomal prostaglandin E_2 synthase (mPGES-1) and the 5-lipoxygenase (5-LOX) pathway (Pellati et al. 2018a, b). In a recent study (Moreau et al. 2019), an unnatural isomer of cannflavin B was found to increase apoptosis in vitro of two pancreatic cancer cell models, particularly when combined with radiotherapy. Additionally, this cannflavin B isomer delayed progression of both primary tumors and metastases of pancreatic cancer in a mouse model, while also increasing survival times in the treatment group. The mechanism of action is not yet clear, but a patent for this treatment and an orphan drug designation has been granted (https://patents.google.com/patent/US10398674B2/en?q=FBL-03G&oq=FBL-03G). As the newest discovered cannflavin, cannflavin C is noted to have strong antioxidant activities, however it did not demonstrate activity as an analgesic (Radwan et al. 2008).

4.14 Chapter Conclusion

To date, more than 20,000 terpenes and 6,000 flavonoids have been identified in many types of plants, with a number of these present in cannabis. These secondary metabolites are considered vital components in numerous pharmaceutical, nutraceutical, and medicinal applications. Terpenes are the largest class of plant chemicals known, with many demonstrated therapeutic effects. Flavonoids are among the largest nutrition families and are most known for their antioxidant and anti-inflammatory effects, particularly in diseases like cancer and metabolic disorders such as diabetes mellitus. Both terpenes and flavonoids found in cannabis contain beneficial properties related to their antioxidative, anti-carcinogenic, and anti-inflammatory effects, even without the presence of tetrahydrocannabinol (THC), and may contribute to the entourage effect, working together with cannabinoids to influence the body's endocannabinoid system. There are many studies of terpenes available, including those molecules that do not appear in large amounts in cannabis, demonstrating their medicinal effects both in vitro and in vivo. Flavonoids, and particularly cannflavins, do not yet have as robust a research library to refer to; illumination of their mechanisms of action and biosynthesis will be important, not only in terms of their medicinal/therapeutic effects, but also to aid the cannabis industry in determining varieties, doses, and synergistic effects of these important, natural, active compounds in cannabis.

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Chapter 5
Cannabinoids for Pain Management

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5.1 Overview of the Pain Pathway and Endocannabinoid System

The endocannabinoid system (ECS) is involved in all aspects of the pathophysiological processes of pain. The nociceptive pathway includes transduction, transmission, modulation and perception, with the ECS playing an essential role in both afferent and efferent pain stimuli.

The ECS operates at all major signaling points on the pain pathway including the periphery, spinal cord, periaqueductal grey matter, and the ventroposterolateral nucleus of the thalamus (Russo and Hohmann 2012; Hohmann and Suplita 2006; Walker et al. 1999). Endocannabinoids, endocannabinoid enzymes, and the two classically defined cannabinoid receptors (CB1 and CB2) are the fundamental components of the ECS. Endocannabinoid ligands and ECS enzymes also help modulate classic receptors associated with pain signaling and response such as opioid receptors, *N*-methyl-D-aspartate (NMDA) and GRP55 receptors, glycine receptors, etc. Some hormones play an integral role in endocannabinoid release and affect, such as oxytocin-dependent endocannabinoid signaling with anandamide (Wei et al. 2015).

Dysregulation or dysfunction of any component of the endocannabinoid system, or the more commonly known signaling pathways or endogenous chemical or

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enzymes production (also referred to as the *endocannabinoidome*) can lead to pain that is pathological in nature (Russo and Hohmann 2012). The CB1 receptor plays an important role centrally and peripherally via direct and indirect mechanisms. CB1 receptors are dispersed presynaptically throughout the nervous system. These receptors work via synaptic transmission inhibition, modulating the release of specific neurotransmitters and neuropeptides depending on physiologic "need" and triggers (Vučković et al. 2018). The inhibition of nociceptive transmission accounts for the analgesic effects in a so-called "retrograde synaptic circuit breaker" fashion (Russo and Hohmann 2012; LaBuda et al. 2005). The CB1 receptors are located at certain synapses of pain signaling, including the peripheral and terminal units of primary afferent neurons, dorsal root ganglion (DRG), dorsal horn of the spinal cord, periaqueductal grey matter (PAG), ventral posterolateral thalamus, and cortical regions (Vučković et al. 2018; Hill et al. 2009; Sharkey and Wiley 2016).

Essentially, any neurotransmitter that is released over a synapse with the prospect of facilitating a pain signal between neurons can be blocked through the inhibitory characteristics of the CB1 receptor. A majority of studies on the ECS focus on two endocannabinoids: anandamide (AEA) and 2-Arachidonoylglycerol (2-AG), with other known endocannabinoids being described in the literature as well. More recently, other CB1 interacting peptides and a series of arachidonic acid derivatives have been found to generate endocannabinoid-like effects (Zou and Kumar 2018).

An example of a phytocannabinoid that specifically acts on central CB1 receptors with full affinity via orthosteric binding is THC, providing some analgesia via inhibition of neurotransmitter release as described above. Due to the psychoactive central side effects (intoxicating effects) of binding to the CB1 receptor, THC can create dose related limitations due to the subsequent potential negative side effects in animal patients. In people, this intoxicating effect can create euphoria with or without true anti-nociceptive effects. This change in cognitive perception of a pain stimulus is profound in people but less advantageous in unknowing animals who may become anxious because of these effects. The CB1 receptors in harmony with other receptor systems, such as the transient receptor potential types (TRPA) and (TRPV), can decrease pain and inflammation and reduce peripheral hypersensitization. The reduction in these nociceptive responses has been described in numerous animal models. Other phytocannabinoids bind to the CB1 receptor via allosteric modulation which do not create intoxicating effects (Manzanares et al. 2006; Vučković et al. 2018; Richardson 2000; Chiou et al. 2013).

CB2 receptors contribute to the expression of pain and inflammation in a slightly different manner from CB1 receptors. As described in Chap. 2, CB2 receptors are highly inducible and their expression is increased after tissue injury or inflammatory events. CB2 receptors play an important role in immune-mediated inflammatory processes. In healthy, unimpaired tissues, minimal numbers of CB2 receptors are present. However, the expression of CB2 receptors increases in these tissues when inflammation or injury occurs (Dhopeshwarker and Mackie 2014). This is similar to the prolific genesis of opioid receptors in traumatized tissue (Wang et al. 2017; Feng et al. 2012).

Rheumatoid Arthritis (RA) is a good example for explaining the function of CB2 receptors. RA is characterized as an immune-mediated inflammatory disease process, with typically an influx of immune-mediated cytokines into the synovia resulting in cartilage and subsequent bone destruction (Scott et al. 2010).

The endocannabinoids 2AG and AEA are both only present in the synovia of patients with RA and the CB2 receptor expression is upregulated due to the proinflammatory mediators. Studies looking at cannabinoids as a treatment of RA show that the activation of CB2 receptors inhibits the production of cytokines, in particular TNF-Alpha and IL6, and suppresses inflammatory antibody B-cell production. CB2 receptor activation further promotes osteoblast differentiation over osteoclast activity, therefore inhibiting some of the bone destruction which occurs in RA. Animal studies for RA show promising results in reducing disease severity, decreasing swelling, reducing bone destruction, lowering leukocyte infiltration, and reducing the numbers of circulating antibodies against collagen, all processes mediated by the CB2 receptor (Turcotte et al. 2016; Fukuda et al. 2014; Starowicz and Finn 2017; Richardson et al. 2008; Gui et al. 2015).

CB2 receptors are predominantly present postsynaptically in the periphery but are also found centrally. Centrally, CB2 receptor expression has been demonstrated in the typical regions associated with pain, including the cerebral cortex, PAG, cerebellum, and hippocampus, as well as on central glial and endothelial cells. The activation of CB1 receptors decreases presynaptic GABA release and eliminates GABAergic inhibitory control of postsynaptic neurons. Conversely, excitement of the postsynaptic neuron is seen through this disinhibition role. The activation of CB2 receptors typically hyperpolarizes the membrane potential and inhibits postsynaptic neuronal function (Chen et al. 2017).

An upregulation of CB2 receptor expression in the dorsal horn and DRG is seen in both neuropathic and inflammatory pain. It is well documented that the peripheral expression of CB2 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 neuroinflammatory based neuropathic pain. Antinociception to thermal stimuli is also seen via CB2 receptor activation with synthetic cannabinoids (Malan et al. 2001; Chiocchetti et al. 2019; Starowicz and Finn 2017).

CB2 receptor activation inhibits cytokine and chemokine release peripherally. Both CB1 and CB2 receptors are located on mast cells and their activation decreases the release of inflammatory agents (Vučković et al. 2018; Small-Howard et al. 2005).

CB2 receptor activation in the periphery inhibits substance P induced mast cell degranulation and plasma extravasation. The activation of CB2 receptors releases endorphins from keratinocytes into the periphery (Mazzari et al. 1996; Petrosino et al. 2019; Ibrahim et al. 2005; Gao et al. 2015).

While not described in human or animal models to date, in vitro data suggests cannabidiol's (CBD) ability to behave as a CB2 receptor inverse agonist may contribute to its documented anti-inflammatory properties (Kano 2014; Thomas et al. 2007).

Pain is modulated by the endocannabinoid system more specifically on a peripheral, spinal and supraspinal level reaffirming the significant role the ECS plays in the ascending and descending nociceptive pathways (Vučković et al. 2018; Starowicz and Finn 2017).

The roles of the endocannabinoids on the mechanisms of pain are multifold. Endocannabinoids are released when an event like tissue injury, inflammation, and/or disproportionate pain signaling occurs. The objective of the endocannabinoids is to decrease sensitization and pain as well as to decrease the inflammatory cascade process (Vučković et al. 2018; Piomelli 2014).

The endocannabinoid anandamide is a partial agonist with a high affinity for CB1 receptors, achieving inhibition of neurotransmitter release as described above. Anandamide was thought to be inactive on the CB2 receptor but has recently been shown to alleviate certain forms of neuroinflammation in vitro in rat microglial cell cultures (Marzo and Petrocellis 2010; Malek et al. 2015). Anandamide is a full transient receptor potential vanilloid 1 (TRPV1) channel agonist, while also active at other G-protein coupled receptors like GPR18 and GPR55 (Guerrero-Alba et al. 2019). When the TRPV1 receptor is activated, desensitization and depolarization occur leading to the inactivation of voltage-dependent sodium and calcium channels. The resulting decrease of this neuron firing or action potential (Liu et al. 1997) adds to its ability to reduce pain and hyper-sensitization. Anandamide is therefore considered to operate as an "endovanilliod", due to its activation of the TRPV1 receptor (Rosenbaum and Simon 2006).

Similar to the mechanism of action of gabapentin, anandamide inhibits the T-voltage gated calcium channels, which are commonly upregulated in chronic pain states. Anandamide's other mechanisms of analgesia are inhibition of 5-HT3A serotonin receptors, activation of peroxisome proliferator-activated receptor (PPARs), and potentiation of the function of glycine receptors.

The analgesic mechanisms of 2-Arachidonoylglycerol (2-AG) include its capacity to act as a full agonist on both cannabinoid receptors, having a low-moderate affinity for CB1 and high affinity for CB2 receptors, as well as its ability to potentiate the $GABA_A$ receptor and operate on the PPAR-γ receptors contributes to decreasing neuronal excitability and the inflammatory response. Moreover, stimulation of endogenous norepinephrine can activate peripheral adrenoceptors (Vučković et al. 2018; Romero et al. 2013).

As explained in other chapters, endocannabinoids are hydrolized by the enzymes FAAH and MAGL. Blocking these enzymes therapeutically would increase the presence of endocannabinoids and decrease endocannabinoid reuptake. Keeping an active endocannabinoid around for possible activation on the different receptor systems may increase its ability to provide analgesia. Unfortunately, trials using synthetic FAAH inhibitors created serious negative side effects not seen with the simple addition of phytocannabinoids (Mallet et al. 2016).

One of the metabolites of the enzymatic breakdown of both primary endocannabinoids is arachidonic acid (AA). Interestingly, some research shows the anti-inflammatory effect of AEA itself in periodontitis models in the rat. The anti-inflammatory effects of terpenes should also not be dismissed and are further

described briefly later in this chapter and more in depth in Chap. 4 (Rettori et al. 2012; Reynoso-Moreno et al. 2017).

A strong and complex interaction between the ECS and the eicosanoid pathway has been identified (Alhouayek et al. 2018; McPartland et al. 2014). The metabolites of endocannabinoids feed directly into the eicosanoid system affecting the AA levels and prostanoid synthesis (Alhouayek et al. 2018). With the help of cyclooxygenase (COX), anandamide produces Prostaglandin (PG)-EA; 2AG will produce PG-G. Different derivatives of various prostaglandins (PGs) are produced depending on the tissue. Both PG-EA and PG-G derivatives play a role in peripheral and central hypersensitization and can have pro-inflammatory properties. Mixed reviews regarding pro-inflammatory, anti-inflammatory, and other beneficial effects have been presented, however the involvement of their roles is not fully understood. For example, PG-EA decreases IL12 and IL23 expression in microglial cells, reduces tissue necrosis factor (TNF)alpha and binds to PGE2 receptors.

The endocannabinoids can also be metabolized by the lipoxygenase (LOX) and cytochrome P450 systems (Alhouayek et al. 2018). The cytochrome P450 metabolites of anandamide (EET-EA) and 2AG (EET-G) have a high affinity for CB1 and CB2 receptors, playing a strong anti-inflammatory role with a high presence in microglia cells (Chen et al. 2017). Furthermore, the LOX (12-LOX and 15-LOX) derived metabolites of anandamide have binding affinity for both CB receptors as well as the TRPV1 receptor. 2-AG is metabolized by 15-LOX to HETE-G, which is an agonist at PPAR-alpha (Kozak et al. 2002; Kanju et al. 2016), again adding to the anti-inflammatory properties of the combined eicosanoid and endocannabinoid pathway (Fig. 5.1).

In general, the response to pain and even the typical analgesics used to alleviate discomfort is patient variable. This is also true with the response to cannabinoids. This response variability can be attributed to the individual's tonic activity of the ECS, also known as *"Endocannabinoid System Tone"*. The base tone of the ECS in an individual would determine how the patient responds to a painful stimulus. For example, tonic activity can influence the level of endocannabinoids produced, affecting the efficiency of hydrolyzing enzymes and the number and distribution of receptors. For a more in-depth review of this concept please see Chaps. 1 and 2.

Another related component is 'genetic profiling', which has been an interesting development in chronic pain management. It is becoming more and more evident that some patients respond favorably to certain analgesics in comparison to other patient populations. These so-called responders and non-responders are recognized in regard to opioid therapy but are also noted in cannabinoid therapy. In part, this is related to not only the metabolic ability of the patient, but also the genetic variants of the ECS receptors, the endocannabinoid degrading enzymes and the various cytochrome producing enzymes. All of those can be variable in different individuals (Smith et al. 2017; Lester et al. 2017; Ishiguro et al. 2013).

The chronic use of opioids and the subsequent effects on pain and analgesia through desensitization of opioid receptors are well documented. This desensitization is also noted with certain cannabinoids. Most notably, the chronic use of THC at higher doses is reported to desensitize and down regulate both CB1 and CB2

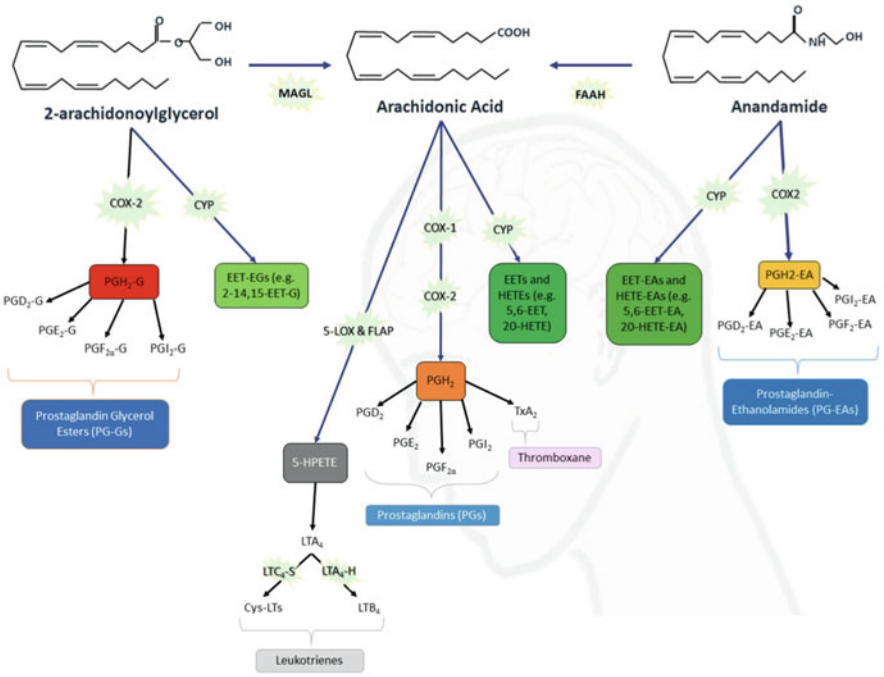

Fig. 5.1 Eicosanoid and endocannabinoid pathways. Arachidonic acid (AA) can be synthesized from 2-arachidonoylglycerol (2-AG) and N-arachidonoyl-ethanolamine (anandamide or AEA) by monoacylglycerol lipase (MAGL) and fatty acid amide hydrolase (FAAH) respectively. Cyclooxygenases (COX-1 and COX-2) enable the conversion of 2-AG, AA and AEA into PGH_2-Gs (prostaglandin H_2-glycerol ester), PGH_2 (prostaglandin H_2), and PGH_2-EA (prostaglandin H_2-ethanolamide) respectively; the various enzyme systems that then convert these precursor molecules into prostanoids (e.g., PGE_2-EA) are not shown in this diagram. (Permission to use with full citation, Cheung, K. A., Peiris, H., Wallace, G., Holland, O. J., & Mitchell, M. D. (2019). The Interplay between the Endocannabinoid System, Epilepsy and Cannabinoids. *International Journal of Molecular Sciences, 20*(23), 6079. doi:10.3390/ijms20236079)

receptors as well as endocannabinoid production; this presents as tolerance to the potential anti-nociceptive effects of phytocannabinoids. A break in treatment will quickly return CB1 density and endocannabinoid production back to normal (Hirvonen et al. 2011; McPartland et al. 2014). Clinical studies on desensitization have focused on humans with heavy, long-term THC usage and it is not clear if this information is clinically significant in the antinociceptive use of cannabinoids in our pet population (Nguyen et al. 2018). Typically, the content of THC in most pet products is low, particularly if those products are derived from hemp. Studies on other phytocannabinoids in companion animals and lab animals on the potential for tolerance are scant. Tolerance to CBD described in the literature so far is lacking. However, CBD can provide antagonistic effects on THC affinity. A phenomenon known as “reverse tolerance” has been postulated, where users require less CBD to achieve the same effects as initial dosing. This has not been described in the

scientific literature but may in fact speak to the better regulation of inflammatory mediators or the homeostatic properties of utilizing phytocannabinoids to support the ECS (Bergamaschi et al. 2011; Hudson et al. 2019).

5.2 Role of Various Phytocannabinoids on Pain Pathways and the ECS

Various phytocannabinoids play a major role in pain and inflammation. Most phytocannabinoids act via the CB receptors, which influence prostaglandin synthesis, the COX and LOX systems, and are agonists or antagonists on the TRPA and TRPV receptor system. An overview of the mechanism of action of phytocannabinoids has been thoroughly explained in Chap. 2, with only pain-specific mechanisms of action of the most common phytocannabinoids being summarized here (Tables 5.1 and 5.2).

5.3 Evidence for Cannabinoids in Chronic Pain

Neuropathic and chronic pain continue to be a source of great discussion amongst the veterinary community but the understanding of each is often elusive. Neuropathic pain is pain related to damage or disease of the somatosensory system. This is both vague and quite complicated resulting in under- recognition. Conditions likely resulting in neuropathic pain in veterinary patients include intervertebral disc disease, radiculopathy, diabetic and other neuropathies, chronic osteoarthritis, and stroke. The concept of chronic pain is simpler but physiologically equally complex. Chronic pain is simply pain of long duration generally greater than 1–3 months. This term, however, does not reflect pathophysiology. Maladaptive pain describes more clearly the effect of longer-term pain. Maladaptive pain relates to activation of NMDA and AMPA receptors (Ferrari et al. 2014; Ultenius et al. 2006; Meymandi et al. 2017).

Conventional therapy of neuropathic pain in both humans and animals typically involves gabapentin, pregabalin, amantadine, and amitriptyline. Because of the difficulty in identifying neuropathic pain, especially in animals, non-steroidal anti-inflammatory drugs are often administered as part of a chronic/maladaptive pain protocol.

An extensive Canadian systematic review of effectiveness of chronic pain treatment in humans showed that medical cannabis may be partially beneficial for the treatment for neuropathic pain, chronic non-cancer pain, and chronic non-cancer, non-neuropathic pain. This overview did not show evidence for efficacy of medical cannabis in the treatment of fibromyalgia, back pain, headache, rheumatoid arthritis, and osteoarthritis in one guideline (Banerjee and McCormack 2019). Another

Table 5.1 Chart of common phytocannabinoids and their receptor activity as it relates to pain signaling

THC (intoxicating)	• Full CB1 agonist • Inhibition of prostaglandin E2 synthesis • Stimulation of the lipoxygenase pathway • Blockage of NMDA receptor, therefore decreasing central sensitization • Serotonin reuptake inhibition and increased serotonin production (Russo and Hohmann 2012) • Stimulation of beta-endorphin production (Manzanares 1998)
THCA (non-intoxicating)	• Direct inhibition of COX1 and COX2
THCV (non-intoxicating)	• CB1 and CB2 antagonist • CB2 agonist at higher doses and therefore may attenuate inflammation and hyperalgesia
CBD	• Perseveration of endocannabinoids • Inhibition of tumor necrosis factor- alpha (TNF-α) • TRPV-1 activation • Inhibition of adenosine transporters • Activation of 5-HT1A serotonin receptors
CBDA	• Direct COX1/COX2 inhibition • TRPV and TRPA agonist • Anandamide reuptake inhibition • HT5 receptor antagonist
CBG	• Activation of CB1 and CB2 receptor (Russo) • Potent alpha2-adrenoceptor agonist • Serotonin receptor antagonist • GABA reuptake inhibitor (Cascio 2010) • TRPV1 and TRPA1 agonist • TRPM8 antagonist • Lipoxygenase inhibitor • Anandamide reuptake inhibitor • Direct COX1/COX2 inhibition
CBGA	• Direct COX1/COX2 inhibition
CBC	• Anandamide reuptake inhibition • Activation of the TRPA1 receptor
CBN[a]	• Weak CB2 and CB1 partial agonist • TRPV2 agonist (high threshold thermosensor receptor), plays a role in burn patients.

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https://www.researchgate.net/publication/51088411_Evaluation_of_the_Cyclooxygenase_Inhibiting_Effects_of_Six_Major_Cannabinoids_Isolated_from_Cannabis_sativa
https://www.researchgate.net/publication/5299182_Cannabidiolic_Acid_as_a_Selective_Cyclooxygenase-2_Inhibitory_Component_in_Cannabis
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3165957/
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6021502/
https://www.jstage.jst.go.jp/article/bpb/34/5/34_5_774/_pdf

(continued)

Table 5.1 (continued)

https://www.frontiersin.org/articles/10.3389/fphar.2018.00482/full, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2828614/
https://www.ncbi.nlm.nih.gov/pubmed/21532172, https://www.nature.com/articles/tp201215
https://www.ncbi.nlm.nih.gov/pubmed/18556441
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1751228/
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2931567/
[a]Formed after degradation of THC from prolonged storage, works best in conjunction with THC

extensive systematic review concluded that there is no high-quality evidence for the efficacy of cannabis-based medicine in any condition characterized by neuropathic pain in humans. The Canadian Pain Society recommends cannabis-based medicine as a third-line therapy for chronic neuropathic pain syndromes when established therapies (anti-convulsant, antidepressants) have failed (Mücke et al. 2018). Despite these reviews, there are emerging publications showing efficacy, or at least the possibility of efficacy, of medical cannabis for back pain (Kim et al. 2020), headache and migraine pain (Baron 2018), signs associated with multiple sclerosis (Rice and Cameron 2018), and rheumatoid arthritis (Lowin et al. 2019).

In regard to neuropathic pain, cannabinoids have been studied in neuropathic pain models in various animal species. In instances of peripheral nerve damage, both ECS receptors and TRPV1 channel receptors are upregulated. It has been recognized in multiple animal models that, when these receptors are blocked, there is improvement of the hyperalgesic state of neuropathic pain. Research is focusing on FAAH and MAGL blocking agents to increase the activity of endocannabinoids. However, prior studies utilizing synthetic FAAH inhibiting drugs proved dangerous in clinical trials (Hossain et al. 2020; Starowicz and Finn 2017).

NMDA and AMPA receptors are also implicated in neuropathic pain states. Functions such as memory formation and processing and behavioral responses to environmental stimuli depend on the activity of NMDA receptors. Endocannabinoids are released to maintain NMDA activity within typical physiological limits. The control of NMDA activity depends on histidine triad nucleotide-binding protein 1 (HINT1) and sigma receptor type 1 (σ1R) assisting in the physical coupling between CB1 and NMDA receptors to dampen their activity. The calcium regulated HINT1/σ1R protein in tandem uncouples CB1 receptors to prevent NMDA receptor hypofunction (Rodríguez-Muñoz et al. 2016).

Cancer pain is a form of chronic pain that has increased complexity particularly due to the presence of peripheral and central hypersensitization including allodynia. Various animal models show that allodynia is attenuated by non-selective agonists, CB1 and CB2 selective agonists, as well as FAAH and MAGL inhibitors (Guerrero-Alba et al. 2019; Khasabova et al. 2012; Johnson et al. 2010).

There is emerging data for the use of medical cannabis in veterinary patients. In a recent survey, Canadian dog owners perceived cannabis products to be equal or more effective than conventional medications for pain and inflammation (Kogan et al. 2020). In another survey, dog owners reported 95% efficacy and cat owners 100% efficacy for pain (Kogan et al. 2016). While survey data is not as strong as

Table 5.2 Chart of common terpenes and their physiological activity as it relates to pain signaling

Analgesic terpenoids	Effects	References
Limonene	• Inhibition of nitric oxide production • Inhibition of gamma-interferon production • Inhibition of IL4 production and other pro-inflammatory cytokines • Inhibition of lipopolysaccharide (LPS)-induced production of nitric oxide • Inhibition of PGE2 • Activation of TRPA1 (when applied topically)	https://www.jstage.jst.go.jp/article/bpb/30/7/30_7_1217/_pdf https://www.ncbi.nlm.nih.gov/pubmed/20625233/ https://onlinelibrary.wiley.com/doi/full/10.1002/ejp.840
Myrcene	• Alpha 2 adrenoreceptor agonist, reversible with yohimbine (Russo) • Modulation of PGE2 to reduce inflammation • Decrease IL-1b-induced nitric oxide (NO) production (Nuutinen, Russo) • Anti-inflammatory and anticatabolic effects to assess its potential to slow down the progression of OA (Rufino, Nuutinen) • Opioid receptor antagonist with opioid-like analgesic effect, reversible with naloxone antagonist (Rao 1990)	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5751100/
α-Pinene	• Inhibition of PGE1 • Inhibition of IL1β and IL6 production • Reduction in TNF-α formation in macrophages	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6920849/ https://reader.elsevier.com/reader/sd/pii/S0024320512004870?token=E1614692B24096464A8E0709F4144D3D86A1B8EC548978FCCBB94869485C28432EDD8040E50FC3EFB70C7F3FCF75FBBE
Linalool	• Agonist on the adenosine A2A receptor • Inhibition of glutamate release at higher doses • Possible inhibition of substance P release • Possible antagonist on neurokinin-1 (NK-1) receptor • Local anesthetic • Anti-inflammatory	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5751100/

double-blinded placebo-controlled data, it can provide insight into efficacy for certain conditions. Part of that insight has been confirmed in one randomized, placebo-controlled, double-blinded, cross-over study of CBD for osteoarthritis (OA) in 16 dogs. This study demonstrated that CBD rich oil at 2 mg/kg based on CBD concentrations, administered orally twice daily, induced a significant decrease in pain and increase in activity as reported by dog owners, coupled with a decrease in the veterinary assessment of pain. It is important to note the formula used is nearly 1:1 CBD to CBDA with a rich terpene profile. Mild elevations in the liver enzyme ALP were noted in nine of the dogs. Some subjects in the study were on concurrent NSAID therapy with no adverse effects. The researchers also noted a noticeable decrease in Hudson scores and rise in CBPI scores during the initiation cross-over placebo treatment suggesting a potential carry over effect from CBD treatment.

No other adverse events were described (Gamble et al. 2018).

In 2020, Kogen et al. found that the addition of a full spectrum CBD rich oil in a dose escalating manner for dogs that suffered from chronic maladaptive pain, primarily associated to OA, was successful in 30/32 dogs, as assessed by one primary observer during the 90 day trial. Dosing started at 0.25 mg/kg, twice a day, and was increased at 0.5–0.75 mg/kg increments until reaching a final 2 mg/kg dose twice a day. Concurrent treatment with gabapentin occurred in 23/32 dogs in the study. Of the 23 dogs on gabapentin, 10 dogs (43.5%) were able to discontinue gabapentin. The 11/13 remaining dogs on gabapentin were able to reduce the gabapentin dose necessary to retain comfort to 20–60% of the original dose. This strongly suggests not only the therapeutic value of CBD for management of maladaptive pain, but also the ability to decrease pharmaceutical burden. Mild elevations in ALP were noted in this study but did not require clinical intervention. This was not a placebo-controlled or blinded study (Kogan et al. 2020).

In March 2020 at the Association of Veterinary Anaesthetists Congress in Dublin, researchers Brioschi et al. described in their poster presentation the use of an oral-transmucosal CBD for dogs with chronic OA. Dogs received oral firocoxib (dose not mentioned) and gabapentin at 10 mg/kg every 12 h. The dogs were then randomly divided into two groups. Dogs in group CBD ($n = 6$) received OTM cannabidiol oil at 2 mg/kg every 12 h. In Group C ($n = 6$), cannabidiol was not administered. Treatments lasted for 3 months. Pain Severity Score (PSS), Pain Interference Score (PIS) and Quality of Life (QoL) were evaluated by owners with the Canine Brief Pain Inventory tool before treatment initiation followed by 1, 2, 4, and 12 weeks thereafter. PSS and PIS were significantly lower in CBD than in C group and QoL was significantly higher in the CBD group. This study suggests significant therapeutic advantage with using CBD as an adjunctive therapy with conventional chronic pain management protocols.

Mejia et al. (2019) presented an abstract at the Veterinary Orthopedic Society meeting that discussed their pilot study for evaluating success using a CBD oil for pain in dogs with radiographically confirmed OA. The cannabinoid and other compound profiles were not clarified for this study. Client-owned dogs were enrolled in this prospective, double-blinded, crossover, placebo-controlled study. Baseline

data were acquired for 4 weeks prior to initiation of the first treatment and patients were randomly assigned to either placebo or oral CBD oil treatment for the first 6 weeks. The subjects were then treated for the subsequent 6 weeks with the opposite treatment. Twenty-three, medium-large breed dogs were enrolled. The researchers found that 14 of the dogs displayed elevation in liver enzymes associated with CBD treatment. Significant differences between treatment groups were identified for several clinical metrology instruments (CMI) and objective gait analysis (OGA) time point comparisons. However, consistency was lacking amongst the different outcome measures. Despite this inconsistency, outcome measures suggest that CBD may benefit dogs with OA associated pain. More adequately powered studies with a larger sample size are needed to confirm this suggestion (Mejia et al. 2019).

Verrico et al. (2020) in a randomized, placebo controlled and blinded study with 20 dogs that had age related OA were divided into four groups. Exclusion criteria included uncontrolled renal, endocrine, neurologic, or neoplastic disease, or were undergoing physical therapy. Medications were discontinued at least 2 weeks before enrollment and subjects were not allowed to receive medications during the 4-week study. The dogs were then assigned 1:1:1:1 to one of four groups: placebo, 20 mg/day (0.5 mg/kg) naked CBD, 50 mg/day (1.2 mg/kg) naked CBD, or 20 mg/day liposomal CBD. The CBD product used in this study was an isolate form. Before study initiation and at day 30 of the study, each dog was evaluated by the study veterinarian. Owners were asked to also evaluate their dogs before treatment and at weeks 4 and 6 using the Helsinki Chronic Pain Index. The results found that neither animals given placebo or animals given the lower doses of naked CBD responded to therapy with any statistical significance. Dogs given a higher dose of naked CBD or a low dose of liposomally encapsulated CBD experienced significant improvements, assessed by the owner and veterinarian. No adverse events were noted, and chemistry values remained within normal limits. Unfortunately, the study did not include pharmacokinetics of liposomally encapsulated CBD, compared to naked CBD.

This study also looked at the inflammatory response to CBD on local and systemic inflammation in two different mouse models. For the local inflammation model topical administration of croton oil, which induces edema, erythema, neutrophil influx, and the production of proinflammatory TNF-α, was applied to the ear of a mouse. After 2 h a topical application of 1 mg CBD was applied to the same area. Local myeloperoxidase (MPO) activity (a proxy for neutrophil influx) was measured, showing activity in the treated ear was reduced over 80%. Four hours after the initial croton oil application, TNF-α levels were assessed. Circulating TNF-α was decreased by 50% among mice in the treatment group. The CBD treatment also reduced the development of edema.

Intraperitoneal administration of lipopolysaccharide (LPS) induces an inflammatory response with expression of pro-inflammatory TNF-a and IL-6. These are two cytokines relevant to the pathogenesis of OA. After 2 h the mice were then treated intraperitoneally with increasing doses of CBD (1, 10, or 100 mg) or topically with a single CBD dose of 100 mg. After yet an additional 2 h the physiological impacts were assessed. CBD treatment on circulating cytokine levels indicated intraperitoneal and topically administered CBD reduced circulating TNF-a and IL-6 levels in a

dose-responsive fashion. Systemic administration of CBD alone increased levels of anti-inflammatory IL-10 in the absence of inflammatory stimulus (Verrico et al. 2020).

In an abstract presented May 6th, 2019 at the 2019 Australian Veterinary Association Innovation, Research and Development Symposium, Dr. Margaret Curtis shared research focused on absorption and changes in inflammatory response when 11 purpose bred dogs were given various doses and concentrations of CBD and THC. Single oral doses were administered to each dog 2.5 h after feeding. The dogs were organized into groups receiving the doses as follows; Group 1 (n = 4) received THC (0.24 mg/kg) and CBD (0.12 mg/kg), Group 2 (n = 4) received THC (0.12 mg/kg) and CBD (0.24 mg/kg), Group 3 (n = 3) received a placebo containing no phytocannabinoids. Plasma and whole blood were drawn at 15 different timepoints within 72 h after dosing. Effects of treatment for pain and inflammatory pathways were assessed by associated gene expression using qPCR on whole blood. No adverse events were noted for any participants. Gene expression was compared at 1.5 h and 72 h time points for each group. A significant change in fold regulation was seen in expression of chemokine ligand 5 gene (CCL5), cerebellar degeneration-related protein 2 gene (CDR2), CB2, and Interleukin 8 gene (CXCL8) in Group 1 compared with placebo and chemokine ligand 5, CB2, and Interleukin 8 in Group 2 compared with placebo. THC and CBD effects on inflammatory cytokines and neurotransmitters were also tested at 7 time points over 24 h for all groups. Notable differences were observed in biomarkers known to be associated with modulation of anti-inflammatory processes in treatment groups compared to placebo. Specific biomarkers that decreased variably depending on the ratio of THC:CBD were Interleukin 15 (IL-15) and granulocyte-macrophage colony-stimulating factor (GM-CSF) (Curtis et al. 2020).

The evidence in chronic animal pain models in pets (dogs and cats) can be problematic to interpret due to commonly inadequate or confusing methods of interpretations of assessment. The other concern with evaluating data and extrapolating it to clinical use is the significant differences in test articles used in these studies. The inconsistency between various products that make up their cannabinoid, terpene and even flavonoid profiles can bring frustration to clients and practitioners in finding products that are similar to what was used in a study with the exception of isolate products. We also lack a valid assessment tool in our companion animals that is without a subjective or flawed component. In particular, force plate gait analysis and questionnaires have interpreter discrepancies that are not always accounted for in study data collection or discussion.

5.4 Evidence and Role for Cannabinoids in Acute Pain

The scientific evidence for the efficacy of cannabinoids in acute pain is less clear. In the published human studies of acute pain, it seems evident that cannabinoids are not as effective as other more commonly used analgesics (Holdcroft et al. 1997; Russo

2008). The effects that are notable seem to be dependent on the product and dosing protocols, and products with higher THC do not seem to dampen the pain itself but rather change the patient's perception of the pain and overall experience, which is less favorable in our animal patients. The methodology of the clinical studies varies significantly and does not help clinicians or researchers in making a comprehensive statement at this point (Beaulieu 2007; Beaulieu and Ware 2007).

On the contrary, acute pain animal models, particularly the tail flick, heat models, or carrageenan injections show significant antinociceptive efficacy of cannabinoids (Starowicz et al. 2013; Tham et al. 2005). The studies do allude to the fact that both CB1 and CB2 receptors are operating in the periphery to decrease and inhibit the sensitizing effects of pain through both local and systemic administration. The beta endorphin release from peripheral keratinocytes is considered one CB2 receptor-mediated mechanism of action in acute inflammatory pain. Other mechanisms of inhibition sensitizing peripheral neurotransmitters have also been suggested. Reduction of edema and swelling is considered to be a CB2 receptor-mediated effect, however there is also CB1 receptor involvement, particularly through down-modulation of mastcell activation and degranulation. The involvement of the eicosanoid pathway further plays a crucial role in the anti-inflammatory mechanism of action (Starowicz and Finn 2017; Hossain et al. 2020; Ibrahim et al. 2006; Lehto et al. 2008).

Nociception at the level of the spinal cord is suppressed by both CB1 and CB2 receptors. These receptors have a role in cannabinoid-mediated analgesia as evidenced in acute pain models showing an increase in CB1 activity after tissue injury (Hama and Sagen 2011; Arevalo-Martin et al. 2012; Monory et al. 2015). CB receptor independent activity is also a crucial component of acute anti-nociception. In general, the involvement of multiple pain targets being initiated by the cannabinoids, instead of one specific receptor, makes it more complex to understand exact mechanisms (Guindon and Hohmann 2009). A synergistic interaction between the mu-opioid receptor and CB1 receptor, as well as a supplementary interaction between cannabinoid receptor activity and the alpha 2-adrenoceptor, has been presented (Cascio et al. 2009; Cathel et al. 2014; Katia 2015).

All three types of receptors are similar in location and structure and share the same signaling pathways, but the exact interaction has yet to be fully explained. The endocannabinoid/endovanilloid-mediated analgesia has been researched for both acute and chronic pain. TRPV1 receptor activation plays an important role in supraspinal modulation of acute pain models in rats (Maione et al. 2007).

Various acute-pain rodent studies validate the relationship between the endocannabinoid system and analgesia. In these studies, analgesia was accomplished through the administration of exogenous endocannabinoids or by increasing the level of endocannabinoids by blocking their degradation (Guindon and Hohmann 2009). Most of these studies not only show a CB1 receptor-mediated antinociception but also non-cannabinoid receptor involvement (TRPV1, PPARα). Despite the lack of more conclusive evidence for using phytocannabinoids for acute pain, they still may be worthwhile in multi-modal protocols to decrease the pharmaceutical burden, given the synergistic and potentiating effects they have with common acute pain medications described in this chapter. Studies using phytocannabinoids for acute

pain in our animal patient population are currently underway and will bring more light into their efficacy in the future.

5.5 Conventional and Traditional Analgesic Agents in Combination with Cannabinoids

Phytocannabinoids, in particular CBD, in a dose dependent manner will inhibit and temporarily deactivate the cytochrome P450 system. The exact dose for this effect is still unknown but is likely patient specific. The addition of CBD to a multimodal analgesia protocol may contribute to delayed metabolism and prolonged activity of many analgesic agents due to the inhibition and temporary deactivation of this system. This may or may not be clinically relevant. However, side effects can be seen due to potentially higher plasma levels of certain analgesic agents. Considering the potential for higher plasma concentrations, in conjunction with the synergy of phytocannabinoids with most analgesic agents, dose reduction of analgesic agents when using multi-modal protocols that include phytocannabinoids might be warranted. It is important to evaluate each case individually and make adjustments based on clinical evaluation. Studies in mice show that the inactivation of the cytochrome P450 system is short lived with brief use but might be clinically relevant in the long term use of phytocannabinoids (Zendulka et al. 2016; Alsherbiny and Li 2018).

Opioids There are areas of overlap and interaction between the endogenous opioid receptor system and the ECS. Cannabinoid and opioid receptors are close in location and have similar mechanisms of action. They interact at the supraspinal and spinal level and are codistributed in areas of the dorsal horn and areas of the brain controlling nociceptive responses. The spinal blockade of pain transmission becomes greater-than-additive as both opioid and cannabinoid receptor types are activated in the dorsal horn (Maguire and France 2016; Reisine and Brownstein 1994; Al-Hasani and Bruchas 2011).

The synergy in analgesic effect observed in clinical trials is more likely related to pharmacodynamic drug interactions than) pharmacokinetic increases in plasma levels (Navarro et al. 2001). Studies in mice show potent synergistic analgesia, even at doses that are considered below efficacy for each class (Manzanares et al. 2006). This has been confirmed in a clinical trial in humans. Other studies show that CBD and THC are allosteric modulators at mu and delta opioid receptors (Kathmann et al. 2006; Bushlin et al. 2012).

Due to the worldwide opioid crisis, research has been focused on prevention of opioid tolerance and reduction of withdrawal symptoms with conflicting results. Release of endogenous opioids stimulates both delta & kappa opioid receptors. Research (clinical and preclinical studies) shows a difference between short- and long-term opioid and cannabis coadministration. Acute opiate administration in conjunction with cannabinoid administration enhances effects of each medication,

but chronic opioid administration alone appears to have) a negative effect on the endocannabinoid system (McPartland et al. 2014, 2015).

Gabapentin The pharmacological mechanism of action of gabapentin has been linked primarily to voltage dependent calcium channels (VDCCs). One of the mechanisms of action of CB receptor-mediated G-protein activation is also the inhibition of VDCCs, which is likely the reason for the synergistic effects observed when using these analgesics in combination. Both agents also have effects on the serotonergic system. Sedation is associated with the administration of high doses of gabapentin in combination with cannabinoids. This adverse side effect could be related to increased gabapentin plasma levels due to the CYP450 system inhibition or due to the agents having similar mechanisms of action. Careful consideration of drug dosage and patient monitoring is essential when both drugs are used simultaneously, particularly in older dogs where the "drunken sailor" static ataxia can be seen with higher gabapentin doses. Pregabalin shows fewer side effects and, better bioavailability in comparison to gabapentin in dogs and has been more popular as an analgesic in chronic pain patients, particularly senior pets. Clinical experience has shown less sedation when combining pregabalin with cannabinoids (Luszczki 2007; Quintero 2017; Bakas et al. 2017).

Non-Steroidal Anti-inflammatory Drugs (NSAIDs) Combining NSAIDs with cannabinoids produces additive or synergistic analgesic effects. This synergy is due to the additional blockage of prostaglandins that are produced via delayed enzymatic endocannabinoid metabolism and the stimulation of COX due to inflammation. COX2 selective inhibition increases endocannabinoid levels, explaining one of the mechanisms of synergistic analgesia (Telleria-Diaz et al. 2010; Takeda et al. 2008). The direct inhibition of COX2 has been shown for THCA, CBDA, CBG, and CBGA. NSAIDs can inhibit the metabolism of endocannabinoids, leading to an increase in anandamide and 2-AG (Ruhaak et al. 2011; Maroon and Bost 2018; Manzanares et al. 2006). This is also the mechanism of action of acetaminophen (paracetamol). Acetaminophen produces a metabolite (N-arachidonoylphenolamine), which inhibits the breakdown of AEA by FAAH, inhibits COX1 and COX2, and is a TRPV1 agonist (McPartland et al. 2014). This acetaminophen-cannabinoid interaction may be species-specific due to different metabolism pharmacology, as well as being dose dependent, but is a promising therapeutic avenue. Acetaminophen is not considered an NSAID and can be given with them, in addition to cannabinoid products (Botting 2000; Klinger-Gratz et al. 2018).

The side effects related to using cannabinoids and NSAIDs together are less clear in patients with renal or hepatic disease although human studies show little cause for concern with appropriate dosing. Some phytocannabinoids are gastroprotective and may counteract some of the NSAID-related GI concerns (Gyires and Zádori 2016; Abdel-Salam 2016). Further, the possibility of reducing NSAID toxicity through the addition of cannabinoid inhibition of the COX pathway may lead to beneficial advantages. It is not uncommon that NSAID doses and intervals can be reduced when phytocannabinoid products are added to pain management protocols (Vanegas et al. 2010; Alexander and Randall 2007; Du et al. 2011).

5.6 Anesthesia

Little information exists on how cannabinoids can affect anesthesia specifically when it comes to animals. Clinical experience suggests little change and, like in most things, with animals under anesthesia it is important to continuously assess the patient and adjust inhalants or injectable anesthetics based on patient status and monitoring trends. Because of the synergistic effect's cannabinoids can have with several medications, dose adjustments will likely be the only noticeable consequence (Gordon et al. 1976; Kumar et al. 2010).

5.7 Conclusion and Practical Guidelines and Clinical Implications

Interest in using cannabinoid products for pain is of continued research and clinical interest. Data in human, lab animals, and now companion animals does show efficacy especially for chronic pain states. The relative therapeutic index appears favorable and the addition of these compounds, at least, gives veterinary practitioners another tool to consider for pain management. Careful consideration is important for product selection, not only quality but cannabinoid and terpene profiles, that may best fit a patient's pain state.

Acute pain: Considering upregulation of CB2 receptors during tissue injury and inflammation, therapy should target CB2 receptors to prevent changes from acute to maladaptive chronic pain. A combination of NSAIDS and cannabinoids may be most advantageous for patients where a NSAID alone is insufficient. Higher doses of acidic forms of cannabinoids (CDBA or CBGA) for NSAID intolerant animals may be more beneficial.

Considering the synergistic effects of cannabinoids and opioids, practitioners should expect to decrease the dose of opioids used in acute pain events when combining with cannabinoids. Product profiles that include CBD, CBDA, THCA, CBG, CBGA, myrcene, and beta-caryophyllene would likely be ideal to take advantage of their anti-inflammatory analgesic properties.

Chronic pain: When treating chronic pain, it is wise to take advantage of not only the major and minor cannabinoids but the terpenes as well. Most chronic pain medications work in synergy with cannabinoids. This can be used to an advantage when using multi-modal approaches in particular for refractory pain cases. Doses of pharmaceuticals can often be reduced when the two are used together, preventing or decreasing side effects. Synergy with gabapentin, for example, can be beneficial but clinicians may need to decrease doses of gabapentin to avoid unwanted side effects in older patients, such as lethargy. Use with long term NSAIDS appears not only to be highly effective but also safe per the authors' experience and the increasing number of OA studies being published, particularly in dogs. Liver enzyme monitoring should be considered for patients that are on cannabinoid products long term,

similarly to what is recommended for chronic NSAID administration.) Interestingly, some data exists showing CBD use can decrease C-reactive protein. While this inflammatory biomarker is not pain specific and is not commonly evaluated in the United States so far, it may become an important monitoring reference point for gaging the success of utilizing cannabinoid products in patients (Alshaarawy 2019; Vuolo et al. 2015).

Tolerance to the analgesic effects of cannabinoids, more specifically THC, has been shown in human patients after a long duration of administration. Tolerance to CBD has not been described with the exception of a percentage of epileptic patients described in Chap. 6.

The clinically relevant science of medical cannabinoids for veterinary patients is in its infancy but is very encouraging as we (delete:. We) now have our first well conducted clinical trials with more underway. The future of veterinary cannabinoid therapy appears promising for the treatment of pain conditions in our patients. Given the numerous nuances of products and the ECS, dosing for both acute and chonic pain) should be done in an escalating manner. Reasonable considerations for CBD dominant or isolate products can start as low as 0.5 mg/kg by mouth twice to three times a day and increase significantly until the therapeutic benefit is appreciated.

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Chapter 6
Cannabinoids for Neurological Conditions

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6.1 Introduction

Neurologic conditions represent some of the most confusing, challenging, and frustrating cases to treat. Because of this, it is not surprising that many pet owners turn to alternative therapies, including cannabis, to treat their pets. This chapter provides information on the use of cannabinoid compounds for the treatment or management of common neurologic diseases and disorders of dogs and cats.

6.2 Seizures

As described by the International League Against Epilepsy, an epileptic seizure is defined as a "transient occurrence of signs and or symptoms due to abnormal, excessive or synchronous neuronal activity in the brain." Seizures are sudden in onset, transient in duration, and are a result of disease or dysfunction in part, or the entirety of, the brain.

The endocannabinoid system (ECS)—most notably the CB1 receptors in the central nervous system, brain, and the endogenous cannabinoids—play a role in modulating neuronal activity including the "initiation, propagation and spread of seizures." Further exploration into the role of the ECS has shown that it is activated by seizures and that the upregulation of CB1 receptors in the brain may have anti-seizure effects. In 1971, Charles Hockman reported increased epileptiform discharges and electrical activity that preceded seizure and epileptiform activity in

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rats, cats, and primates in response to exposure to low doses of THC (Hockman et al. 1971). Only two years later in 1973, Juhn Atsushi Wada (the famed neurologist known for creating the Wada test for determining side dominance for epilepsy surgery) reported that THC had a protective effect in cats that were exposed to experimental cortical electrical stimulation, which in turn resulted in decreased seizures (Wada et al. 1973). Wada's team continued their research and in 1975 demonstrated that these same protective effects were seen in rats and primates (Wada et al. 1975). Numerous scientific reports continued to be published throughout the latter half of the twentieth century that both supported the use of cannabis as a treatment for seizures and the possibility that cannabis and its constituents triggered or precipitated seizures. The conflicting reports can be attributed, at least in part, to the lack of randomized, placebo-controlled scientific studies and confounding variables. An example of one such study that indicated the pro-epileptic properties of cannabis was published by Labrecque et al. in 1978; dogs were forced to "smoke" four marijuana cigarettes per day via tracheostomy tubes for ten weeks. Electrocorticography (ECoG) was used to detect any epileptiform complexes. Epileptiform discharges and generalized seizures were only seen in those dogs that were exposed to both cannabis and penicillin injections. Those dogs that were exposed to marijuana smoke only or penicillin injections only did not exhibit epileptiform discharges nor generalized seizures. Most of the past research into the use of cannabis as an anti-seizure treatment has been based on human subjects via surveys and case studies, calling into question the effect of cannabinoid ratios as well as terpene and flavonoid profiles. Surveys and case studies fail to adequately control for confounding factors including biases, doses, lifestyle, socioeconomic, and environmental variables. More recently, an increasing number of randomized, placebo-controlled studies are being conducted using companion animals to evaluate both the therapeutic potential and limitations of the use of cannabis in the treatment of epilepsy. Seizure syndromes in veterinary medicine have not been as well-defined or extensively studied as they are in human medicine which presents some limitations in the interpretation of some scientific literature.

6.2.1 Cannabidiol (CBD) in Seizure Disorders

CBD is the most abundant, non-intoxicating cannabinoid in the cannabis plant and is thought to be superior to THC for seizure control given its lack of intoxicating effects, safety, and relative demonstrated efficacy in seizure control. CBD's anticonvulsive properties have been demonstrated in both in vitro and in vivo experiments—specifically seizures induced by electrical, surgical, or chemical means. The mechanism of action is not fully understood. It is thought that CBD's anti-seizure properties are not only through direct interaction with the ECS but also association with other parallel receptors and signaling pathways that intermingle with the ECS (Cheung et al. 2019).

In a 2020 review article investigating the proposed mechanisms of the anti-convulsive action of CBD, authors describe the receptor promiscuity of CBD resulting in functional modulation of neuronal excitability. This contributes to the pathophysiology of several diseases. Researchers' attention is now focused on the modulation of intracellular calcium (Ca^{2+}). This includes effects on neuronal Ca^{2+} mobilization via GPR55 and influx via TRPV1 receptor. There is also interest looking at the modulation of adenosine mediated signaling.

It is also possible that the anti-seizure and neuromodulation properties of CBD are exerted through intracellular calcium regulation via activation of T-type calcium channels. CBD may also inhibit presynaptic release of glutamate and the reuptake of adenosine. Other mechanisms of action currently under investigation include transporter molecules, enzymes, and other channel targets (Cheung et al. 2019).

The FDA approval of Epidiolex® for two seizure syndromes— Lennox-Gastaut and Dravet Syndromes in children—has brought to light the efficacious application of CBD as an adjunctive anti-epileptic agent for the treatment of refractory seizure syndromes. Jones et al. in 2010 showed that 100 mg/kg of CBD in humans was successful in exerting anti-convulsive effects and decreased the incidence of seizures; epileptiform activity in vitro and severity in vivo were both reduced. Preclinical studies show that CBD is effective in mitigating seizure frequency and severity in mouse and rat models with experimentally induced seizures.

Theile and GW pharmaceuticals in 2018 published the first double-blind, randomized, placebo-controlled study investigating the effect of pure (isolate) CBD in seizure syndromes in humans. The study showed that a CBD isolate administered at 20 mg/kg/day, as an adjunct treatment to traditional anti-epileptic medication, was effective in reducing seizure frequency and was well tolerated by most participants.

McGrath et al. and Colorado State University (CSU) in 2019 published the first randomized, double-blind, placebo-controlled pilot study evaluating the efficacy and safety of CBD as an adjunct treatment for seizures in dogs. The study used a CBD-dominant hemp oil product manufactured by Applied Basic Science Corp. The oil was administered to canine participants at a dose of 2.5 mg/kg (total cannabinoids) orally every 12 h for a period of 12 weeks. All patients included in the study were epileptics whose seizures were refractory to other conventional anti-seizure therapies; the oil was administered concomitantly with other anti-epileptic drugs (AEDs). The researchers found the dogs in the CBD group had a median decrease in seizure frequency of 33%. This result, though statistically significant, failed to meet the definition of a CBD "responder" which is defined by a 50% decrease in seizure frequency in this study. There was no statistical difference between "responders" in the CBD and placebo groups, per the study conclusion. Even if the 33% decrease in seizure frequency did not meet the threshold of "responder", it should be noted that this result was not only statistically significant but likely clinically significant to the owners of the dogs experiencing a decrease in seizure frequency. Another interesting finding from this study showed the plasma concentrations of CBD in the dogs in the CBD group were positively correlated with reduced seizure frequency: the higher the plasma concentration, the greater the reduction in frequency of the seizures. A more recent human study of Epidiolex®

by Szaflarski et al. (2019) found that higher CBD plasma concentrations correlated with better responses; each 100 mg/ml increase in CBD plasma concentration was associated with a reduction in seizure frequency. The maximum dose for the study was 50 mg/kg/day for the efficacy analysis group. This data leaves open the possibility that, in order to increase the number of responders to treatment (defined as a greater than 50% reduction in seizure frequency), doses greater than 2.5 mg/kg of CBD orally every 12 h require further study. Other studies using CBD-dominant, CBD isolate, and 1:1 CBD:THC products are being conducted as of the writing of this chapter.

6.2.2 Side Effects

CBD is generally well tolerated with few side effects, as demonstrated in studies involving human and canine participants. The most common reported side effects of CBD administration in dogs were lethargy and increased alkaline phosphatase (ALP). The increase in ALP was not associated with liver failure based on other tests to evaluate liver function (e.g., bile acids testing). Ultrasound examinations of dogs with elevated ALP did not demonstrate any functional or structural changes to the liver. As of yet, the functional significance of ALP elevation is unknown, though may be related to the hepatic metabolism of cannabinoids. Deabold et al. in 2019 found that 12-week dosing of CBD at 2 mg/kg in dogs and cats did not induce liver enzyme increase outside of normal reference ranges in healthy animals. One cat (out of eight total subjects) had a transient spike in ALT at week 4 of the study. Longer term studies submitted to the FDA for approval of Epidiolex® in dogs (39 and 56 weeks, respectively), showed elevations in ALP and only slight elevations in ALT at CBD doses greater than 10 mg/kg. The researchers also noted hepatocellular hypertrophy which resolved after cessation of the CBD product. Typical clinical dosing is less than 10 mg/kg in most cases. In people, the side effects of CBD administration include transient increase in liver enzymes, somnolence, ataxia, and decreased appetite. Rebound seizures, which are appreciated with the sudden cessation of THC products, have not been associated with the use or sudden cessation of CBD.

6.2.3 Drug Interactions Between CBD and Traditional Anti-Seizure Medications

With the availability and popularity of hemp and marijuana derived CBD products increasing, the concern for safety as well as the potential for drug interactions is ever present, especially in patients with neurological conditions who are often taking multiple pharmaceutical agents. In the 2019 CSU study, McGrath, et al. found no

drug interactions or changes in serum blood levels of either potassium bromide or phenobarbital. The serum drug levels of other AEDs, and interactions with other medications generally, have not been fully evaluated or published in the companion animal population. Currently, researchers at Auburn University School of Veterinary Medicine are collecting representative population blood samples of patients on various AEDs and CBD products to evaluate potential adverse effects and interactions that may change blood levels of conventional pharmaceuticals. In human studies, co-administration of CBD and zonisamide resulted in elevated serum levels of zonisamide. This is thought to be a result of CBD's inhibitory effect on the enzymes involved in the metabolism of zonisamide. Also in humans, co-administration of CBD with clobazam showed an average increase in clobazam serum levels of 60% and an increase in its metabolite N-desmethyl-clobazam of an average of 500%, resulting in drowsiness and sedation. These effects subsided once the dose of clobazam was decreased. Clobazam is not routinely prescribed nor administered to veterinary patients, but this effect should be considered in patients that are prescribed benzodiazepines as either primary or adjunctive seizure control agents.

In humans, CBD is a potent CYP isoenzyme inducer. The CYP enzymes metabolize both cannabinoids and AEDs. Therefore, CBD's induction of the enzymatic pathway may increase metabolism of AEDs, leading to changes in blood levels of these medications. Thus far, no published veterinary research has demonstrated any change in phenobarbital levels when it is co-administered with CBD. More study on interactions with this and other AEDs is warranted. Additionally, CYP inducing medications administered alongside CBD, such as phenytoin and carbamazepine, may result in increased clearance of CBD.

There are many hemp and marijuana derived CBD products now available to both veterinarians and pet owners. Most cannabis products within the United States are considered supplements and are not regulated by any governmental agency (the issue of quality control and regulation is more fully discussed in Chap. 13). This lack of regulation leaves a wide variation in quality, actual CBD concentrations, and the potential presence of harmful contaminants.

There is also the potential for other drug interactions that have been demonstrated in the human literature, but not yet explored in veterinary medicine, including the co-administration of ketoconazole, topiramate, rifampicin, and benzodiazepines. Clinical experience suggests dosing is fluid and patient dependent. A starting dose of 1 mg/kg of a CBD-dominant product is reasonable but will likely need to be dose escalated over a period of a few weeks. Dosing of Epidiolex®, which was tested in canine models prior to FDA approval, and in the two Colorado State University studies—using a different CBD dominant product— is 5 mg/kg/day, split into two oral doses, up to 20 mg/kg/day, split into two oral doses. GW Pharmaceuticals' package insert for Epidiolex® also suggests obtaining baseline transaminases (ALT and AST) and total bilirubin levels prior to administration. Based on the veterinary studies we have to date, this suggestion, along with alkaline phosphatase (ALP) measurement, is warranted. Elevations of ALP alone are not suggestive of hepatic insult in dogs, but are of concern in cats. Dosage adjustments are recommended for

veterinary patients with moderate to severe hepatic impairment as with any medication metabolized by the liver (EPIDIOLEX® Full Prescribing Information 2019).

In an abstract presented at the 2018 American Epilepsy Society's annual meeting, an Israeli group led by Dr. Shimrit Uliel-Sibony presented data showing tolerance in some human patients (~32.6%) to CBD over time, with tolerance evident after 7 months. This has yet to be described in veterinary patients (Uliel-Sibony et al. 2020).

6.2.4 Δ^9-Tetrahydrocannabinol (THC)

THC is the main intoxicating cannabinoid present in cannabis. This intoxicating property which makes cannabis (marijuana especially) popular for recreational use in humans makes it a less attractive choice for seizure control, due to the negative effects of intoxication, including symptoms of marijuana toxicity in pets (see Chap. 3). Nonetheless, numerous animal studies have demonstrated the potential anti-seizure effects of THC (Rosenberg et al. 2015).

Successful suppression of seizure activity using THC is thought to be related to its interaction with the CB1 and CB2 receptors in the brain, most notably in the hippocampus. Studies have shown mixed results with the activation of CB1 receptors with the use of THC or synthetic CB1 agonists. Most studies show that agonism of CB1 receptors results in a decrease in seizures. Other studies have shown no effect on seizures, while a minority of studies show that CB1 receptor activation had a pro-convulsive effect. Some preclinical and preliminary data suggest that CB1 receptor antagonism may decrease seizure threshold (Ali et al. 2018; Malyshevskaya et al. 2017).

THC has been shown to decrease the incidence and severity of many experimentally induced seizures. This includes audiogenic seizures in mice and rats, as well as seizures secondary to electrical stimulation of the brain. Epileptic chickens receiving THC showed a decrease in incidence and severity of seizures as well as a decrease in inter-ictal EEG recordings of activity commonly preceding seizure (Boggan et al. 1973; Johnson et al. 1974, 1975; Consroe and Man 1973).

In their 1973 study, Wada, et al. found that THC had a neuroprotective effect and decreased seizure activity in cats with experimentally induced seizures by electrostimulation of cortical structures. Further research by Wada, et al. in 1975 showed that THC was effective in suppressing the kindling effect of seizures in cats with amygdaloid seizures. Safety and dosing guidance for THC is still lacking, yet clinical use suggests relative safety and efficacy particularly for patients with seizures that are less responsive to treatment, including CBD. Dosing of products with higher concentrations of THC should be calculated based on the concentration of THC to avoid negative side effects.

6.2.5 *Pro-Epileptic?*

Some studies have shown that THC may aggravate seizure conditions in humans. Furthermore, there is evidence that sudden cessation of THC products may have a rebound seizure effect in epileptic patients (Perucca 2017). For this reason, preparations that contain high levels of THC, and the use of recreational marijuana to treat seizure conditions, may not be suitable. In a study published by Whalley et al. in 2018 (sponsored by GW Pharmaceuticals) the researchers attempted to induce seizure activity in rat and canine models. Interestingly, the researchers preferred female to male rats as they were reported in a previous study to have an increased frequency of THC-induced convulsions. The study also used 20 male and 20 female purpose bred Beagles for the 52-week steady state treatment. The dogs received oral doses up to 27 mg/kg of THC with 25 mg/kg of CBD. This long term, high-dose study failed to produce convulsions in the canine model. Clinical signs observed in the treatment group of dogs included: ptyalism, hypoactivity, ataxia, tremor, abdominal breathing, tachypnea, lateral recumbency, reflux at dosing, vomiting, soft or liquid feces, and dehydration.

6.2.6 *Other Cannabinoids*

Tetrahydrocannabinolic Acid (THCA) and Cannabidiolic Acid (CBDA) have been shown in preliminary studies to exert some anti-convulsive effect and have been used for their anti-epileptic properties in some parts of the world. These acidic cannabinoids have no intoxicating effects, however, their anti-seizure properties have not been investigated as fully as CBD or THC. Care should be taken when handling THCA as exposure to heat or arid conditions, even if accidental, can decarboxylate the molecule, converting it to THC. CBDA can also convert to CBD under these same conditions but there are fewer adverse effects secondary to this conversion (Gaston and Friedman 2017; Anderson et al. 2019).

Cannabivarin (CBV) is another non-intoxicating compound found in small amounts in cannabis. It has been shown to exert anti-seizure effects in experimentally induced seizure models. Like CBD, its mechanism of action is not believed to be via direct interaction with the ECS. Its interaction with transient receptor channels and interaction with the synthesis of the endocannabinoid 2AG is not fully understood, nor is it known if or how these actions contribute any anti-seizure effects (Hill et al. 2012).

6.3 The Role of Cannabinoids in Neuroinflammation

Neuroinflammation is a unique process that differs in many ways from peripheral inflammation. Inflammation in the periphery is composed mainly of hematopoietic cells, macrophages, and lymphocytes; these cells undergo extravasation and invade the harmed tissues. In contrast, in neuroinflammation glial cells are the predominant cell type and the process is primarily mediated through microglia. Glial cells have several phenotypes and are involved with multiple facets of neuroinflammation including cytotoxicity, repair, regeneration, and immunosuppression. M1 activation has a predominantly cytotoxic profile while M2 activation results in release of anti-inflammatory cytokines, such as IL-4 or IL-10, and expression molecules which repair cellular damage (Martinez and Gordon 2014; Orihuela et al. 2015). Due to glial activation, a large number of cytotoxic molecules (i.e. cytokines, chemokines, glutamate, interleukins, nitric oxide, and reactive oxygen species) are released, which are exceedingly detrimental to neurons. The ultimate goal of this process is to eliminate possible pathogens or cellular debris (e.g., from dead neurons), culminating in brain tissue repair. To achieve this goal, several anti-inflammatory cytokines, growth factors, and tissue repairing molecules are released, restoring brain homeostasis. This is a dynamic process and its success depends on a variety of factors including the trigger of the injury and subsequent inflammation, as well as the brain regions affected. However, it is also a process limited by both space and time. Astrocytes play a key role in maintenance of brain homeostasis through: removal of ions and neurotransmitters such as glutamate; regulation of synaptic transmission; and antagonism of excitotoxic compounds (Verkhratsky and Butt 2007). Additionally, perivascular astrocytes are crucially located and involved in supplying energy to neurons and inflammatory conditions that alter the blood-brain barrier could integrally disrupt intermediate metabolic processes. Astrocytes respond to inflammation by releasing proinflammatory cytokines and NO (nitric oxide). Many neurological diseases involve some kind of neuroinflammation—whether primary or secondary; therefore, the use of anti-inflammatory treatments is desired. Due to their neuroprotective and anti-inflammatory properties, cannabinoids may have a key role in treatment (Grundy et al. 2001; van der Stelt and Di Marzo 2005; Gowran et al. 2011; Correa et al. 2005; Croxford and Yamamura 2005; Klein 2005).

The ECS is involved in immunomodulation, neuroprotection, and control of inflammation in the CNS. All cell types present in the brain express CB1 receptors and, due to their high density and widespread distribution, were initially named “central cannabinoid receptors”. CB2 receptors were called “peripheral cannabinoid receptors” due to their predominance on immune cells and organs; however, there is increasing evidence that some neurons can also express CB2 receptors (van Sickle et al. 2005; Atwood and Mackie 2010; Lanciego et al. 2011; Ando et al. 2012; den Boon et al. 2012). CB2 receptor activation can reduce the release of pro-inflammatory factors and enhance secretion of anti-inflammatory cytokines. Glial cells express both CB1 and CB22 receptors and astrocytic endocannabinoids

may modulate other cell types (Cabral and Marciano-Cabral 2005; Cabral and Griffin-Thomas 2008; Stella 2004; Molina-Holgado et al. 1998).

Various studies have evaluated the effects of cannabinoids on microglial proliferation; to date, there is no proof that cannabinoids induce microglial proliferation. Cannabinoids have been shown to prevent microglial activation, decrease NO production, and decrease both the production and release of TNF-alpha (Waksman et al. 1999; Puffenbarger et al. 2000; Facchinetti et al. 2003). Astrocytes in culture have shown the ability to synthesize endocannabinoids, which they may release upon stimulation of different neurotransmitter receptors (Stella 2004, 2010; Walter et al. 2002). Cannabinoids have been shown to reduce the release of proinflammatory cytokines and NO from astrocytes through CB1 and/or CB2 receptor-mediated action. Anandamide (AEA) has been shown to reduce endotoxin release and viral release of NO and TNF-alpha in culture. AEA also potentiates the release of IL-6 in a CB1 receptor-dependent manner (Molina-Holgado et al. 1998). Furthermore, in human astrocytes activated by IL-1B, the addition of cannabinoids inhibited the expression of NO and NOS release, along with the production and release of several chemokines and TNF-alpha (Sheng et al. 2005). In rats, activation of astrocytic CB1 receptors increased energy supply to the brain via ketogenesis and increased glucose oxidation (Sanchez et al. 1998). Cannabinoids have also been shown to regulate glutamate uptake and release from nerve terminals, resulting in reduction of synaptic transmission and modulation of glutamate-induced excitotoxicity (Brown et al. 2003). Astrocytes are key elements in neuroinflammation, and cannabinoids may offer some regulation of neuroinflammation; studies are limited regarding the effect of cannabinoids on neuroinflammation and the effects of cannabinoids under neuroinflammatory conditions have not yet been studied.

6.3.1 Neuroinflammatory Diseases of Companion Animals

Dogs and cats are affected by numerous inflammatory conditions affecting both the central and peripheral nervous systems. Neuro-inflammatory disease may be the result of infectious organisms or immune-mediated disease. Classically, these patients present with multifocal or diffuse encephalopathic signs; however, many patients will not follow this pattern. Infectious causes of meningoencephalomyelitis include bacterial, fungal, viral, protozoal, rickettsial, and other miscellaneous agents. Treatment of infectious neuroinflammatory diseases include antimicrobial therapy and anti-inflammatory medications. Immune-mediated inflammatory diseases of the CNS are mostly diagnosed in dogs (they are extremely rare in cats). These diseases are classified as immune-mediated because all attempts to demonstrate or isolate infectious agents from associated lesions have failed. This category of diseases includes granulomatous meningoencephalitis (GME), necrotizing meningoencephalitis (NME), necrotizing leukoencephalitis (NLE), eosinophilic meningoencephalitis (EME), steroid responsive meningitis-arteritis (SRMA), and corticosteroid

responsive tremor syndrome (White Shaker disease). Histopathologically, these diseases present with a non-suppurative inflammatory process and variable degrees of granulomatous inflammation. Treatment classically involves immunosuppression using a variety of medications including glucocorticoids, chemotherapy, and other immunomodulatory drugs. Disease outcomes can be variable with some patients completely responding to therapy while others are resistant to treatment and succumb to the disease.

Of these diseases, there is a single study of the ECS in dogs with steroid responsive meningitis-arteritis (SRMA) or CNS infection (Freundt-Revilla et al. 2018). SRMA, also known as Beagle pain syndrome due to its initial diagnosis in laboratory colonies of beagles, is a sporadic disease noted to occur more frequently in certain breeds of dogs (e.g., Boxer, Bernese Mountain Dog, Labrador Retriever) and may exhibit a familial pattern in some breeds (Boxer and Nova Scotia Duck Tolling Retriever). Young adult dogs are typically affected and classically present with a relapsing fever and neck pain. With severe or chronic cases, paresis, ataxia, polyarthropathy, and heart arrhythmias may be noted. The underlying cause of SRMA is excessive IgA production, likely due to immune dysregulation and an abnormal Th2 response. Other signaling biomarkers (interleukin-6, vascular endothelial growth factor, and transforming growth factor beta 1), have been shown to play a role in the disease process and may be involved in the development of the autoimmune response (Maiolini et al. 2013).

Freundt-Revilla et al. (2018) evaluated the ECS in canine SRMA and intraspinal spirocercosis. The endocannabinoids anandamide (AEA) and 2-arachidonoyl glycerol (2-AG) were quantified in CSF and serum samples of dogs with acute phase SRMA, SRMA under treatment, intraspinal spirocercosis, and healthy dogs. Endocannabinoid concentrations were highest in dogs with intraspinal spirocercosis, followed by dogs with untreated SRMA. Cerebrospinal fluid samples demonstrating an eosinophilic pleocytosis had higher levels of endocannabinoids compared to dogs with neutrophilic CSF samples. Additionally, while healthy dogs demonstrated CB2 receptor expression in glial cells, dogs with SRMA and intraspinal spirocercosis also expressed CB2 receptors on the surface of infiltrating leukocytes at lesion sites. Based on this study, the ECS may be a potential target for treatment of inflammatory CNS disease.

While there is substantial evidence detailing the role of the ECS in neuroinflammation, information is lacking on the specific role of the ECS in canine and inflammatory disease. As the underlying pathogenesis of neuroinflammation varies by disease, directing treatment to specific parts of the ECS may be necessary for effective therapy. In conclusion, while more information and research are warranted, the ECS appears to be a possible therapeutic target in neuroinflammatory diseases. Dosing has not been elucidated for these conditions but is likely consistent with other dosing suggestions and the patient's clinical response.

6.4 Traumatic Brain Injury

Traumatic brain injury (TBI)—sustained when the head is struck by an external force causing the brain to either move violently within an intact skull or causing skull fractures to impact the brain—involves an intricate web of acute injury and delayed secondary injury processes culminating in structural and functional damage (Blennow et al. 2012; Bolouri and Zetterberg 2015; Braun et al. 2018; Kuo et al. 2018; Namas et al. 2009). Primary injury processes are those which occur at the moment of impact, resulting in physical disarrangements of intraparenchymal brain structures. Concussion (loss of consciousness without histopathologic changes), contusion (bruising of intraparenchymal brain structures), laceration (physical severance of brain structures), hemorrhage, and hematoma formation may occur as primary injury processes (Kuo et al. 2018; Namas et al. 2009). Acute injury processes include an immediate release of excitatory neurotransmitters, rapid depolarization of neurons, glucose metabolism derangements, disruption in cerebral blood flow from vascular trauma, and axonal dysfunction, all of which collectively result in excitotoxicity. Delayed secondary injury processes occur minutes to days—and possibly months—following the acute trauma and include cerebral hypotension, hypoxia, edema, and elevations in intracranial pressures further adversely affecting brain neurometabolism and function. The massive accumulation of excitatory neurotransmitters, like glutamate, induces sodium influxes from depolarization shifts, increasing intraparenchymal edema; calcium influxes further activate damaging proteases, lipases, and endonucleases, leading to profound excitotoxicity effects potentiating neuronal cell injury and even death. These secondary injury processes, no matter how minor, result in blood-brain barrier (BBB) disruption and amassing of peripheral blood borne substances, such as fibrin and nitric oxide. Production of reactive oxygen species (ROS) contribute to the breakdown of BBB tight junction proteins, as well as lactic acidosis and adenosine triphosphate depletion, accelerating intraparenchymal neuronal inflammation and injury processes. These may result in cell death, further compounding deleterious effects of higher brain functions subsequent to the TBI. An ever-growing body of preclinical evidence indicates that the ECS plays a critical role in regulating adult neurogenesis. However, there is still more research needed before clinicians can conclusively recommend phytocannabinoids, like CBD, as a therapy (Oddi et al. 2020).

TBI-induced BBB disruption may persist even after apparent clinical recovery. BBB disruption increases the risk of continued cognitive decline over months to years. Damaging neurofibrillary tangles (NFTs), amyloid-β (Aβ) plaque formation, and elevations of inflammatory mediators (interleukins, cytokines, tumor necrosis factor/TNF, etc.) have all been identified intraparenchymally as part of the secondary injury processes following a TBI (Blennow et al. 2012; Bolouri and Zetterberg 2015; Kuo et al. 2018; Mayer et al. 2019; Namas et al. 2009; Szarka et al. 2019). Vasoconstriction, coagulation cascade activation, paradoxical hypo-coagulation, and cytotoxic edema all potentially add to neuronal cell and glial cell injury and death (Mayer et al. 2019). Primary injury processes, while outside the control of any

caregiver, initiate delayed secondary injury mechanisms; interventions between the primary and secondary injuries may provide opportunities for caregivers to intervene to change the course of injury progression via various therapeutic modalities (Kuo et al. 2018) (Fig. 6.1).

TBIs in veterinary patients are most likely to occur secondary to traumatic events like being hit or crushed by motor vehicles (50%), being dropped, gunshot injuries, falling off ledges, injurious encounters with other animals, or intentional harm inflicted by humans. Approximately 96% of dogs and cats with severe TBI had evidence of intracranial hemorrhage, while 89% of dogs had severe skull fractures. Head trauma injuries carry an estimated 18–24% mortality rate in dogs (Kuo et al. 2018). Cognitive dysfunction, epilepsy, endocrine imbalances, tremors, and paresis are just a few of the possible neurological issues which may follow TBIs (Caine et al. 2018; Hecht and Adams 2010; Kostic et al. 2019; Kuo et al. 2018; Murtagh et al. 2015; Parratt et al. 2018). Animal models are being used to learn more about TBIs and to help develop targeted therapies to reverse or slow the acute and secondary injury processes which are so devastating to humans and animals alike (Bolouri and Zetterberg 2015; Braun et al. 2018; Kostic et al. 2019; Mayer et al. 2019; Namas et al. 2009; Szarka et al. 2019; Williams et al. 2019).

Aggressive supportive care aimed at maintaining normal cellular homeostatic processes like normovolemia, normothermia, neuroprotective mechanisms, and ventilatory support are critical to achieve stabilization and provide the best chance for recovery (Farrell and Bendo 2018). Modulation of the ECS may play an integral part in those life saving measures. (Biegon 2004; Braun et al. 2018; Elliott et al. 2011; Fernández-Ruiz et al. 2005; Liu et al. 2017; Lopez-Rodriguez et al. 2016; Mechoulam et al. 2002; Panikashvili et al. 2001). Endocannabinoids, primarily AEA and 2-AG, are physiological ligands for two known cannabinoid receptors, CB1 and CB2. Exogenous cannabinoids, primarily THC and CBD, have both direct and indirect modulating effects on the ECS (Braun et al. 2018). Phytocannabinoid administration can play a role in effectively modulating the ECS, accentuating its neuroprotective effects following TBI, resulting in improved outcomes (Fig. 6.2).

As previously stated, TBI initiates a cascade of release of detrimental mediators such as glutamate, cytokines, ROS, TNF- α, interleukins, and others, all potentiating neuronal cell injury and death. Ischemic and inflammatory mediators are also increased intraparenchymally following TBI and lead to the accumulation of endothelin (ET), thromboxane, sodium and calcium influxes, and free radical development within the neuronal and glial structures. It has been well documented that endocannabinoids, such as 2-AG and AEA, are increased within the brain following TBIs. These increases are thought to be natural neuroprotective mechanisms as both 2-AG and AEA inhibit the release of glutamate, ROS, cytokines, TNF-α, interleukins, and ET (Biegon 2014; Mayer et al. 2019; Mechoulam et al. 2002; Panikashvili et al. 2001; Piro et al. 2018; Russo 2018). In vitro studies have shown AEA to be neuroprotective against ischemia-induced TBI injury in rat brains and in vitro studies have shown 2-AG decreases TNF-α production, interferes with fibrin formation, and counteracts endothelial vasoconstriction effects which further provides neural and glial cell protection following TBI (Mechoulam et al. 2002). Administering synthetic

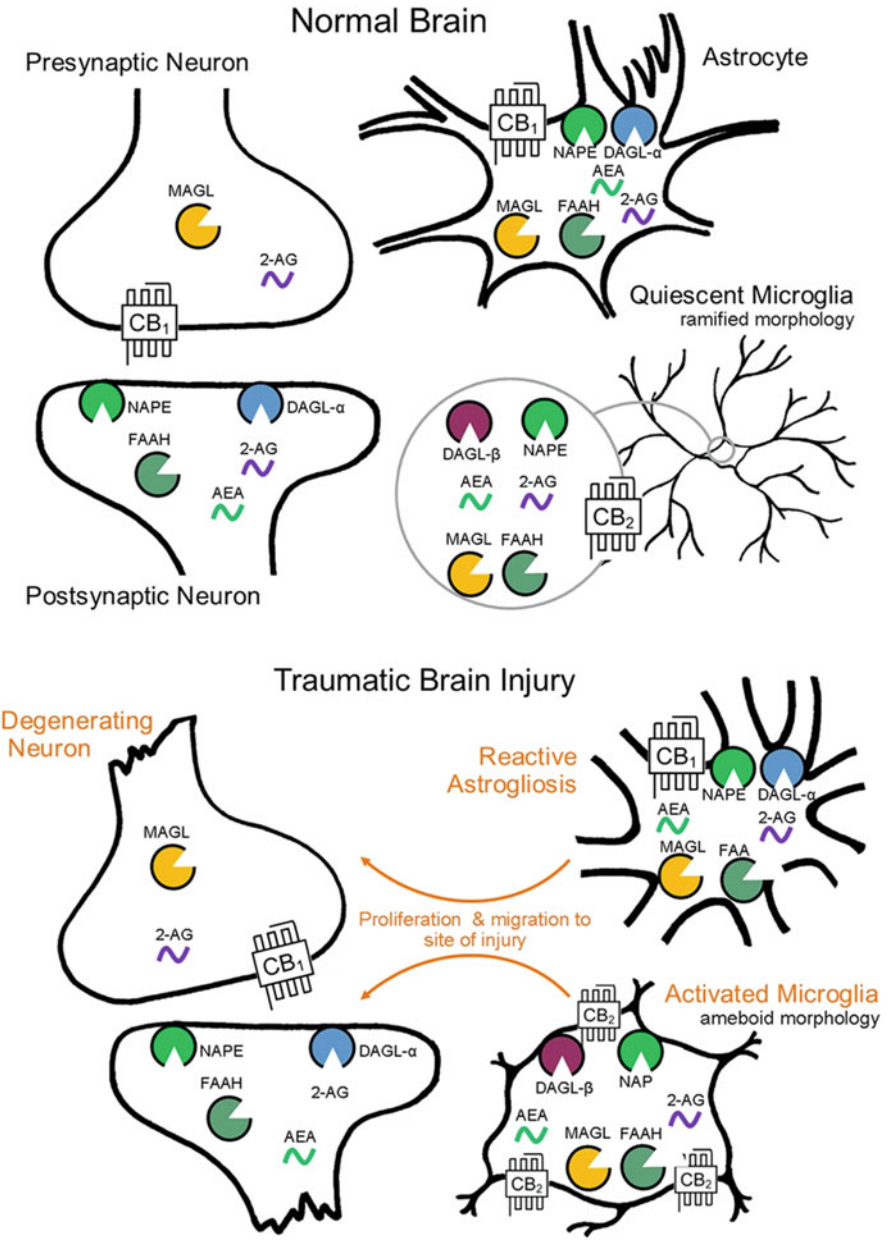

Fig. 6.1 Postulated changes in endocannabinoid biosynthetic and catabolic enzymes in brain injury. (Permission to reprint with full citation made, Schurman, L. D., & Lichtman, A. H. (2017). Endocannabinoids: A Promising Impact for Traumatic Brain Injury. *Frontiers in Pharmacology, 8*. doi:https://doi.org/10.3389/fphar.2017.00069)

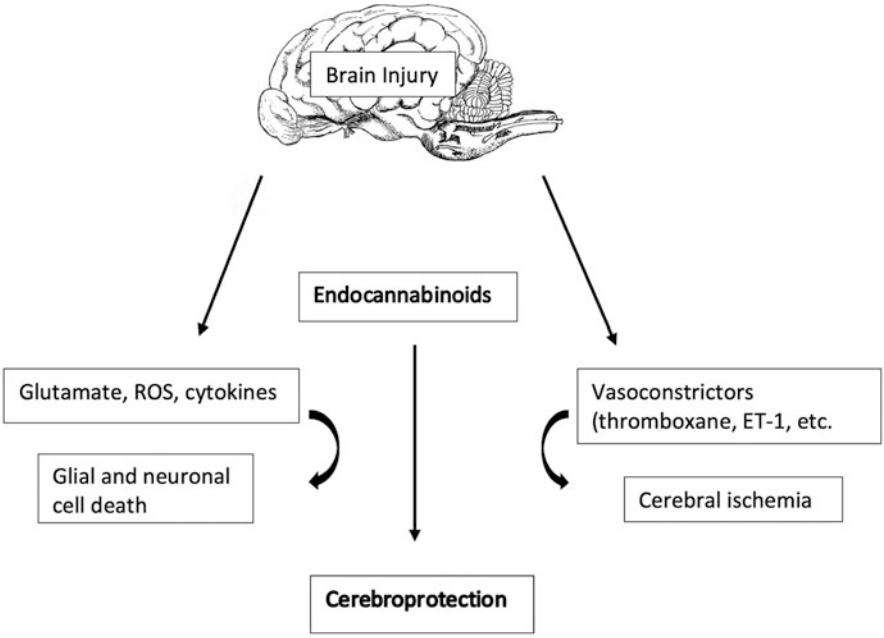

Fig. 6.2 Brain response in TBI

2-AG to mice after TBI resulted in significantly less brain edema (47% smaller cytotoxic edema volume 7 days post administration), improved recovery times, reduced ischemic territories (67% smaller infarcts 7 days post administration), and reduced hippocampal cell death (Mechoulam 2016; Mechoulam et al. 2002). Exogenous cannabinoids are being researched to determine if they may hold therapeutic benefits for patients suffering from TBI. HU-211 (Dexanabinol), a synthetic cannabinoid, acts as a glutaminergic receptor agonist and also inhibits TNF-α, ROS, and free radical production. It is currently in phase III clinical trials against TBI and is showing promising outcomes with regards to improving neurologic recovery in humans. THC has demonstrated minor neuroprotective effects in a dose dependent manner in rodent studies but may be of limited use clinically given its intoxicating effects (Braun et al. 2018; Mechoulam et al. 2002). A recent murine study of a controlled cortical impact model demonstrated improvements in neuroinflammation, reduced neurovascular injury, decreased BBB permeability, and reduced macrophage/microglial activation limiting neuronal degeneration when selective CB2 agonists were administered 72 h following TBI. CBD also has been shown to decrease glutamate toxicity, reduce BBB permeability, and is a powerful antioxidant in its own right (Braun et al. 2018; Fernández-Ruiz et al. 2005).

TBI initiates a robust and complex cascade of acute and secondary injury processes within the brain which can be summarized as causing hemodynamic, metabolic, neuro-endocrine, and inflammatory responses. While these processes, especially inflammation, are needed to optimize recovery processes and cellular

homeostasis, if left unchecked deleterious and perhaps continual neuronal and glial cellular injury and death can occur (Fernández-Ruiz et al. 2005; Namas et al. 2009). Mouse models with TBI developed chronic pain associated with anxious and aggressive behavior. Further more, the injury also resulted in a late depressive-like behavior and impaired social interaction. Treatment with oral CBD restored the behavioral alterations and partially normalized the cortical biochemical changes (Belardo et al. 2019). It stands to reason that developing new therapeutic strategies utilizing the ECS, exogenous phytocannabinoids, and synthetic counterparts may provide novel approaches to improved TBI outcomes in both humans and animals. Further studies are needed but the future looks very promising regarding cannabinoids and improved TBI outcomes given their promising neuroprotective mechanisms.

6.5 The Role of CBD in Neurodegenerative Diseases

A variety of neurodegenerative diseases exist in human and veterinary medicine. Some of these, such as Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS) along with their canine equivalents canine cognitive dysfunction and canine degenerative myelopathy, are secondary to protein misfolding (Rakhit and Chakrabartty 2006; Bruijn et al. 1998; Yu et al. 2011). Other degenerative processes are due to errors on a genetic level, rendering a specific protein or enzyme nonfunctional resulting in neuronal death, as occurs in cases of neuronal ceroid lipofuscinosis (NCL) or the gangliosidoses. Additionally, neurodegenerative processes typically result in low-grade sustained inflammation of the nervous system which further contributes to the degenerative process (Minghetti 2005). Because these diseases cause a fatal progressive deterioration, they are the focus of much research to develop novel treatments. Since many of these diseases also occur in animals, much of the research is focused on large vertebrate models for the development and testing of novel therapies. Luckily, with the exception of canine cognitive dysfunction and canine degenerative myelopathy, most of these diseases are rare and occur primarily in genetically modified populations developed for research purposes.

6.5.1 Alzheimer's Disease and Canine Cognitive Dysfunction

Canine cognitive dysfunction (CCD) has a prevalence of 14–35% in companion dogs and the risk of developing CCD increases exponentially as dogs age (Azkona et al. 2009). Dogs show cognitive deficits such as disorientation, memory loss, and changes in behavior. In their brains, beta amyloid plaques are commonly detected both in the extracellular space, as senile plaques, and around the blood vessels (Prpar Mihevc and Majdič 2019). Current treatment options for CCD target

prevention along with slowing and/or improving the cognitive decline in dogs. Some drugs or food supplements are available for senior dogs and might act as neuroprotective agents. Some enhance the blood flow into the brain while others work as antioxidants. More effort is now directed to slowing the progression of the disease instead of providing only symptomatic treatment. The ECS' ability to regulate neuronal function via such mechanisms as Ca^{2+} buffering, modulating metabolic activity, neurotransmission, and the inflammatory response (via CB1 and CB2 receptors) has been shown to be beneficial in some diseases, such as Alzheimer's Disease (AD), and may prove beneficial for CCD. Several studies have also demonstrated that drugs targeting the ECS may improve a variety of clinical signs such as agitation, aggression, pain, and memory, thereby enhancing our patients' quality of life (Walther et al. 2006; Rikkert 2014a, 2014b; Passmore 2008).

6.5.2 *Amyotrophic Lateral Sclerosis and Canine Degenerative Myelopathy*

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disorder caused by selective and progressive injury and death of motor neurons. There are two forms recognized in humans: familial ALS and sporadic ALS (Ahmed and Wicklund 2011). Familial ALS occurs in only about 5–10% of patients and only in those patients with an autosomal dominant pattern of inheritance (Renton et al. 2014). In contrast, nearly all cases of canine degenerative myelopathy undergo a familial pattern of inheritance. Certain breeds, such as Boxers, German Shepherds, Bernese Mountain Dogs, and Chesapeake Bay Retrievers, appear overrepresented (Zeng et al. 2014). The complete pathogenic process of ALS remains mostly unknown, but some proposed mechanisms include processes similar to other chronic neurodegenerative disorders, such as oxidative stress, excitotoxicity, defects in glial-neuron crosstalk, neuroinflammation, mitochondrial dysfunction, protein aggregation and deposition, and dysregulation of RNA transcription and processing. Since 2004, studies have suggested that cannabinoids may be a potential disease-modifying therapy in ALS due to their ability to modify microglial activation and neuroinflammation (via CB2 receptor targeting), decrease excitotoxicity, and reduce oxidative injury (Carter et al. 2010; Rossi et al. 2010). Furthermore, cannabinoids may help control the toxicity of protein aggregates in ALS due to their ability to enhance autophagy, though this has not been tested at this time (Salazar et al. 2009). Unfortunately, little information exists about ECS changes occurring within the affected CNS areas in ALS patients. SOD-1 mutant mice display elevated levels of AEA and 2-AG in the spinal cord and CB2 receptor upregulation in microglia within the lesion (Shoemaker et al. 2007; Moreno-Martet et al. 2014; Witting et al. 2004; Bisland et al. 2006). Another study demonstrated presymptomatic downregulation of CB1 receptors in conjunction with an upregulation of glutamate receptors in

SOD-1 mutant mice. This is a key observation given that CB1 receptors play an important role in glutamate homeostasis and excitotoxicity is thought to be an important factor in ALS pathogenesis; however, other studies have challenged this finding (Moreno-Martet et al. 2014; Bisland et al. 2006).

Preclinical treatment of ALS in SOD-1 mutant mice has shown promising pharmacologic evidence that cannabinoids may provide neuroprotective effects. The first studies performed (using THC) showed delayed onset of motor impairment and prolonged survival. This effect was most pronounced when treatment was initiated prior to symptom onset, but effects were also seen if treatment occurred after the appearance of clinical signs (Raman et al. 2004). However, when SOD-1 mutant mice were treated with Sativex® only a small delay in symptom onset and no change in survival time was noted (Moreno-Martet et al. 2014). Additionally, studies using FAAH enzyme-ablated mice, with subsequently increased AEA levels, demonstrated delayed evidence of disease signs but not prolonged survival times (Bisland et al. 2006). Conversely, CB1 receptor-ablated mice did not show any change in time to disease onset but did experience a significantly extended lifespan (Bisland et al. 2006). To date, no studies have examined CB2 receptor-ablated SOD-1 mutant mice. As a whole, these studies suggest that cannabinoids may have neuroprotective effects in ALS, mediated by mechanisms other than CB1 receptor activation. These mechanisms may involve antioxidant and anti-inflammatory properties of certain cannabinoids. At this time, there are no large studies that have thoroughly researched the potential of cannabinoids as disease-modifying therapies, even though such therapies have been implied and supported by preclinical studies.

6.5.3 Conclusions

Neurodegenerative diseases are some of the most challenging to treat in both veterinary and human medicine. Current medications do little to slow the progression or alleviate symptoms for many of these diseases. However, based on the ability of the ECS to regulate neuronal function, buffering, metabolic activity, neurotransmission, and the inflammatory response, it follows that therapeutic targeting of this system could be beneficial in treatment of neurodegenerative diseases. As we are just beginning to fully understand many of these diseases in veterinary medicine, the therapeutic use of cannabinoids has not been adequately explored. The authors recommend caution when applying cross-species research and proof of efficacy, as many medications, including several for ALS, have shown promise in one species only to show minimal effect in another.

6.6 Brain Tumors

Beyond alleviation of cancer associated symptoms, research in humans is taking a much closer look at how targeting the ECS may be used to complement targeted anti-cancer therapies (Dumitru et al. 2018; Hausman and Guerriero 2016; Hermanson and Marnett 2011; Hinz and Ramer 2019; López-Valero et al. 2017; Moreno et al. 2019; Pellati et al. 2018; Ramer et al. 2019; Russo 2018; Schwarz et al. 2018).

THC, CBD and a few other cannabinoids (CBDA, CBGA, CBG, CBC, CBNA, CBN, etc.) have been shown to exhibit anti-cancer effects both in vitro and in vivo (Dumitru et al. 2018; Hermanson and Marnett 2011; López-Valero et al. 2017; Moreno et al. 2019; Pellati et al. 2018; Ramer and Hinz 2017; Schlein et al. 2019). CBD has received a great deal of attention as a possible anti-cancer therapy, capitalizing on its antioxidant and anti-inflammatory activity. Additionally, CBD exerts antibacterial, neuroprotective, anxiolytic, and anticonvulsant effects, all with minimal to no intoxicating effects (Dumitru et al. 2018; Hermanson and Marnett 2011; Hinz and Ramer 2019; Moreno et al. 2019; Pellati et al. 2018; Ramer and Hinz 2017; Ramer et al. 2019; Schlein et al. 2019; Schwarz et al. 2018). Flavonoids and terpenes found in cannabis are also known to exert anti-cancer, anti-inflammatory, and neuroprotective biological effects, thus amplifying the potential benefits of cannabinoids in anti-cancer therapies (Echigo et al. 2012; Pellati et al. 2018; Ramer et al. 2019; Schwarz et al. 2018).

Approximately 4.5% of both human and dog populations will have a primary brain tumor during their lifetime. Gliomas and meningiomas are overrepresented in both species. In the dog, meningiomas account for nearly 47% of all brain tumors while gliomas account for 55–70%, and choroid plexus cancers account for approximately 6% (Dickinson 2014; Hicks et al. 2017, 2019; Snyder et al. 2016). Traditional therapies such as radiation therapy, surgical resection, chemotherapy, and combinations of modalities provide a wide range of survival times for dogs. In dogs with meningiomas:

- Following surgical resection: median survival time (MST) 7–10 months
- After radiation therapy: MST 8–11.7 months
- After combination therapy: MST of 16 months

Other therapeutic modalities such as gene therapy and immunotherapies are currently being investigated for dogs. In humans, meningiomas are often treated with both radiation therapy and surgery, yielding MSTs of 5 years (Dickinson 2014; Dolera et al. 2018; Hicks et al. 2017; Hu et al. 2015; Snyder et al. 2016).

Gliomas—consisting of astrocytoma, oligodendroglioma, ependymoma, and glioblastoma—of varying grades often carry much lower MSTs in both humans and dogs. MSTs approaching 6 months in dogs and approximately 2 years in humans have been reported with aggressive anti-cancer therapies including combinations of radiation, surgery, and chemotherapy (Dickinson 2014; Dickinson et al. 2010; Dolera et al. 2018; Hicks et al. 2017; Kato et al. 2014; Schlein et al. 2019).

It is hoped that benefits similar to those being seen in human medicine will occur in veterinary medicine, but more research is needed. Many canine brain tumors have similar occurrence rates, microscopic traits, and genetic characteristics to those found in humans, making dogs nearly perfect translational models. There are, however, some exceptions. Meningioma COX-2 expression is used in humans as a correlate to the proliferative index and grade of the tumor. This does not translate to the dog. Likewise, canine meningiomas do not exhibit the correlation between NF2 expression and tumor grade found in human meningiomas. Finally, canine astrocytomas do not exhibit an exonic p53 mutation commonly seen in human astrocytomas. It stands to reason that identifying common therapeutic targets could improve prognoses, especially in regard to non-surgical or recurrent brain tumors and thus extend quality of lives in both species (Dickinson 2014; Dickinson et al. 2009; Dolera et al. 2018; Hicks et al. 2017; Joshi et al. 2017; Russo 2018). Veterinary research aimed at manipulating the ECS and utilizing cannabinoids is warranted as this arena of targeted therapy appears to have nearly limitless potential.

The regulatory mechanisms and actions of the ECS modulate virtually every cancer biology route. The primary aim of the ECS is to counteract endogenous and environmental influences that threaten internal homeostasis (Dumitru et al. 2018; Hermanson and Marnett 2011; Hinz and Ramer 2019; Moreno et al. 2019; Ramer et al. 2019). Cancers naturally fall into this paradigm (Kitchell and Dervisis 2010). Endocannabinoid anti-cancer actions include apoptosis induction, autophagy, and arrest of the cell cycle process within tumor cells (Dumitru et al. 2018; Hermanson and Marnett 2011; Hinz and Ramer 2019; Pellati et al. 2018; Ramer and Hinz 2017). Manipulating any facet along this metastatic cascade may prove successful in slowing down or preventing cancer growth (Kitchell and Dervisis 2010). Endocannabinoids are able to influence antiproliferative, antiangiogenic, and antimetastatic effects by enhancing tumor immune surveillance and inhibiting tumor cell migration (Dumitru et al. 2018; Hermanson and Marnett 2011; Hinz and Ramer 2019; Pellati et al. 2018; Ramer and Hinz 2017; Petersen et al. 2005). Endocannabinoids also have profound anti-inflammatory effects by acting as substrates for cycloxygenase-2 (COX-2), lipoxygenases (LOXs), and cytochrome P450 (CYP450s), thus influencing inflammatory mediators (Hermanson and Marnett 2011; Hinz and Ramer 2019; Pellati et al. 2018; Ramer and Hinz 2017; Petersen et al. 2005). Cannabinoids are known to prevent epithelial-to-mesenchymal transition and enhance chemotherapeutic effects on drug-resistant cancer cells (Echigo et al. 2012; Hermanson and Marnett 2011; Hinz and Ramer 2019; Pellati et al. 2018; Ramer et al. 2019; Schwarz et al. 2018). Endocannabinoids, cannabinoid receptors, and lipid modulators are up-regulated in many tumors with a direct parallel between degree of expression and tumor severity and aggressiveness (Hermanson and Marnett 2011; Petersen et al. 2005); mechanisms of action are dependent on receptor type (CB1, CB2, transient receptor potential vanilloid type I (TRPV1)), and may be independent of receptors, depending instead on the interaction between the cannabinoid or endocannabinoid with the tissue or tumor cell (Hermanson and Marnett 2011; Hinz and Ramer 2019). Studies to determine possible correlations between endocannabinoid metabolizing enzymes FAAH and MAGL are currently underway and may provide further insight as to how best manipulate the balance between

endocannabinoids, phytocannabinoid supplementation, and their metabolizing enzymes in cancer therapies.

Of particular interest are studies investigating human glioma tumor cells using animal models. These studies demonstrate the antineoplastic activity of phytocannabinoids (specifically CBD and THC) via inhibitory effects on the proliferation and survival of glioma cells. Elevated levels of endocannabinoids AEA and 2-AG have been identified in glioblastomas, meningiomas, pituitary adenomas, prostate and colon carcinomas, endometrial sarcomas, and other invasive human cancers. The effects of AEA and 2-AG are dictated by the enzymes synthesizing and metabolizing them, thus regulating their levels (Echigo et al. 2012; Hermanson and Marnett 2011; Hinz and Ramer 2019; López-Valero et al. 2017; Pellati et al. 2018; Petersen et al. 2005; Ramer et al. 2019; Schlein et al. 2019; Schwarz et al. 2018). CBD has virtually no direct action at the CB_1 or CB_2 receptors, but it has been shown to suppress glioma and breast tumor growth in vitro and in vivo by stimulating apoptosis and suppressing cell migration and angiogenesis, regardless of CB and TRPV1 receptor activity (Hermanson and Marnett 2011; López-Valero et al. 2017; Schlein et al. 2019). Gross et al. in 2020 presented an abstract looking specifically at canine and human glioma cells' response to CBD. While questionable concentrations of systemic CBD might be pharmacologically difficult to achieve in living animals, more feasible systemic concentrations did show an increase in glioma cell sensitivity, with CBD distressing mitochondrial function. They also found that the addition of an autophagy inhibitor increased this sensitivity, suggesting autophagy as a pathway for mediating CBD induced cell death. More on this can be found in Chap. 10 (Gross et al. 2020).

THC and CBD are known to increase the cytotoxic effects of several chemotherapeutics including: cytarabine (leukemia); doxorubricin (leukemia); mitoxantrone (embryonic fibroblasts); carmustine; temosolomide; bortezomib; vinblastine (leukemia); carfilzomib; cisplatin (glioblastoma); vincristine (leukemia); and carfilzomib (multiple myeloma) (Dumitru et al. 2018; López-Valero et al. 2017; Moreno et al. 2019).

A distinctive feature of tumorigenesis is inhibition of normal cellular apoptosis. There are many intracellular proteins (death receptors, mitochondrial pore proteins, TP53) that work to upregulate and downregulate apoptosis of cancer cells. A recent study has shown encouraging results indicating cannabinoid-based medicines with a higher level of CBD, in combination with temozolomide, (TMZ) may have enhanced antitumor action, as compared to therapies with higher levels of either THC and TMZ or TMZ alone (López-Valero et al. 2017).

Manipulating these processes with phytocannabinoids may hold the key to future anti-cancer therapies for the central nervous system. While clinical veterinary data is still lacking, reviewing the research currently available, and coupling that research with clinical anecdote, it appears that phytocannabinoids are safe and possibly effective in many neurological types of cancer.

6.7 Conclusions

Current therapies for many neurological and neurodegenerative diseases, such as canine cognitive dysfunction, canine degenerative myelopathy, pharmaco-resistant epilepsy, and neuroinflammatory disorders, may be limited in their effectiveness and in some cases, standard treatment options do not exist at this time. This underscores the need for further research and new medications for preventing or reversing the disease process. There is ample evidence that the ECS plays a role in regulating numerous pathological processes, including alteration of neuroinflammation, excitotoxicity, mitochondrial dysfunction, and oxidative stress. Many studies have indicated that targeting CB1 and CB2 receptors may have clinical benefits. However, it has also been documented that CB2 receptor gene structures and expression patterns differ between mice, rats, and humans (Kong et al. 2014). These subtle differences between rodent and human disease models may also have significant impacts on canine and feline therapies. At this time, the authors cannot advocate for the use of cannabinoids as a primary disease treatment modality; however, it may have a beneficial adjunctive treatment role, especially in cases that have been refractory to standard treatments. Dosing has not been elucidated for these conditions but is likely consistent with other dosing suggestions and the patient's clinical response.

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Chapter 7
Well Being

Jamie Peyton, Katherine Kramer, Brook Quesnell, and Stephen Cital

7.1 Introduction

The concept of well-being is a common topic in both human and veterinary medicine with a wide-ranging and highly debated definition (Zainuddin and Russell-Bennett 2017; Charlemagne-Badal et al. 2015). In its broadest sense, "well-being" encompasses all aspects of an individual's life and experiences. It can be viewed as a multidimensional coordination of "an intersecting triumvirate of emotional, physical, and cognitive self" (Charlemagne-Badal et al. 2015; Gillett-Swan and Sargeant 2015).

The physical and cognitive aspects of well-being are the ones most commonly addressed in medicine by evaluating the presence/absence of disease, environmental factors, resources, memory, and the ability to learn. Yet the emotional self may be the most synonymous with well-being, since it can include happiness, worry and anxiety, and its effects on the family unit or social interactions.

In children, the definition of well-being also includes a "state of readiness", where an individual can thrive, repel, confront, and cope with information presented to the brain (Gillett-Swan and Sargeant 2015). In animals, this emotional aspect of well-being can be seen in behavior issues such as fear, anxiety, and related physiologic

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disorders such as feline lower urinary tract disease. This chapter will investigate the current knowledge and potential application of cannabinoid medicine in animal "well-being disorders" based on preclinical animal and human studies.

7.2 Behavior

7.2.1 Anxiety Disorders

While behavior modification is the preferred means of managing patients with stress or anxiety, this does not mean that it is without challenges or always successful. Management of any problem behavior requires recognizing and avoiding the stimuli that triggers the behavior and relying on the animal to behave differently, based on training, in response to the stimulus. "*Differently*" is intentionally vague because it is personalized to the individual animal and context in which the problem behavior occurs. Obviously, there are some significant tribulations with the success of this model, especially if the owner or caregiver is unable to perform counterconditioning for a fearful animal, provide alternative behavior to a cue, or desensitize a separation anxiety distressed pet when no one is around. One of the largest concerns with relying solely on behavior modification techniques is that they may be unrealistic for most owners given the amount of time required and the need for consistent enforcement of the techniques required. This is where augmentation of behavior modification, historically with pharmaceutical interventions, comes into perspective. Traditional pharmaceutical approaches come with their own risks and challenges. Depending on the type of medication used, pets may experience only short-lived effects, no effects, or adverse effects. Another confounding factor to the dilemma of managing stress related behaviors with pharmaceutical approaches (either acutely or chronically) stems from owner reservations of using behavioral medication. These reservations include the pet's personality changing, the perception and fact of pharmaceuticals potentially causing harm, and most often, the owner not wanting the pet to be drowsy all the time. There is also the potential concern of owner diversion and abuse of these medications. These challenges elucidate not only the interest in alternative options but the necessity.

One area of cannabis research in human medicine is its role in emotional well-being, particularly in regard to the treatment or management of anxiety. Anxiety is one of the most prevalent mental disorders in people within the U.S.A. (Crippa et al. 2009; Blessing et al. 2015) and it is also a common behavioral issue noted in companion animals (Tiira et al. 2015). While anxiety is an adaptive, protective response to perceived environmental threats, it can lead to a maladaptive disability if the anxiety is excessive or persistent. This ensuing condition can significantly affect an individual's well-being (Blessing et al. 2015; Tambaro and Bortolato 2012). There are several classifications of anxiety disorders in both people and in animals with differing clinical presentations and neurobiological alterations. These classifications are further defined in other resources. This chapter will focus on

general anxiety and the regulation of anxiety in the central nervous system in relation to the endocannabinoid system. Ongoing research has recognized cannabinoids as a potent modulator of emotions and anxiety and thus warrants further evaluation as a potential therapy in animals (Moreira and Lutz 2008; Tambaro and Bortolato 2012). There has long been evidence that the endocannabinoid system plays an integral role in the regulation of the stress response. This stress response includes the activation of an autonomic response via the sympathetic nervous system, in addition to a neuroendocrine response through the hypothalamic-pituitary-adrenal (HPA) axis. The stress response does act to restore homeostasis. However, chronic triggering of the response and even disease can lead to exaggerated and unbalanced release of hormones, unfavorable behaviors, and decrease in quality of life.

The use of *Cannabis sativa* has been documented since ancient civilization for its medicinal value, relaxation effects, and mood-enhancing properties (Tambaro and Bortolato 2012). Despite its controversial and illicit status during recent decades, it is one of the most frequently utilized drugs for relaxation, euphoria, and tension relief, as described by cannabis users (Arendt et al. 2007; Steward et al. 1997). Further investigation has revealed the interaction of cannabis and the endocannabinoid system to be a complex issue and often paradoxical.

The ability of cannabis to increase or decrease anxiety and emotional reactivity is highly influenced by the types of cannabinoids and terpenes utilized, the dosage, time of administration (prior or during an anxiety triggering event), and the setting in which it is administered (highly stimulatory versus a more neutral setting). Additional components include the individual and species genetic variability, developmental influence, and contextual variables (Bortolato et al. 2010).

7.2.2 Anxiety Physiology of CB1 Receptors and Δ^9-THC

The more obvious psychogenic effects seen with cannabis are often related to the involvement of the endocannabinoid receptor CB_1 and its infamous cannabinoid agonist Δ^9-THC. The CB1 receptors are localized on nerve terminals expressed on both γ-aminobutryic acid-ergic (GABA) and glutamatergic neurons (Wilson and Nicoll 2002). CB1 receptors have been shown to play a critical role in the balancing and regulation of neurochemical substances associated with anxiety (such as serotonin, norepinephrine, dopamine, acetylcholine, and other stress hormones) (Szabo and Schlicker 2001). The activation of the CB1 receptors can exercise a bidirectional influence on anxiety depending on the dose of the agonist (Tambaro and Bortolato 2012). In human clinical studies, low to moderate doses of Δ^9-THC are primarily associated with anxiolytic effects, such as relaxation, euphoria, heightened perception, and creativity. This is in marked contrast to high doses that can result in increased anxiety, paranoia, and fear. This biphasic effect has been confirmed in multiple preclinical rodent studies (Ruehle et al. 2012; Viveros et al. 2005; Tambaro and Bortolato 2012). In both human and animal models the definition of a "low" or "high" dose is not clearly defined and will largely depend on the individual's

sensitivity to cannabinoids or *endocannabinoid system tone.* We must also understand, just like all the other complex diseases and conditions mentioned throughout the text, that we are likely influencing systems within the endocannabinoidome rather than just the CB1 or CB2 receptors.

One explanation that suggests complex multi-signal and pathway crossing is that with lower doses of THC, CB1 receptors influence the neurotransmitter balance to decrease anxiety. However, at higher doses these receptors can stimulate TRPV1 receptors (Rubino et al. 2008). TRPV1 receptors are postsynaptic cation channels and are expressed both in the periphery and within the amygdala, periaqueductal grey, hippocampus, and other areas within the brain (Kauer and Gibson 2009). Activation of these receptors is associated with increased levels of anxiety and may be responsible for some of the biphasic effects noted. Therefore, it is essential in the treatment of anxiety or fear behavior to assess the balance of CB1 and TRPV1 activation through patient dose monitoring (Moreira et al. 2012).

Another difference affecting CB1 receptor activation in studies is the presence of an unconditioned versus conditioned fear response and how that is coded in behavioral studies. In unconditioned fear situations, the receptor activation results in an anxiolytic effect, while in long-term conditioned fear, receptor activation can result in a decreased or increased fear response depending on the cannabinoid agonist and the area of the brain stimulated (Blessing et al. 2015). This differing response can also be dependent on individual genetic and developmental variability. For example, individuals with an endocannabinoid system gene polypmorphism can show an increased fear response and emotional dysregulation more specifically to THC (Dincheva et al. 2015). In addition, chronic stress has also been associated with causing impairment of endocannabinoid signaling within the hippocampus and amygdala resulting in increased anxiety (Neumeister et al. 2014). Thus, CB1 receptor activation can result in both anxiolytic and anxiogenic effects, depending on an individual's ECS tone, genetic variability, and the stimulus presented.

7.2.3 Anxiety Physiology of CBD and Serotonergic System

The endocannabinoid system also has a complex relationship with the serotonergic system that is continuing to be elucidated (Blessing et al. 2015). 5-HT1A receptors are a primary target for anxiolytic therapy. In animal models, activation of this receptor has been shown to decrease general anxiety (Roncon et al. 2013), mitigate chronic stress (Zhou et al. 2014), and increase reduction of fear (Saito et al. 2013). Cannabidiol (CBD) has been shown to have broad therapeutic central anxiolytic activity as a potent antagonist/inverse agonist on CB1 receptors and TRPV1 receptors, while also acting on 5-HT1A receptors (Blessing et al. 2015). CBD has a low affinity for CB1 receptors and may function by augmenting the regulator or baseline status of CB1 receptors and increasing the effects of endogenous endorphins (Blessing et al. 2015). The exact effect on 5-HT1A receptors is unclear but studies have shown CBD can act as a direct agonist to increase the activity of 5-HT1A

receptors in the dorsal periaqueductal gray. This area is vital for regulating emotional reactivity during times of stress (Campos and Guimaraes 2008; de Paula Soares et al. 2010). In addition, CBD has also been shown to reduce amygdala responses to fearful stimuli (Blessing et al. 2015).

Similar to the studies on THC, doses of CBD can affect the response to anxiety. In several preclinical rodent studies various doses were administered, and consistently lower doses were noted to have the most anxiolytic effect. In contrast, higher doses did not exert the same anxiolytic property and were not as effective (Guimaraes et al. 1990; Onaivi et al. 1990). Unlike THC, CBD does not appear to worsen anxiety at the higher doses but instead has no discernable anxiolytic effect. One theory is that CBD may interact with TRPV1 receptors at higher doses, resulting in the loss of anxiolytic effects but not strong enough to cause anxiety (Blessing et al. 2015). Overall the preclinical studies are supportive of the potential of using CBD as a treatment for anxiety. It is important to note that responses of CBD may also be contingent on prior stress, activity in the brain, and species variability.

In human clinical studies, CBD has shown some effectiveness in treating anticipatory anxiety and removing fear memories such as in PTSD. Two studies involving both healthy individuals and those suffering from social anxiety disorder showed a significant decrease in anxiety in regard to both public speaking (Zuardi et al. 1982; Bergamaschi et al. 2011) and a stressful diagnostic procedure (Crippa et al. 2004, 2011).

Other clinical studies have shown that doses ranging from 300 to 600 mg of CBD in an average sized adult person decreased experimentally induced anxiety in healthy controls without affecting baseline anxiety levels (Blessing et al. 2015). Neuroimaging findings also suggest that CBD may reduce anxiety by decreasing amygdala activation and altering medial prefrontal amygdala connectivity, although these studies have been hindered by small sample size and additional investigation is warranted (Blessing et al. 2015). In another human study, published in 2020, Appiah-Kusi et al. found that CBD potentially attenuates the acute stress response in high risk individuals for severe anxiety, positively affecting not only the neuroendocrine response but the psychological perception of stressors.

Due to the entourage effect, there are also variable results in using a combination of cannabinoids for the treatment of anxiety. The combination of CBD with THC has been shown to reduce THC-induced anxiety and improve the sensation of well-being induced by an acute high THC dose in healthy volunteers (Zuardi et al. 1982). Other effective combinations of cannabinoids are also possible but further studies are needed.

7.2.4 Comorbidities and Long-Term Sequelae

At this time there is relatively little information on the role of chronic administration of cannabis for the treatment of anxiety in humans. Long-term administration of cannabinoids has been associated with neuroplastic changes such as downregulation

of CB receptors (Tambaro and Bortolato 2012). It is still unclear if there is a valid causal relationship between cannabis use and long-term anxiety disorders (Gilder et al. 2006; Crippa et al. 2009). Factors that may increase the risk include biological, neurodevelopmental, environmental, and social influences (Crippa et al. 2009).

Extensive investigations are being conducted looking into the role of anxiety leading to cannabis dependence and the development of anxiety due to cannabis use, especially during developmental phases in childhood and pregnancy. Anxiety may also be a symptom of withdrawal in users with dependence (Crippa et al. 2009).

7.2.5 Applications in Animals

According to a recent survey of veterinarians by Kogan, et al, anxiety is one of the most common reasons owners are asking about the use of cannabidiol for their pets. It is also noted as one of the conditions with the most perceived clinical impact by veterinarians, yet there are currently no published clinical studies evaluating this condition and the use of cannabis (Kogan et al. 2019). Therefore, at this time, the role of cannabis for anxiety in companion animals must be extrapolated from the established preclinical and human clinical studies. Based on the regulatory role of emotional well-being by the endocannabinoid system, the use of cannabis clearly holds potential for application in anxiety disorders in animals. Currently there are many unanswered questions as to the appropriate dose and combination of cannabinoids for different anxiety conditions and triggers, as well as in species and individual variability.

Cannabidiol appears to be ideal for use in animal anxiety due to the mild psychogenic properties and that it does not have the potential for directly increasing anxiety. It is important to note that the lower doses in the preclinical studies were more effective for anxiety relief (Guimaraes et al. 1990; Onaivi et al. 1990). This demonstrates the need to start a CBD-based medication at a low dose, have an open discussion with owners that more is not necessarily better, and carefully monitor the response to therapy. In addition, there is little known about the potential for drug interactions of CBD in animals with concurrent administration of other behavior modification medications and their hepatic metabolism (Greb and Puschner 2018). Several of the authors in this text have noted the co-administration of CBD products with certain medications can dramatically decrease the overall doses of typical anxiolytics needed in some animals and eliminate the need altogether in others, particularly for long term anxiety. This is most relevant when it comes to adverse events related to typical pharmaceutical approaches such as heavy sedation, behavioral changes extending past 12–24 h, inappetence, organ damage, and concerns over owner diversion. However, owners with pets on benzodiazepines may want to proceed with more caution. This class of medications, which includes diazepam (Valium®) and alprazolam (Xanax®), may have more profound effects if both the medication and a moderate to large (>2 mg/kg) dose of CBD are used. We recommend veterinarians decrease the dose of the pharmaceutical medication by

half or three-quarters or start with a quarter of the dose of the CBD product and gauge the patient's response.

Despite the evidence of acute anxiety seen with increased consumption of Δ^9-THC, low doses may have value in the direct agonist effects on CB1 receptors. Due to the risk of creating anxiety, this dosing should be made with caution and more intense patient monitoring. Based on the potential interactions, CBD use may also be an effective treatment in patients with THC intoxication and suffering from acute anxiety. Therapeutically, the combination and different ratios of THC/CBD and terpenes are also suggestive for having marked advantages for improving anxiety, but additional investigation needs to be conducted before definitive guidance can be provided. However, many veterinary practitioners are already using hemp CBD products (with <0.3% THC) for chronic anxiety starting at 0.1–0.5 mg/kg, once to twice daily. For more acute forms of anxiety, one may want to try 0.5–3 mg/kg at least 1 h prior to a trigger if possible. A repeat dose may also need to be considered depending on the response of the pet.

While studies using phytocannabinoids in companion animals are still in their infancy, there are now two studies, albeit with multiple limitations and questionable dosing, we can learn from. In 2020, Morris et al. looked at using CBD treats with and without trazodone to reduce noise-induced fear response in dogs. A total of 16 healthy dogs of mixed breeds and sexes weighing ~12–20 kg from a shelter were acquired for this study. The dogs were assessed and void of major behavioral issues, including aggression. The dogs were split into multiple cohorts that received either a placebo, CBD alone at ~1.4 mg/kg/day, ~1.4 mg/kg/day CBD with trazadone (100 mg for dogs 10–20 kg, 200 mg for dogs >20.1 kg) or trazodone alone at the same dosing used with the CBD. The shelter dogs were only given three days of acclimation to the testing room which is a point of concern. The dogs were then subjected to a loud recording of thunderstorms or fireworks. Cortisol levels were measured with no significant changes in the CBD groups. However, a decrease in the trazodone group was noted. Interestingly, CBD appears to have attenuated the effects of trazodone on plasma cortisol levels. The conclusion of this study found inadequate evidence of the anxiolytic effects of CBD on noise-induced fear in dogs. A more recent study using 24 healthy shelter dogs, ages 1 to 10 years old, were used to assess CBD's effect on aggressive behaviors towards people. The dogs were split evenly into a treatment group and a placebo group. The researchers found little benefit in reducing aggressive behaviors after 45 days of treatment. This study had several limitations, most profound being the lack of clarity on the phytocannabinoid, and terpene profile of the product used- presumably an isolate. The second was a curious dosing regimen that was given only once a day and likely inappropriate for these dogs (Corsetti et al. 2021).

The type of fear stimulus and individual variability are other issues that need to be considered with the use of cannabinoids for anxiolytic therapy. As noted in the chapter, responses can vary depending on the fear conditioning. Therefore, additional behavioral modification techniques and pharmaceuticals with continued monitoring would need to be included in an overall plan in which cannabis therapy is instituted. Currently individual variability can be challenging to identify in the

veterinary patient without trial and error but, similar to people, pharmacogenetics may play a role in the future for understanding the differing responses.

In animals, the effects of chronic cannabinoid (>1 year) use are virtually unknown. Most preclinical studies have been evaluated only through 28 days of treatment. The issue of dependence is also unknown but should be considered if a patient has been on a cannabinoid product for an extended period of time. Long term effects will need to be monitored, possibly including serum chemistry profiles similar to pets on long term NSAIDS.

In conclusion, there is strong preclinical evidence for the role of cannabis in treating anxiety in animals. Future studies are needed and will establish the dosing, benefits, and chronic risks to ensure a safe and effective treatment for veterinary patients.

7.3 Feline Idiopathic Cystitis

Feline Idiopathic Cystitis (FIC) is a complex disease that remains a puzzle despite decades of research. It is the most common form of feline lower urinary tract disease and is characterized by clinical signs that occur without an obvious underlying or inciting cause (Grauer 2013). Clinical signs include dysuria, pollakiuria, periuria, stranguria, and hematuria and can cause urinary tract obstruction in male cats (Dorsch 2017). The resulting house soiling and repeated veterinary visits can have a devastating effect on the quality of life for both cat and guardian.

As the term idiopathic implies, the inciting mechanisms of FIC remain elusive, as do reliable preventive measures or treatments. A majority of affected cats will show an improvement of clinical signs within a few days regardless of whether or not they receive therapy (Westropp and Buffington 2010). Recurrent episodes are quite common with up to 50% of cats having a relapse within 1 year while some cats have multiple recurrences, and for a small population of severely affected cats clinical signs become chronic and never resolve (Westropp and Buffington 2010). A variety of treatment options, including diet, nutraceuticals, analgesic and behavioral medications, are proposed for this chronic pain syndrome but responses to therapy vary. Current therapeutic recommendations are aimed at minimizing pain, clinical signs, and recurrent episodes. Acute episodes of FIC are treated with systemic analgesia agents, usually opioids or nonsteroidal anti-inflammatories. Tricyclic antidepressants, such as amitriptyline or clomipramine, are recommended for chronic cases that have not responded to previous therapies. It is important to note that these behavioral medications are not approved for use in felines and carry multiple potential adverse effects and drug interactions (Buffington 2019; Carney et al. 2014). To date, the only therapeutic option that a majority of cats respond to is Multimodal Environmental Modification (MEMO) (Westropp and Buffington 2010). This involves reducing stress while enriching the cat's environment.

The research to date suggests that FIC involves not only complex interactions between the urinary bladder, the nervous system, and the adrenal glands but is also

influenced by the cat's care, environment, and genetics (Forrester and Towell 2015; Westropp et al. 2019). Many cats with FIC often exhibit chronic comorbid conditions involving multiple organ systems and have a history of early or severe stressful events (Buffington 2019). This has led to the concept of 'Pandora Syndrome', implying that FIC appears to be one component of a multisystem disease. Pandora Syndrome is described as an 'anxiopathy', a pathologic condition resulting from chronic activation of the central stress response system (Buffington 2019; Westropp et al. 2019). The fact that multimodal environmental modification appears to be the most effective treatment for cats with FIC supports this theory (Westropp et al. 2019).

Interstitial Cystitis/Painful Bladder Syndrome (IC/PBS), a similar disease in humans, is also a chronic disease of unknown etiology (Kullmann et al. 2018). This chronic condition is diagnosed in seven to eight million patients annually in the United States alone (Wang et al. 2015). Multiple studies have noted the similarities of FIC and IC/PBS in levels of pro-inflammatory cytokines and abnormal protein expression in cell adhesion, barrier function and the GAG layer of the urinary bladder (Hauser et al. 2015; Parys et al. 2018). Due to the close parallels, FIC has served as the animal model for IC/PBS in humans since the early 1990s.

FIC and IC/PBS share several characteristics with other chronic pain syndromes that appear to share a common pathophysiology of endocannabinoid deficiency. The concept of 'Clinical Endocannabinoid Deficiency Syndrome' has been proposed as an explanation for numerous human subjective pain syndromes, such as migraine, fibromyalgia, and irritable bowel syndrome (Russo 2008). These maladies share many common clinical, biochemical and pathophysiology characteristics, as well as the lack of clear preventive or treatment options (Russo 2008). As the endocannabinoid system has been shown to reduce nociception, mediate inflammation, and provide analgesia, much research is now devoted to this messenger system in hopes of finding a novel therapy for cystitis and other chronic pain syndromes.

While a complete discussion of FIC is beyond the scope of this text, a review of the abnormalities that occur in the urinary bladder and the neurohormonal messenger system is necessary to elucidate areas of potential intervention with cannabinoid therapy. There are a growing number of recognized inflammatory changes that occur in FIC. Affected cats have been found to have increased bladder permeability, most likely due to deficiencies in the mucopolysaccharide layer, that lead to hemorrhage and ulceration of the urothelial layer (Schoeman 2018). Once initiated, inflammation increases and results in further bladder permeability. Studies of FIC cats have found an increased number of degranulated mast cells, leukocytes, and increased expression of cyclooxygenase (COX)-1 in the bladder and COX-2 in the urethra (Kullmann et al. 2018). Levels of other inflammatory mediators, including nerve growth factor (NGF), substance P, and a host of prostaglandins, cytokines, chemokines, and potassium ions, have also been shown to be increased in FIC patients compared to healthy cats (Farquhar-Smith et al. 2001). NGF is of particular importance since it can sensitize afferent bladder nerves, leading to increased hyperalgesia and hyperreflexia (Farquhar-Smith et al. 2001). Chronic inflammation appears to cause remodeling of the urinary bladder that is theorized to contribute to alterations in

nerve signalling (Farquhar-Smith et al. 2001). Sensory nerves are also stimulated by irritating protons and potassium ions that are able to seep out of the bladder due to the increased permeability (Grauer 2013).

In addition to serving as a barrier, the urothelium functions as part of a sensory network that includes neuroendocrine cells (paraneurons) (Kullmann et al. 2018). These cells react to chemical and mechanical stimulation by releasing neurotransmitters, such as acetylcholine, adenosine triphosphate, and nitric oxide, and actively communicate with bladder dorsal root ganglion, smooth muscle, and cells of the immune and inflammatory system (Birder et al. 2010). This communication extends to the grey matter astrocytes of the spinal cord (Birder et al. 2010). Studies suggest that this process is significantly augmented in FIC, causing a continuous cycle of increasing inflammation and hyperalgesia (Westropp and Buffington 2010; Ikeda et al. 2009). FIC cats have been found to be deficient in Trefoil Factor 2, which may impair the immune response of the urothelium, leading to greater inflammation (Lemberger et al. 2011).

Other neurohormonal abnormalities have been noted in FIC patients as well that indicate an abnormal stress response with increased sympathetic stimulation but suppressed adrenocortical responses (Schoeman 2018). Significantly higher levels of tyrosine hydroxylase, the rate limiting enzyme of catecholamine synthesis, have been found in the brains of FIC cats (Grauer 2013). This enzyme is associated with prolonged stress and increased sympathetic nervous system activity. FIC patients have also been documented to have increased levels of circulating norepinephrine and dihydroxyphenylalanine (the precursor of dopamine) (Grauer 2013). Large amounts of norepinephrine are thought to further increase urothelium permeability thus increasing nociceptive nerve fiber (C-fiber) activity and further activating local neurogenic bladder inflammatory responses (Grauer 2013).

FIC cats appear also to have increased levels of corticotropin-releasing factor and adrenocorticotropic hormone but instead of corresponding increased levels of cortisol these cats have been found to have low levels (Grauer 2013). This finding suggests a disconnected response to stress between the sympathetic nervous system and the hypothalamus-pituitary-adrenal axis (HPA) (Grauer 2013). Histopathology has noted smaller adrenal glands in FIC cats which, combined with the lower cortisol levels, suggests a primary adrenocortical insufficiency or a diminished adrenocortical reserve (Chew 2012; Westropp et al. 2003). Decreased cortisol levels may also contribute to further urothelium permeability in that cortisol is necessary to maintain cellular junction integrity (Grauer 2013).

Serotonergic signaling appears to be altered in FIC patients as well (Ikeda et al. 2018). Urinary bladder 5-hydroxytryptamine (5-HT) receptors have been shown to change expression in disease states and FIC cats have been shown to have increased bladder contractile responses to serotonin (Ikeda et al. 2018).

7.3.1 Role of Endocannabinoids in Cystitis

Numerous studies indicate that cannabinoid medicine may offer beneficial treatment options for FIC and IC/PBS through multiple pathways. It is well established that one of the many functions of the ECS is to modulate nociception and provide analgesia both peripherally and centrally. There is growing evidence that manipulating the endocannabinoid system can reduce bladder pain by suppressing inflammation through several mechanisms. The endocannabinoid anandamide (AEA) has been shown to inhibit activation of proteins (specifically NFkB) that activate inflammatory cytokines and has also been found in particular to increase apoptosis which inhibits mitogen-induced T- and B-cell lymphocyte proliferations (Bjorling and Wang 2018). The endocannabinoid 2-Arachidonoylglycerol (2-AG) binding to cannabinoid receptor type 1 (CB1) receptors has been shown to suppress MAPK/NFkB signaling by inhibiting COX-2. Multiple studies have shown that activation of cannabinoid receptor type 2 (CB2) receptors suppresses general tissue inflammation (Wang et al. 2013). Both AEA and palmitoylethanolamide (PEA), a putative CB2 receptor agonist, have been found to reduce hyperreflexia induced by urinary bladder inflammation (Jagger et al. 1998).

One avenue of therapy may be inhibiting the enzymes primarily responsible for degrading the endocannabinoids, specifically fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL). One study found that limiting FAAH resulted in increased AEA and suppression of bladder pain in a rodent model (Bjorling and Wang 2018).

Activating cannabinoid receptors in the bladder also shows potential for pain relief. Multiple studies have elucidated the presence of CB1 and CB2 receptors in the urinary bladder of rodents, monkeys, and humans (Merriam et al. 2008). These receptors are found primarily within the urothelium and the afferent and cholinergic nerves within the bladder (Bjorling and Wang 2018). The positive effects of systemic CB1 and CB2 agonists on bladder inflammation (specifically decreased hyperalgesia and increased micturition threshold and voiding interval) have been confirmed with cystometric studies (Walczak and Cervero 2011). Murine studies have shown that CB2 receptors in the bladder detrusor appear to upregulate and increase expression in the presence of both acute and chronic inflammation (Merriam et al. 2008). Both CB1 and CB2 receptors appear to play a role in the pathophysiology of detrusor overactivity associated with bladder outlet obstruction (Kin et al. 2017). Activation of CB1 receptors has been shown to inhibit increased bladder activity induced by NGF (Wang et al. 2015).

A theoretical pathway of intervention may be through the interaction of cannabinoids, specifically cannabidiol, with serotonin receptors. Cannabidiol has been shown to modulate serotonergic transmission, decrease allodynia in neuropathic pain, and normalize 5-HT function (De Gregorio et al. 2019). More research is necessary to assess whether cannabinoids can modulate the abnormal serotonin signaling present in FIC patients.

Another theoretical option for therapy involves the effects of cannabinoids on chronic stress. In addition to the effects of providing analgesia and reducing inflammation, the endocannabinoid system appears to have an extensive association with HPA axis function and stress responses. Studies have suggested that the HPA axis is inhibited by the ECS, specifically by CB1 receptors on glutamatergic neurons (Hill et al. 2010). Disruptions of the ECS result in the activation of the HPA axis. Acute stess appears to increase hypothalamic endocannabinoid signaling through a glucocorticoid mediated pathway. Multiple studies suggest that chronic stress decreases endocannabinoid signaling within the brain (Hill et al. 2010).

Since FIC patients have lower levels of cortisol and abnormal HPA axis responses it can be assumed that FIC patients have decreased hypothalamic endocannabinoid signaling. However, it is unclear whether chronic stress has resulted in endocannabinoid deficiencies or the opposite scenario. More studies will be needed to clearly elucidate the role of the ECS on the HPA axis in FIC patients.

7.3.2 Conclusion

Although FIC remains a frustrating and painful disease, the ongoing studies of the ECS may offer future solutions. It is possible that further research in this area may not only provide novel analgesic therapies with minimal systemic side effects but may also lead to a better understanding of the pathophysiology of FIC. Anecdotal evidence is mounting of FIC patients who have had a reduction of clinical signs when given cannabidiol rich products. While the use of cannabinoids for treating FIC appear to have immense potential, more research and clinical trials in cats are needed.

7.4 Bone Health

As discussed throughout this text, the Endocannabinoid System (ECS) is intricately involved in nearly every part of vertebrate physiology. Bone physiology is no different, with evidence that the endocannabinoids 2-AG and anandamide are also produced endogenously in the bone marrow and within the metabolically active trabecular compartments (Tam et al. 2007; Bab et al. 2008). Other studies show that osteoblasts and osteoclasts are capable of producing anandamide and 2-AG in culture (Whyte et al. 2009). This same group of researchers also found that differentiation of human osteoclasts from monocytes is associated with a reduction in 2-AG levels and an increase in anandamide levels (Whyte et al. 2012). Detectable quantities of 2-AG and anandamide have been reported within cultured human osteoclasts (Rossi et al. 2009). In a human study neither 2-AG or anandamide were detected in synovial fluid from normal subjects, but both endocannabinoids

were detectable in humans with osteoarthritis and in a study using canines (Valastro et al. 2017).

The cannabinoid receptors (CB1 and CB2) and the GPR55 receptor play critical roles in the regulation of osteoclast function and bone resorption. In rodent studies, the CB1 selective antagonist/inverse agonist AM251 (1–3 mg/kg/day) prevents ovariectomy-induced bone loss in wild type mice. Additionally, CB1 deficient mice are resistant to ovariectomy-induced bone loss (Idris and Ralston 2012; Idris et al. 2005).

Models of bone fracture healing using rats found that cannabidiol (CBD), with the potentiating effects of Δ^9-tetrahydrocannabinol (THC), significantly increased the area under the force displacement curve up to the maximum load, meaning CBD leads to improved femur fracture healing. Stiffness was defined as the slope of the linear part of the curve prior to yielding but was not decreased in this study. CBD was found to stimulate mRNA expression of Plod1 in osteoblast cultures with encoding enzymes that catalyze lysine hydroxylation. This in turn incorporated collagen crosslinking and stabilization that can improve biomechanical fracture callus properties (Kogan et al. 2015).

In another study using rats to assess CBD for patients with spinal cord injury (SCI), researchers found CBD given for 14 consecutive days after the spinal cord injury increased serum levels of osteocalcin and reduced serum levels of collagen type-I cross-linked C-telopeptide. This study also showed enhanced bone density in CBD-treated rats. Interestingly, the study found that CBD upregulated mRNA expression of alkaline phosphatases (ALP) (Li et al. 2017).

A similar study found several phytocannabinoids, including cannabinol (CBN), cannabidivarin (CBDV), CBD, THC, and tetrahydrocannabivarin (THCV) stimulate bone nodule formation, collagen production, and ALP activity in cultures of bone marrow stromal cells (Scutt and Williamson 2007). CBD has been found to upregulate the mRNA expression of osteoprotegerin and downregulate expression of receptor activator of NK-kB ligand, along with tartrate-resistant acid phosphatase (Li et al. 2017).

These findings have sparked interest in utilizing the ECS as a target for bone protective therapies utilizing both synthetic and phytocannabinoids. Although currently theoretical, the percentage of animals mentioned in recent companion animal studies with increases in serum ALP could possibly be occurring due to the effects described above, rather than the postulated drug metabolizing enzyme theory via the CYP450 pathway and increased stress on hepatocytes. The studies where ALP was increased did not look at the specific isoform of ALP that was elevated in their study subjects.

7.5 Palliative Care

Palliative care is of vital importance in the field of oncology, perhaps even more so than the cancer treatment itself. Many owners are interested in treating the cancer directly, if possible, but all pet owners want their animal to have the best quality of

life possible. Instituting palliative measures early after the diagnosis can significantly improve outcome, even if only temporarily, and significantly improve quality of life.

Cannabis use for medicinal purposes has been documented for thousands of years. The Chinese Pharmacopeia, Pen Ts'ao Ching, documents medicinal use of cannabis as far back as 2700 B.C. Noted uses include rheumatic pain, constipation, female reproductive disorders, and malaria. Documentation from India, circa 1000 B.C., notes use of cannabis for analgesic, anticonvulsant, hypnotic, tranquilizer, anesthetic, anti-inflammatory, antibiotic, antiparasitic, antispasmodic, appetite stimulant, diuretic, aphrodisiac, and antitussive and expectorant properties (Bouquie et al. 2018). Although no scientific evaluation and documented scientific evidence as we currently understand it was available during these time periods, the use of cannabis was commonplace.

The use of cannabis for palliative care is also commonplace in our current society, both for ourselves and our pets, especially in cancer patients. Anecdotal and scientific evidence suggests that cannabis can assist in alleviating nausea, vomiting, pain, and decreased appetite associated with cancer and chemotherapy in humans. Unfortunately, very little scientific evidence exists that is specific to oncology in veterinary medicine, although many preclinical studies use rodents or other animal models to elucidate potential therapeutic advantages. Until further research is reported, we must extrapolate evidence from human and rodent studies and the small handful of companion animal studies, while exercising caution with our animal patients. This is not to say that cannabis use in veterinary medicine should be avoided, but that its use should be closely monitored and documented as more research is performed, particularly in the palliative care patient.

Assisting clients in choosing a quality product and tailoring a dose specific to their pets' needs is important to maximize efficacy and reduce harm. As with many supplements used throughout the world, there are no regulatory guidelines holding cannabis companies responsible for ensuring that their product contains what it claims. Helping to guide clients to a quality product that has undergone third party, independent laboratory testing and includes quality control measures and documents for all lot/batch numbers is key to providing the highest quality product. By working to assist our clientele in choosing a quality cannabis product we increase our chances of developing a successful palliative care plan.

7.5.1 Pain and Mobility

Pain and mobility issues within a palliative care setting are of huge concern. The ability to reduce pain and maintain or improve mobility of their pet is high on the list of goals for most clients. Cancer-related pain can be a significant problem as well as pain issues not necessarily directly related to cancer (e.g. osteoarthritis, age-related changes). Pain related to the cancer itself can be direct, as in the case of space-occupying masses, or chemotherapy related, which can cause different forms of neuropathic pain. Exploring current evidence regarding cannabis and pain, as well as

what the future may hold, is of paramount importance to the goal of improving the quality of life for our patients. For a more in-depth discussion on cannabis for pain, please refer to Chap. 5.

7.5.2 Tumor-Related Pain

Many tumors can cause discomfort whether due to direct nociceptive stimuli due to physical tumor presence or secondary to the tumor's location. One study examined the analgesic properties of THC-dominant cannabis in rats exposed to various painful stimuli. The study examined four different cannabis compounds: a full-spectrum extract, a THC isolate, a broad-spectrum cannabinoid extract without terpenes, and a terpene isolate oil. Results regarding thermal nociception showed that THC-containing compounds provided substantial analgesic effects, with the terpene isolate oil providing no relief. The THC isolate showed equivalent analgesic effects as the full-spectrum preparations indicating that THC is the main component providing analgesic qualities. The THC-containing compounds showed equivalent analgesic efficacy when compared to the gold standard of pain relief, 18 mg/kg morphine. These same results were noted on the inflammatory assay portion of the study. Had analgesic effects only been noted in one test and not the other, differentiation between spinal and supraspinal pain pathways could have been determined. Based on these results it was discovered that cannabis, specifically THC, can affect the nociceptive pathways by both spinal and supraspinal mechanisms providing analgesic effects in a multimodal way (Rousseau 2018). In contradiction to this study, another found that only THC:CBD compounds provided significant pain relief, while THC alone provided no pain relief compared to the placebo group (Abrams and Guzman 2015).

Other studies exhibit the use of cannabinoids besides THC to treat inflammatory and neuropathic pain with great success. Glycinergic cannabinoids were shown to effectively attenuate pathological pain without causing significant psychoactive side effects or analgesic tolerance, as can be seen with THC-containing compounds and many other analgesic medications (Xiong et al. 2012). Many other cannabinoids have been shown to decrease hyperalgesia in various models and types of pain (Starowicz et al. 2017).

More recent unpublished data, from a study investigating the utility of cannabinoids as an anti-cancer agent, found success in EC50 tests with three different canine cancer cell lines (mammary carcinoma, lymphoma, and osteosarcoma). The study revealed affected cell killing effects. As with many aspects of cannabis and its medical use in small animals, additional studies are needed to further delineate specific therapeutic uses. However, the versatility of using cannabinoids as treatment and symptom management is intriguing.

7.5.3 Neuropathic Pain (Chemotherapy Induced)

Neuropathic pain secondary to chemotherapy administration is a commonly noted side effect in humans. Chemotherapy-induced neuropathic pain (CINP) is seen with vinca alkaloids, platinum agents, and taxanes. CBD alone or in conjunction with THC has demonstrated viable use for treating CINP in various types of chemotherapy in human and rodent models (Masocha 2018).

7.5.3.1 Vinca Alkaloids

The mechanism of vinca alkaloid-induced CINP control is one of the least studied among cannabinoid-based pain control options. Vinca alkaloid administration is known to cause cold and mechanical allodynia in both human and rodent models.

Mechanical allodynia, a painful sensation caused by innocuous stimuli such as light touch, seen with CINP is reported to be most severe 1-week post vincristine administration. This effect returned to pre-infusion levels by the fourth week post treatment in rodent models. Evidence was also found that many standard pain and anti-inflammatory drugs provided little relief of the mechanical allodynia that accompanies vinca alkaloid CINP.

Significant cold allodynia, a painful sensation caused by cold temperature, was seen 2-weeks post-infusion and resolved when vincristine treatment was ceased. Thermal nociception remained stable over the same 5-week period (Lynch 2004).

7.5.3.2 Platinum Agents

Studies using rodent models have shown that CBD alone alleviates mechanical allodynia related to cisplatin administration. Evidence also suggests that THC has been shown to reverse mechanical and cold allodynia and heat hyperalgesia in cisplatin-induced neuropathy (Blanton et al. 2019).

7.5.3.3 Taxanes

Taxanes are rarely used in veterinary medicine in comparison to platinum agents and vinca alkaloids. Evidence does support that CBD has an antinociceptive effect in mechanical and cold allodynia induced by CINP due to paclitaxel administration. A combination of CBD and THC may enhance the antinociceptive effects of THC in cases of CINP. Antinociceptive effects of cannabinoids are not limited to one type of chemotherapy treatment and can be a valuable addition to various chemotherapeutic and palliative pain-management protocols (Blanton et al. 2019).

7.5.4 Gastrointestinal

Chemotherapy-induced gastrointestinal issues such as nausea, vomiting, and diarrhea have long been a target of scientific research as the human medical communities search for a viable treatment option for these side effects.

7.5.5 Nausea

In 1986 dronabinol, a synthetic THC medication, was approved by the FDA for use as an antiemetic for chemotherapy-induced nausea and vomiting. Clinical studies proved the drug to be as, if not more, effective than available antiemetic agents. It is important to note that these studies did not compare synthetic THC medications to smoked marijuana or to today's mainstay antiemetic therapies (e.g. serotonin receptor antagonists such as ondansetron, or the neurokinin-1 antagonist maropitant) (Abrams and Guzman 2015). More recently (2011), researchers using synthetic cannabinoid agonists (Δ^9-THC, HU-210) and the fatty acid amide hydrolase (FAAH) inhibitor URB-597 were successful in suppressing conditioned nausea in rats. CBD also suppresses nausea and vomiting within a limited dose range that will likely vary among species. The anti-nausea/anti-emetic effects of CBD may be mediated by indirect activation of somatodendritic 5-HT1A receptors (Parker et al. 2011). Another study from 2013 showed in rats that THCA suppressed lithium chloride induced conditioned gaping (nausea) (Rock et al. 2013). Finally, in 2015 a meta-analysis was performed looking at the efficacy of cannabinoids for nausea and vomiting in human adults undergoing chemotherapy. The conclusion stated that cannabinoids may be a useful option for treating refractory chemotherapy induced nausea and vomiting (Rock et al. 2013). To date, no studies have been performed to investigate clinical use of specific cannabinoids for antiemetic effect in small companion animals.

7.5.6 Appetite

Multiple human studies have looked at the effect of cannabis on appetite when compared to commercially available appetite stimulants. Some studies showed promising results while others showed no discernable difference between cannabis and the mainstay appetite stimulants. Many animal studies have shown that cannabinoids contribute to increased appetite and food intake. Anandamide, an endocannabinoid, significantly increased food intake in mice. It is important to note that throughout the many human and animal studies performed there is insufficient evidence that appetite stimulation secondary to cannabinoid administration has any effect on cancer cachexia. There is emerging evidence that non-cancer

cannabis consumers have a lower prevalence of obesity and have lower insulin levels, even with appetite stimulation, than non-users (Abrams and Guzman 2015). As with all aspects regarding palliative cancer care and cannabis, more research is necessary to provide concrete evidence in small animals.

7.5.7 Diarrhea

Most studies looking at the effects of cannabinoids regarding gastrointestinal function are related to irritable bowel syndrome and its symptoms. Currently there is insufficient evidence that cannabinoids are an effective treatment option for symptoms related to Inflammatory Bowel Disease (IBD) or diarrhea in general. There is evidence that cannabis can have anti-inflammatory effects in the GI tract, but additional studies are required to further characterize its therapeutic uses (Reichenbach and Schey 2016). Please see Chap. 8 for more information on use for gastrointestinal health.

7.5.8 Conclusion

Cannabis use in a palliative care setting for veterinary cancer patients appears to be a promising treatment modality. Additional studies to examine the effects of cannabis and its constituents on the varied side effects of both cancer and chemotherapy is warranted to further explore the therapeutic benefit.

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Chapter 8
Cannabinoids for Gastrointestinal Health

Micki McCabe and Stephen Cital

8.1 Introduction

Multiple studies have explored the use of cannabinoids in a large array of GI conditions, including non-chemotherapy-induced nausea and vomiting, inappetence, gastric reflux, abdominal pain (visceral hypersensitivity), diarrhea, inflammatory bowel disease (IBD), and irritable bowel syndrome (IBS) (Izzo and Sharkey 2010; Trautmann and Sharkey 2015; Reichenbach and Schey 2016; Esposito et al. 2013). Given the paucity of companion animal studies clinical use has been extrapolated largely from other species.

This is a review of what is known about the GI tract and cannabinoids, both endogenous and exogenous, and the more newly termed *endocannabinoidome*.

8.2 The Endocannabinoid System, Encannabinoidome, and Enteric Nervous System

The body's largest sensory organ is the gut (Hoffman and Lumpkin 2018). Recent identification and elucidation of the endocannabinoid system (ECS) has been revolutionary. We now understand that the ECS overlaps with the enteric nervous system (ENS) which are both extensive anatomically and functionally. Interactions between the two systems appear to be elegant and complicated. The ENS has long been

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considered the 'Second Brain' (Gershon 1998), as it is the only part of the peripheral nervous system that is able to function without input from the central nervous system (CNS) (Hoffman and Lumpkin 2018). The ENS and the ECS exist throughout the entire GI tract, and together are responsible for motility modulation, secretory activity, satiety, maintenance of the epithelial barrier, and gut immunity (Izzo and Sharkey 2010; Trautmann and Sharkey 2015; Reichenbach and Schey 2016; Di Patrizio 2016). The ECS also regulates nausea, vomiting, visceral sensation, intestinal inflammation, and enteric cell proliferation (Izzo and Sharkey 2010; Trautmann and Sharkey 2015; Reichenbach and Schey 2016). Recognition of the existence of the ECS and its importance has led to tremendous interest in the use of exogenous cannabinoids, such as CBD, to effect positive change in a myriad of gut diseases.

To date, the classic ECS is known to be composed of specific cannabinoid receptors (CB1 and CB2), endocannabinoids that bind to these receptors, and endocannabinoid synthesizing and hydrolyzing enzymes.

Endocannabinoids are not stored molecules. They are rapidly synthesized on-demand from the remodeling of membrane lipids. Anandamide is made from arachidonic acid and phosphatidylethanolamine. 2-AG is made from phosphatidic acid and phosphatidylinositol (Izzo and Sharkey 2010; Hasenoehrl et al. 2016). Anandamide and 2-AG are rapidly broken down by their respective intracellular enzymes, fatty acid amide hydrolase (FAAH) for anandamide, and monoacylglycerol lipase (MAGL) for 2-AG (Trautmann and Sharkey 2015; Reichenbach and Schey 2016; Esposito et al. 2013; Hasenoehrl et al. 2016). This rapid production and breakdown of endocannabinoids and similar ligands (binding molecules) allow the ECS to react to physiologic needs to maintain homeostasis in the gut and elsewhere in the body (Hasenoehrl et al. 2016). Endocannabinoids can also be broken down by cyclooxygenase-2 (COX-2) and lipoxygenase (LOX), as well as the cytochrome P450 (CP450) enzymatic pathways (Hasenoehrl et al. 2016).

ECS receptors in the GI tract are integrally associated with the ENS; they are present on neurons, nerve fibers, and at ENS terminals (Izzo and Sharkey 2010; Trautmann and Sharkey 2015; Reichenbach and Schey 2016). CB1 and CB2 are G-coupled protein receptors which transmit signals from outside the cells to their interior to effect a variety of changes (Landa et al. 2016; Purves et al. 2001). Anandamide is a partial agonist of both CB1 and CB2 and 2-AG is a full agonist of both CB1 and CB2. In low concentrations, 2-AG is the primary ligand for CB1 (Di Patrizio 2016; Uranga 2018; Masanobu 2014). CB1 receptors in mice and humans are almost identical while CB2 homology is over 80% between the two species (Reichenbach and Schey 2016).

The ECS has been shown to modulate ENS signaling by *retrograde communication*, with impulses traveling backwards across the synapse to affect neurotransmitter release. The ECS is unique as it is one of the few places in the body where retrograde communication occurs (Trautmann and Sharkey 2015; Hoffman and Lumpkin 2018; Furness 2012). As an example, CB1 activation can block acetylcholine release as well as the release of several other substances. Blocking acetylcholine release inhibits GI motility (Izzo and Sharkey 2010; Trautmann and Sharkey 2015; Reichenbach and Schey 2016; Di Patrizio 2016). Conversely, ATP interacts

with the ECS to stimulate contractility (Di Patrizio 2016). These interactions have been described as 'synaptic plasticity' by Izzo et al. The ECS communicates bidirectionally via the vagus nerve (Trautmann and Sharkey 2015; Reichenbach and Schey 2016; Di Patrizio 2016). During fasting, acetylcholine release via the vagus causes increases of 2-AG by activating muscarinic acetylcholine receptors, thereby promoting motility. In fasting, anandamide and CB1 are upregulated at the vagal efferents, and anandamide binding at CB1 in small intestinal enteroendocrine cells blocks release of the satiety hormone, cholecystokinin (CCK). After feeding, CCK blocks endocannabinoid appetite stimulation, as do CB1 antagonists (Izzo and Sharkey 2010; Trautmann and Sharkey 2015; Reichenbach and Schey 2016; Di Patrizio 2016).

Exogenously, both synthetic and natural CB1 agonists lead to slower gastric emptying and decreased intragastric pressure, pyloric contraction, and intestinal transit (Uranga 2018). Chronic steroid administration (and stress-induced cortisol production) down-regulate CB1 sites, lower anandamide levels, and enhance anandamide breakdown in the limbic system; interestingly steroids can even be potential negative regulators of the ECS. This suggests that steroids can interact with the ECS both positively and negatively (Bowles et al. 2012).

CB1 receptors are widely distributed throughout the GI tract, with regional and species variations and organ-specific actions (Izzo and Sharkey 2010; Trautmann and Sharkey 2015; Reichenbach and Schey 2016; Landa et al. 2016). Both CB1 and CB2 are expressed on B cells, natural killer cells, and mast cells, the predominant surveillance components of the immune system. CB2 receptors are involved in inflammatory processes primarily via immune cell inhibition of pro-inflammatory cytokine production and enhancement of anti-inflammatory cytokine release (Landa et al. 2016). CB2 receptors are present in the esophagus, stomach, and ileum only in times of GI pathology, indicating the ECS, via CB2, provides protection during states of GI inflammation (Izzo and Sharkey 2010; Reichenbach and Schey 2016; Esposito et al. 2013; Di Patrizio 2016).

Research has also identified other G- protein coupled receptors where endocannabinoid binding exists, two of which include G-protein receptors 55 and 119 (GPR55 and GPR119). These receptors bind endocannabinoids as well as other related lipid molecules (Izzo and Sharkey 2010; Trautmann and Sharkey 2015; Landa et al. 2016). Anandamide and its cousin, palmitoylethanolamide (PEA), are natural ligands to GPR55. GPR55 was recently confirmed in the macrophages of canine lamina propria and smooth muscle cells (Galiazzo et al. 2018). They have also been identified in feline tissues-also present in the lamina propria, enteric neurons, and also scattered along the pylorus, small and large intestine (Stanzani et al. 2020).

The exact function of GPR55 in the GI has not yet been well described and is also somewhat controversial. The GPR55 receptor is likely involved in GI motility and may explain many "non-CB receptor" activities of cannabinoids (Izzo and Sharkey 2010; Uranga 2018; Hasenoehrl et al. 2016). It has also been hypothesized that this receptor may even have pro-inflammatory effects, but more work is needed to confirm (Stančić et al. 2015). A physiologic impact the GPR55 receptor more

conclusively has is the regulation of islet physiology which may influence GI hormone secretion via intestinal enteroendocrine cells and well as a role in analgesia (Liu et al. 2016).

GPR119 is expressed on enteroendocrine cells and pancreatic beta cells and is involved in glycemic control via an anti-diabetic peptide named glucagon-like peptide-1. Oleoylethanolamide (OEA, another lipid molecule related to anandamide) binds to GPR119, but amandamide has no affinity for GPR119. Despite similar origins to anandamide, OEA and PEA do not activate CB1 and CB2, so are not technically considered endocannabinoids (Izzo and Sharkey 2010; Hasenoehrl et al. 2016; Sun and Bennett 2007).

Nuclear peroxisome proliferator-activated receptor-α (PPARα) is a receptor that is expressed by enterocytes throughout the small intestine and enteric neurons of the myenteric plexus within blood vessels, smooth muscle cells, glial cells, and the submucosal plexus (Izzo and Sharkey 2010; Hasenoehrl et al. 2016; Galiazzo et al. 2018). It also is present on vagal afferent nerves. PPARα is important for lipid metabolism and glycemic control (Sun and Bennett 2007). OEA binding of PPARα induces satiety. Anandamide and its phytocannabinoid twin, THC, are PPARα agonists (Izzo and Sharkey 2010; Hasenoehrl et al. 2016). PPARα activation may mediate analgesic and anti-inflammatory effects of cannabinoid treatment (Hasenoehrl et al. 2016).

Transient receptor potential vanilloid 1 (TRPV1) is the receptor for chili pepper or capsaicin (Izzo and Sharkey 2010; Landa et al. 2016). TRPV1 agonists include anandamide and OEA. The receptor is prevalent throughout the GI tract as well as on some immune cells. Interestingly, activation of TRPV1 in the colon is anti-inflammatory but leads to neurogenic inflammation in the ileum, possibly from Substance P release. This local inflammation may be a contributor to chronic pain experienced by IBD patients (Izzo and Sharkey 2010).

Interactions between anandamide and its receptors, CB2 and TRPV1, help regulate and maintain the health and immunity of the gut. These interactions promote immunosuppressive macrophages (CX3CR1) to allow for tolerance of certain foreign proteins and confirm an important link between the immune and nervous systems of the gut and maintenance of gut tolerance (Acharya et al. 2017). Natural killer (NK) cells are an example of the body's elegant response to potential cancerous seeding cells and the body's natural defenses via interaction with the intricate GI microbiome. Indeed, dysbiosis may be secondary to disruption of the healthy endocannabinoidome given that both pre- and probiotic activity are affected by ligands and receptors in the endocannabinoidome. Recent evidence shows that some bacteria of the normal gut flora produce compounds that act like endocannabinoids (Di Marzo and Silvestri 2019). A recent study by Al-Ghezi et al. (2019) suggests that cannabinoids can help prevent dysbiosis and promote healthy gut microflora. Benefits were seen in both intra- as well as extra-gastrointestinal diseases, including muscular sclerosis and experimental autoimmune encephalitis (Uranga 2018; Landa et al. 2016; Hasenoehrl et al. 2016).

Another transient receptor potential receptor type (TRPA1), more recently described in feline gut tissues, is also highly expressed in human and rat

enterochromaffin (EC) cells. TRPA1 agonists cause calcium influxes and 5-HT release in EC cells. Contraction of parts of the intestine via the 5-HT3 receptor has also been described with TRPA1 agonists. This has suggested that TRPA1 acts as a sensor molecule critical for the regulation of gastrointestinal motility functions (Stanzani et al. 2020; Nozawa et al. 2009).

The term *endocannabinoidome* has been coined to describe an expanded definition of the ECS, now encompassing other ligands and receptors and their complex interactions with the ECS as well as with receptors outside the ECS. The endocannabinoidome's overall homeostatic responsibilities encompasses glucose metabolism and regulates weight gain, gut immunity, circulating lipids, and blood pressure. It is modulated by vitamin D levels, diet, exercise, GI flora, and many medications (Di Marzo and Silvestri 2019). A handful of these receptors have now been mapped in the canine and feline GI tract which may help clarify anatomically and physiologically how exogenous cannabinoids can be used therapeutically in enteropathies (Galiazzo et al. 2018).

8.3 Appetite Modulation

The ECS and ENS regulate both energy balance and appetite (Izzo and Sharkey 2010; Di Patrizio 2016). Interaction between the gut and the brain to maintain satiety or stimulate appetite are modulated in part by the vagus nerve, as discussed above (Izzo and Sharkey 2010; Hoffman and Lumpkin 2018). Ghrelin, the orexigenic (appetite stimulating) peptide mimicked by capromorelin (Entyce®, Elanco), has receptors on vagal afferents, and activation of these receptors blocks cholecystokinin (CCK) down-regulation of CB1 (Izzo and Sharkey 2010; Lim et al. 2013). In mice, it was determined that an intact ghrelin signaling pathway is necessary for cannabinoids to act on the hypothalamus and vice versa (Lim et al. 2013).

In a study using rats genetically prone to obesity (Zucker rats), basal levels of anandamide were found to be twofold , and 2-AG levels were measured at nine times the normal basal level (Izzo and Sharkey 2010). Rimonabant, a CB1 blocker, was briefly commercially available in Europe as a dieting aid to help suppress hunger in obese patients, but it was quickly removed from the market in 2008 when it was noted to cause depression in a large number of patients, despite its effectiveness in producing satiety and weight loss (Moreira and Crippa 2009).

Not surprisingly, THC stimulates hunger with a preference toward high fat foods by binding to CB1 receptors in the brain, increasing ghrelin release, and leading to the release of dopamine. CBD, in contrast, does not stimulate hunger, per se, but can help resolve nausea, abdominal pain, and anxiety, which can indirectly support a healthy appetite (Parker et al. 2011).

8.4 Nausea and Vomiting

Humans more easily express the difference between nausea and vomiting than animals do. Cannabinoid action at CB1, CB2 receptors, and TRPV1 have been shown to have both anti-nausea and anti-emetic effects (Izzo and Sharkey 2010; Uranga 2018). In people, cannabinoids may resolve nausea better than 5HT3 antagonists like ondansetron (Zofran, Novartis). Cannabinoids may also work synergistically with HT3 antagonists to decrease nausea and vomiting (Izzo and Sharkey 2010; Reichenbach and Schey 2016). In humans, the anti-nausea effects of dexamethasone and aprepitant, an NK1 antagonist and relative of maropitant (Cerenia®, Zoetis), are also surpassed by cannabinoid administration (Izzo and Sharkey 2010; Uranga 2018). Studies have confirmed that CBD was effective in rats and house musk shrews against anticipatory nausea (Uranga 2018). In one study looking at cannabidiolic acid (CBDA) in conjunction with low dose ondansetron, the combination was found to be superior to administration of higher doses of either CBDA or ondansetron alone for chemotherapy-induced vomiting in experimental rat models. In the same study, CBDA also showed inhibition of breast cancer cell migration (Rock et al. 2013).

Cannabinoids can also act centrally to reduce nausea and vomiting. CB1, TRPV1 receptors, and to a lesser degree CB2, are also located in the brainstem and inhibit vomiting at the chemoreceptor trigger zone, where the dopamine antagonist metoclopramide also acts (Izzo and Sharkey 2010; Hasenoehrl et al. 2016).

8.5 Visceral Pain/Hypersensitivity

The CB2 receptor has a suspected role in decreasing visceral hypersensitivity, and Dronabinol® has been shown to relax the colon and decrease colonic motility after meals (Reichenbach et al. 2015). In human patients with post-infectious IBS pain, 5HT levels were increased and anandamide was decreased in duodenal biopsies; lower anandamide levels were associated with increased pain severity (Feng et al. 2014). The microbiome appears to also interact with the ECS. In rodent studies, *Lactobacillus acidophilus* induces CB2 expression as well as *mu*-opioid receptors, allowing for improvement in pain scores without central side effects (Reichenbach and Schey 2016; Kikuchi et al. 2008). Some cannabinoids have been shown to decrease lower esophageal sphincter relaxation, slow gastric emptying and gastric acid production, decrease bowel motility and secretion, decrease visceral pain, and possibly decrease inflammation. Please see Chap. 5 for more on pain management (Quezada and Cross 2019).

8.6 Esophageal Reflux

In rodent stomachs, CB1 stimulation of vagal efferent pathways blocks gastric acid production separate from H2 blockade. Conversely, humans have CB1 on parietal cells, where cannabinoids may have direct action similar to H2 blockers (Uranga 2018). Cannabinoid agonists also inhibited gastric acid secretion with significant improvement in gastro-esophageal reflux (GERD) and lower incidence of gastric ulcer formation secondary to NSAIDs and stress in several studies (Reichenbach and Schey 2016; Uranga 2018; Pertwee 2001). GERD models using guinea pigs showed that CB2 upregulation decreased hydrochloric acid damage without CB1 involvement (Reichenbach and Schey 2016). In ferrets, transient lower esophageal sphincter relaxations (TLESR) were diminished in one study, suspected to be due to CB1 activation in the brain (Uranga 2018). CB1 activation inhibits lower esophageal sphincter relaxation as a vasovagal reflex in dogs and ferrets, which may make exogenous phytocannabinoids beneficial for GERD (Reichenbach and Schey 2016).

8.7 Diarrhea/Peristalsis

As stated previously, acetylcholine is an excitatory vagal neurotransmitter that stimulates GI motility and has been shown in numerous animal and human models to be down-regulated via activation of the CB1 by THC and other cannabinoids (Izzo and Sharkey 2010; Di Patrizio 2016). Also, agents that block FAAH are potent modulators of GI motility and inflammation by maintaining anandamide levels. Those that inactivate MAGL, thereby preserving 2-AG presence and activity, inhibit gut motility (Izzo and Sharkey 2010). CB1 appears to regulate normal modulation of motility, while CB2 appears important in inflammatory conditions. Currently there is substantial research into the ability of phytocannabinoids to medically slow GI transit time in human patients with diarrhea (Trautmann and Sharkey 2015; Reichenbach and Schey 2016; Furness 2012; Di Patrizio 2016).

8.8 Inflammatory Bowel Disease and Irritable Bowel Syndrome

IBD in humans and animals is a considered a chronic idiopathic, multifactorial disease (Cerquetella et al. 2010). In humans, IBD is defined as ulcerative colitis (UC) and Crohn's disease (CD), specifically (Izzo and Sharkey 2010; Trautmann and Sharkey 2015; Reichenbach and Schey 2016). IBD criteria for diagnosis with UC includes abdominal pain and bloody diarrhea with eventual blood loss anemia (Izzo and Sharkey 2010; Trautmann and Sharkey 2015; Reichenbach and Schey 2016). Crohn's disease criteria include chronic weight loss and diarrhea that is often

associated with granuloma formation and fibrosis. CD is considered a 'mouth-to-anus' disease in people. Both UC and CD can be associated with systemic inflammatory arthritis signs (Izzo and Sharkey 2010; Trautmann and Sharkey 2015; Reichenbach and Schey 2016). IBD is suspected to affect 1–2% of the human population, and activation of cannabinoid receptors by both anandamide and 2-AG have protective effects against this inflammatory process (Izzo and Sharkey 2010; Trautmann and Sharkey 2015; Reichenbach and Schey 2016; Uranga 2018). Histologically, increased CB2 expression and increased endocannabinoid levels were noted in IBD patients with UC and CD, as well as diverticulitis and celiac disease (Izzo and Sharkey 2010). Cannabinoids are known to promote epithelial wound healing via CB1 in humans. Interleukin-8 (IL-8), an inflammatory cytokine often used as a marker of GI inflammation, is inhibited by CB2 activation (Izzo and Sharkey 2010).

In veterinary patients, IBD encompasses a broader definition of chronic GI signs that involves infiltration of inflammatory cells within the stomach, small, and/or large intestine, therefore it may also be considered a 'mouth-to-anus' syndrome (Landa et al. 2016). The etiology is often unclear, but is generally defined by the cell type and location involved, e.g., lymphoplasmacytic gastroenteritis or eosinophilic lymphoplasmacytic duodenitis, etc. (Cerquetella et al. 2010). Diagnosis is made based on histologic changes and ruling out other potential causes of inflammation, including food allergy, parasites, etc. Human and veterinary IBD patients are frequently managed with diet change, immunosuppression, appetite support, antidiarrheal agents, and anti-nausea medications (Cerquetella et al. 2010).

As a third comparison, IBS criteria in humans involves several subsets of patients with a multitude of specific clinical signs including pain, diarrhea, constipation, nausea, and inappetence. Defining characteristics of IBS include periodic abdominal pain for at least 3 days per month that improves with defecation, and episodic changes in stool frequency or consistency (Reichenbach and Schey 2016). IBS affects an estimated 10–20% of adult humans throughout the world. Four subtypes of IBS exist that may overlap with veterinary patients: IBS-D (diarrhea), IBS-C (constipation), IBS-M (mixed), and IBS-U (unclassified) (Reichenbach and Schey 2016). While IBS has not historically been associated with specific histopathologic or biochemical changes, IBS patients have recently been shown to have low grade GI inflammation, changes in the mucosal barrier, and bacterial overgrowth (Reichenbach and Schey 2016; Uranga 2018; Hasenoehrl et al. 2016). New research suggests possible genetic causes as well as low endocannabinoid levels as contributors to the development of IBS (Esposito et al. 2013). CB1 along the gut-brain axis may become a promising target for therapeutic use of exogenous cannabinoids for IBS (Hasenoehrl et al. 2016).

In several recent studies, less common phytocannabinoids have shown promising therapeutic benefits for GI disease. In one study, cannabigerol (CBG), a non-intoxicating phytocannabinoid, decreased inflammation in experimental murine models of ulcerative colitis and Crohn's disease via several models (cytokine modulation, down-regulation of NO production, and as an antioxidant) and may show promise in clinical inflammatory bowel disease (Romano et al. 2013).

In another study, cannabichromene (CBC) is another non-intoxicating cannabinoid that has shown significant protective effects on murine experimental colitis by lowering nitric oxide (NO) production and thus decreasing tissue destruction via anti-inflammatory action on activated macrophages. CBC also had curative effects in experimental murine colitis at doses more than 100 times lower than its LD50. CBC improved (slowed) transit times in experimental intestinal inflammation. CBC action appears to be at TRPA1, a receptor related to TRPV1, rather than via direct action at CB receptors. Interestingly, activated CB1 modulates CBC activity. CBC selectively inhibits intestinal motility during experimentally induced intestinal inflammation but does not affect motility in healthy control animals. The mechanism of action has not been elucidated (Romano et al. 2013). Intrarectal or intraperitoneal administration of CBD, which bypasses hepatic metabolism, also improved murine colitis and induced regeneration of diseased tissue of the colon (Borrelli et al. 2013).

8.9 Gastric and Intestinal Cancer Research

Chronic inflammation has been identified as a trigger for the development of human colon cancer (Uranga 2018). CB1 are down-regulated and CB2 are up-regulated. In human colorectal cancer, cannabinoids appear to have antiproliferative, antimetastatic, and pro-apoptotic activity via both CB1 and, likely, CB2 (Izzo and Sharkey 2010). Cannabinoids may also inhibit tumor migration via CB1, which was noted in human colon carcinoma cell lines in vitro. In gastric cancer cell lines, anandamide was synergistic with the chemotherapeutic agent paclitaxel, causing apoptosis. In multiple colorectal cancer cell lines, both CB1 and CB2 activation had apoptotic effects (Izzo and Sharkey 2010).

8.10 Acute Pancreatitis

There have been minimal changes in the treatment or management of pancreatitis for people or animals noted in medical literature over the past 50 years despite its high morbidity and mortality. The disease varies in severity of clinical signs, from local pancreatic edema to severe pancreatic necrosis and subsequent sepsis or multiple organ failure (Li et al. 2013). A recent study identified protective activity of CBD as a GPR55 antagonist (Li et al. 2013). CBD has also been shown to antagonize CB1 agonists, inhibit FAAH and activate PPARα, which are all involved in inflammatory modulation of experimentally induced acute pancreatitis in the mouse model. Additionally, CBD demonstrated the ability to lower lipase, amylase, IL6, and TNFα levels (Li et al. 2013). In a study using rats with experimentally induced acute pancreatitis, subjects had improved outcomes when treated with CB1 and CB2 agonists (Cao et al. 2012). Conversely, in a small number of human patients, acute

pancreatitis has been documented following chronic THC use. The etiology is unknown to date (Nunez Herrero et al. 2016).

8.11 Conclusion

As more research emerges, we will have much more information at our fingertips for condition specific dosing and best practices for recommending cannabinoid profiles for our patients. CBD's preferential affinity on cannabinoid-related receptors, such as GPR55 (antagonist), TRPA1 (agonist), TRPV1 (agonist), and serotoninergic receptors 5-HT1a (agonist), 5-HT2a (partial agonist), and 5-HT3 (antagonist) leaves little question as to the therapeutic potential in veterinary species.

Another important factor is cytochrome P450 enzymes. These enzymes are present in the endoplasmic reticulum or mitochondrial membrane-bound enzymes in the liver and intestine and are tasked with breaking down steroids, cholesterol, vitamin D, bile acids, and eicosanoids. Endocannabinoids are also broken down by CP450 enzymes, so cannabinoids may present concerns over interactions with other substances degraded by CP450, although clinical experience and human meta-analysis studies have found little clinical concern. Doses of CBD necessary for significant perturbation of CYP450 enzymes appear to be well over the therapeutic doses used medicinally (>30 mg/kg) (Zendulka et al. 2016).

In humans, cannabis adverse effects include a cannabinoid hyperemesis syndrome that can occur in some individuals with using marijuana chronically. The syndrome is paradoxically responsive to hot showers but recurs with resumption of cannabis use. This may be due to a dysregulation of cannabinoid receptors that appears to be permanent in these individuals (Izzo and Sharkey 2010; Uranga 2018). This has yet to be described in veterinary species.

When extrapolating research data to clinical use it is important to recognize species differences in cannabinoid activity and receptor locations throughout the gut: rats and mice have CB1 receptors in smooth muscle of the stomach, guinea pigs and humans in the ileum, and humans in the colon, as a few examples (Morales et al. 2017; Almeida and Devi 2020; Laun et al. 2018).

Ongoing research includes the use of cannabinoids to reduce opioid-induced constipation and gastroparesis, lowering NSAID doses to help reduce toxicity, possible decrease in immunosuppressive doses of steroids, and improvement in antiemetic therapy when combined with ondansetron or other antiemetic agents (Reichenbach and Schey 2016; Alhouayek and Muccioli 2014). Therapies directed at slowing endocannabinoid degradation may also be of benefit in treating GI disease. In canines and felines the identification of ECS and endocannabinoidome related receptors expressed in multiple layers of the GI tract with functional evidences of these receptors leaves little question as to the therapeutic benefits of exogenous cannabinoids for gastrointestinal related diseases, most notably inflammatory disease. As mentioned, dosing for specific conditions is yet to be published. A common strategy already being used is starting low (~0.5 mg/kg of a CBD

dominant product) and increasing the dose over time until the therapeutic effect is achieved. This appears to be a safe and effective method of utilizing phytocannabinoid rich products in veterinary patients.

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Chapter 9
Dermatology: Endocannabinoids and Related N-Acylethanolamines in the Skin

Vincenzo Miragliotta and Chiara Noli

9.1 Introduction

The endocannabinoid system—or the broader endocannabinoidome—is an emerging key player in skin homeostasis to the extent that it has recently been termed "c(ut)annabinoid system". Receptors, mediators, and regulatory molecules are produced/expressed by most skin cellular elements and a plethora of intricate mechanisms are increasingly being acknowledged to explain the endocannabinoidome role in skin homeostasis. Among skin cells, mast cell function is regulated by N-acylethanolamines, among which palmitoylethanolamide (PEA) is the most studied molecule. The purpose of this chapter is to review the body of evidence on the endocannabinoidome's role in skin physiology and pathology, with particular reference to canine and feline species. The detection of mediators and receptors in the skin will be reviewed. The main dermatological preclinical and clinical data from studies involving animals treated with endocannabinoids and related N-acylethanolamines will also be presented since there is a paucity of clinical evidence utilizing exogenous cannabinoids thus far. Skin allergy represents a promising target for endocannabinoid treatment in the field of veterinary dermatology.

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9.2 The Skin Endocannabinoidome

The skin is the largest mammalian organ and the primary interface between the body and the environment. It is composed of epidermis and dermis. Epidermis, of ectodermal origin, is mainly composed by keratinocytes, but also melanocytes, Langerhans cells and Merkel cells; keratinocytes are not a single cell population since from the basal proliferative compartment they differentiate in a spinous layer, a granular layer, and a cornified or "horny" layer mainly implicated in the "epidermal barrier" formation. Dermis, of mesodermal origin, houses a plethora of cell populations including fibroblasts that make collagen and other extracellular matrix molecules that provide skin mechanical toughness. Adipocytes, macrophages, mast cells, plasma cells, and different types of T cells are further key elements of the dermal compartment (Jatana and Delouise 2014). The dermis also houses the pilosebaceous units, sweat glands, nerves, blood and lymphatic vessels. The hair follicle itself (as a part of the pilosebaceous unit) develops from a surprisingly intricate set of interactions involving ectodermal, mesodermal, and neuroectodermal components, which further elaborate concentric cycling structures (Welle and Wiener 2016). This complexity provides the first line of defense against invading pathogens and trauma (outside-in barrier), through physical barrier properties and cellular protective mechanisms orchestrated by bidirectional interactions between epithelial and immune cells. In addition, skin regulates body temperature and prevents excessive loss of water and electrolytes (inside-out barrier). Also, skin is a major sensory surface and senses changes in the environment through cutaneous nerve endings (nociceptors), which translate the received information into chemical, physical, and biological messengers which then help regulate global and local homeostasis through the neuroimmune system. Lastly, an exocrine (sweat and sebum), an endocrine (synthesis of a wide-array of hormones, e.g. vitamin D, steroids, and peptide hormones), and a regenerative (wound healing) function can be attributed to the skin (Veiga-Fernandes and Mucida 2016; Biro et al. 2009). The above-mentioned functions are possible through fine-tuned cell-to-cell interactions that are under the tight control of several signaling systems, among which the most remarkable is the recently discovered cutaneous cannabinoid ["c(ut)annabinoid"] system (Toth et al. 2019).

Although detailed in other chapters, it is worth remembering that the endocannabinoid system is classically comprised of two primary endogenous ligands arachidonoylethanolamide (AEA, also referred to as anandamide) and 2-arachidonoyl glycerol (2-AG), the two receptors CB1 and CB2 and a group of enzymes and transport proteins. Since Maione et al. (2013) first introduced the term "endocannabinoidome", it has been broadly used to refer to the biochemical and pharmacologic complexity of endocannabinoids and endocannabinoid-like mediators (Maione et al. 2013; Di Marzo and Wang 2015). The current view of the endocannabinoid system includes the classical mediators (AEA and 2-AG) as well as different non-endocannabinoid N-acylethanolamines (NAEs) and monoacylglycerols (2-MAGs), related biosynthetic and degradative enzymes,

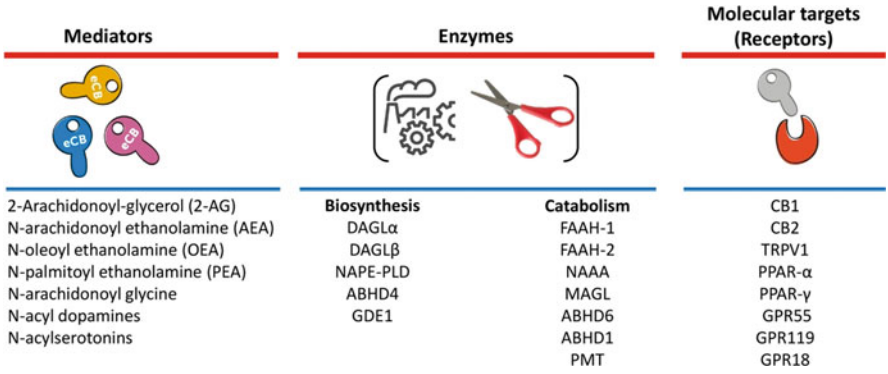

Fig. 9.1 Figure depicting a common endocannabinoid (2-AG), with non-endocannabinoid N-acylethanolamines (NAEs) and monoacylglycerols (2-MAGs), related enzymes, transporters, and known receptors. ABHD, α,β-hydrolase; DAGL, diacylglycerol lipase; FAAH, fatty acid amide hydrolase; GDE1, glycer-ophosphodiesterase-1; GPR, orphan G protein-coupled receptor; MAGL, monoacylglycerol lipase; NAAA, N-acylethanolamine-hydrolyzing acid amidase; NAPE-PLD, N-acyl-phosphatidylethanolamine-specific phospholipase D; PMT, putative membrane transporter; PPAR, peroxisome proliferator-activated receptor; TRPM8, transient receptor potential melastatin type-8 cation channel; TRPV1, transient receptor potential vanilloid type-1 cation channel

cellular transporters and an ever increasing family of receptors, i.e. the classical receptors (CB1 and CB2) as well as members of the transient receptor potential cation channels (TRP channels), isoforms of the nuclear peroxisome proliferator-activated receptor (PPARs) and members of the G-protein coupled receptor (GPCR/GPR) superfamily (Fig. 9.1).

The skin cannabinoid system is functional and has been verified in several species. Notably, a recent study demonstrated that human patients abusing synthetic cannabinoids experienced dramatic dermatological changes such as premature skin aging, hair loss and pigmentation changes (Inci et al. 2017). In the last decade, several reviews have focused on the role of cannabinoids in dermatology and the skin endocannabinoidome has emerged as an important player that critically influences skin general homeostasis (Pucci et al. 2011; Biro et al. 2009), epidermal permeability (Roelandt et al. 2012), hair growth (Szabo et al. 2019; Telek et al. 2007), inflammation (Olah and Biro 2017), wound healing (Wang et al. 2016), itch (Caterina and Pang 2016), pain (Caterina 2014), and even skin tumours (Milando and Friedman 2019).

9.3 The C(ut)annabinoid System in Companion Animals

9.3.1 Mediators: Endocannabinoids and Related N-Acylethanolamines

The most extensively studied canonical endocannabinoids are AEA and 2-AG, the latter being isolated first in canine tissues (Mechoulam et al. 1995). Despite both endocannabinoids being derived from arachidonic acid, the first is an amide while the second is an ester of this long-chain fatty acid (Bisogno 2008). Chemically speaking, AEA belongs to the NAEs family of endogenous lipids, which also includes, among others, N-palmitoylethanolamine (PEA) and N-oleoylethanolamine (OEA). The latter two compounds were originally referred to as "endocannabinoid-like" mediators given their lack of affinity for the classical G protein-coupled transmembrane cannabinoid receptors CB1 and CB2 (Pertwee et al. 2010; Petrosino and Di Marzo 2017).

The first detection of NAEs in the skin was first described when a British dermatologist identified phosphatidyl-(N-acyl)-ethanolamine in granular cells and stratum corneum of the porcine skin (Gray 1976). These mediators were then identified in isolated epidermal cells and found to greatly increase in response to UVB irradiation; it was thus speculated that skin responds to injury through increased cellular production of NAEs and this could be part of an intracellular signaling system reactive to stress (Berdyshev et al. 2000). This was later confirmed in vivo, when stress-induced increase of PEA levels was found in rodent skin (Darmani et al. 2005). The protective role of PEA was further substantiated by Petrosino and colleagues who demonstrated that PEA levels were increased in two models of contact allergic dermatitis (in vitro and in vivo) and PEA administration decreased 2,4-dinitrofluorobenzene-induced ear inflammation in mice (Petrosino et al. 2010). The same line of evidence was provided in canine skin where AEA, OEA, PEA, and 2-AG were found constitutively present and significantly elevated in lesional atopic skin compared to non-lesional skin of privately-owned dogs: PEA levels showed the highest increase (more than 30-fold) while OEA, 2-AG and AEA were 30-, 14- and 6-fold higher respectively (Abramo et al. 2014).

In summary, the skin levels of NAEs, and among them mostly PEA, were found to be increased in different experimental and naturally occurring dermatological conditions.

For this reason, it was proposed that the c(ut)annabinoid system exerts a protective role against skin inflammation, itch and pain (Re et al. 2007). A schematic diagram depicting such protective hypotheses is illustrated in Fig. 9.2.

Today, it is generally acknowledged that skin is an important site of endocannabinoid production and research in the veterinary field is strongly warranted; AEA, 2-AG and PEA were detected in human, canine and rodent skin (Abramo et al. 2014; Beaulieu et al. 2000; Calignano et al. 1998; Guindon et al. 2006; Karsak et al. 2007; Petrosino et al. 2010). In particular, endocannabinoids and related NAEs are produced and released by skin cell populations such as

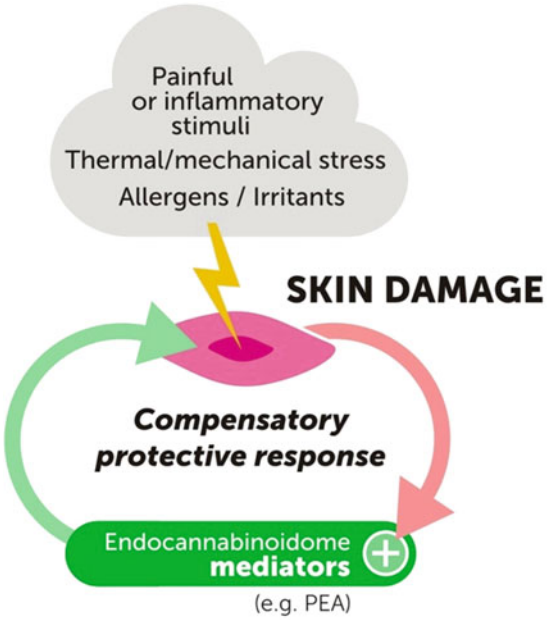

Fig. 9.2 The hypothesized protective mechanism of action of the endocannabinoid system for skin

keratinocytes, sebocytes, melanocytes, sweat gland epithelial cells, and macrophages, under basal and stimulated conditions (Oddi and Maccarrone 2016). The whole biochemical machinery to metabolize endocannabinoids and related compounds has been found in human keratinocytes (Maccarrone et al. 2003) and AEA plays a role in keratinocyte differentiation being able to modulate cytokeratin gene expression (Paradisi et al. 2008).

Receptors: Classical, Ionotropic, Nuclear, Novel As mentioned above, the current concept of the endocannabinoidome includes several players, both as mediators and particularly receptors. Beside the "classical" CB1 and CB2 receptors, the "ionotropic" receptors of the TRP channels superfamily, the "nuclear" receptors PPARs, and the "novel" orphan receptors of the GPCR superfamily are included; their identification in domestic animal skin is a further key step toward the so called "c(ut)annabinoid" system (Toth et al. 2019). Both CB1 and CB2 receptors were shown on several human and murine skin cell populations, e.g., cutaneous nerve fibers, mast cells, keratinocytes, and adnexal tissues (Dobrosi et al. 2008; Karsak et al. 2007; Maccarrone et al. 2003; Stander et al. 2005; Casanova et al. 2003). TRPV1 was also found on several human skin cells, like keratinocytes, mast cells, and epithelial cells of appendage structures (Denda et al. 2001; Stander et al. 2004). Different PPAR isoforms were detected in various cell population of human and murine skin as well. PPAR-α is down-regulated in atopic dermatitis and some skin cancers in humans (Sertznig et al. 2008). In a murine wound model PPAR was described to play a role in re-epithelialization (Michalik et al. 2001). GPRs were shown in humans to be expressed by melanocytes and to be involved in

Table 9.1 Expression and localization of endocannabinoidome molecular targets in the skin of dogs and cats

Species	Receptor	Cell type	Reference
Dog	CB1	Keratinocytes embryo, Keratinocytes, Hair follicles, Sebaceous glands, Sweat glands, Mast cells, Fibroblasts	Campora et al. (2012), Mercati et al. (2012), Pirone et al. (2015)
	CB2	Keratinocytes, Hair follicles, Sweat glands, Sebaceous glands, Mast cells, Fibroblasts, Endothelial cells	Campora et al. (2012)
	TRPV1	Keratinocytes	Barbero et al. (2018)
Cat	CB1	Keratinocytes, Sebocytes, Hair bulb cells	Miragliotta et al. (2018)
	CB2	Keratinocytes, Sebocytes, Hair bulb cells, Sweat glands	
	PPAR-α	Basal keratinocytes, Hair follicle, Dermal papillae	
Horse	PPAR-α	Basal keratinocytes, Endothelial, Perivascular cells	Jorgensen et al. (2018)

pigmentation disorders as well as carcinogenesis (Toth et al. 2019); moreover, GPR55 has been found in human fibroblasts (Gegotek et al. 2017) and GPR119 receptors have been identified on human sebocytes (Dobrosi et al. 2008).

Moving to the veterinary side, CB1 and CB2 were found in the skin of both dogs and cats (Campora et al. 2012; Miragliotta et al. 2018; Mercati et al. 2012). A preferential epithelial expression (including epidermis) of CB1 was described in the developing canine embryo (Pirone et al. 2015). A functional TRPV1 receptor was described in primary culture of canine keratinocytes (Barbero et al. 2018) while PPARα immunolocalization was described both in feline and equine skin (Jorgensen et al. 2018; Miragliotta et al. 2018). The cellular localization of endocannabinoid receptors in domestic animals is summarized in Table 9.1.

Although scarce, data obtained in domestic animals may lead to the conclusion that different cellular elements of the canine, feline, and equine skin, both at epidermal and dermal level, are able to "sense" endocannabinoid mediators (and related compounds) and react accordingly once the ligand has bound to its molecular target. It is thus possible to conclude that a functional c(ut)annabinoid system exists in animals and may represent a therapeutic area in the veterinary field. A simplified overview of the skin endocannabinoidome in domestic animals is depicted in Fig. 9.3.

N-Acylethanolamines (NAEs) and ALIAmides: The Role of Mast Cells To the abovementioned NAEs belongs a set of fatty acid amides such as PEA, OEA, and stearoylethanolamide (SEA) that are particularly present in animal tissues (Hansen and Diep 2009) and are also classified as "Autacoid Local Injury Antagonist amides" (ALIAmides) (Chiurchiu et al. 2018). PEA is the most prominent among ALIAmides; its anti-inflammatory and immune-modulating properties were identified following its isolation in 1957 from soy lecithin, peanut meal, and egg yolk. (Kuehl et al. 1957). More than 30 years later, it was the seminal work of the Nobel

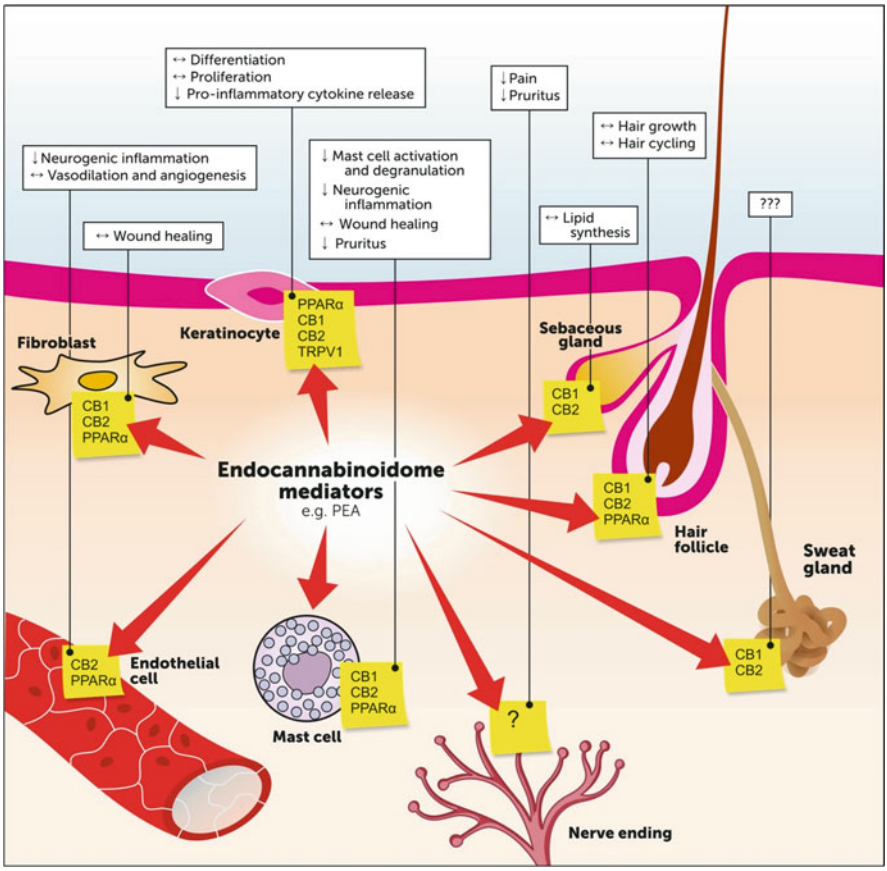

Fig. 9.3 The skin endocannabinoidome in domestic animals

laureate Rita Levi Montalcini that renewed interest in this molecule demonstrating its anti-inflammatory and antinociceptive effects (Aloe et al. 1993). The finding was later confirmed and considered to be mainly dependent upon mast cell down-modulation (Mazzari et al. 1996). Skin mast cells are long-lived tissue-resident cells, located in the dermis, adjacent to blood vessels and nerve endings. Thanks to their localization, sensing capability, and immediate response mast cells are perfectly poised to regulate neuroimmune responses in the skin (Steinhoff et al. 2018; Sumpter et al. 2019; Zoumakis et al. 2007). They are involved in maintenance of skin homeostasis as they promptly react to any local disturbance (Galli et al. 2005; Maurer et al. 2003; Shiota et al. 2010). Indeed, mast cells can sense and respond to antigens, invading pathogens, neuropeptides, and tissue damage, through a wide array of pattern recognition receptors (Dudeck et al. 2019). Following activation, they readily undergo degranulation within seconds, followed by the release of a plethora of de novo synthesized soluble mediators (Abraham and St. John 2010;

Matsuguchi 2012). The cutaneous mast cell can either amplify or limit skin responses, depending on the type, severity, and duration of the stimulus (Frossi et al. 2017; Reber et al. 2017). From a pathophysiological point of view, the skin mast cell is currently considered a key element in wound healing (Komi et al. 2019; Noli and Miolo 2001; Noli and Miolo 2010) and a "central cellular switchboard" of pruritogenic and neurogenic skin inflammation (Arck and Paus 2006; Steinhoff et al. 2018; Choi and Di Nardo 2018). Moreover, mast cells were shown to regulate epidermal barrier function and the development of allergic skin inflammation (Sehra et al. 2016; Germundson et al. 2018). Mast cells are abundantly located in the canine dermis and at the dermal–epidermal junction (Auxilia and Hill 2000; Noviana et al. 2004). Large numbers of mast cells are also found in the skin of healthy and allergic cats (Foster 1994; Noviana et al. 2001; Noli et al. 2003). Mast cell hyperplasia and/or hyperreactivity have been linked to canine atopic dermatitis and flea bite hypersensitivity (Abramo et al. 2014; Brazis et al. 1998; Demora et al. 1996; Hammerberg et al. 2001; Von Ruedorffer et al. 2003; Wuersch et al. 2006). In addition, intradermal injection of anti-IgE in cats is associated with a transient wheal response, development of superficial dermal edema, and increased number of degranulated mast cells (Seals et al. 2014). Once hyperactivated by intensive and/or repeated agonistic stimulation, the mast cell may become hyperactive and contribute to disease pathology (Heger et al. 2014). For this reason, mast cells are finely controlled by systemic and local mechanisms, the former depending on corticosteroids (Lewis and Whittle 1977), while the latter being mainly based on endocannabinoid function (De Filippis et al. 2008). Mast cells, indeed, metabolize endocannabinoids (Bisogno et al. 1997), uptake them (Rakhshan et al. 2000), and express different types of cannabinoid receptors (Cantarella et al. 2011; Facci et al. 1995; Hegde et al. 2015; Sugawara et al. 2012; Sugawara et al. 2013) which may act either individually or in close crosstalk (e.g., CB2 receptor-GPR55 heteromerization) (Cruz et al. 2018).

After Rita Levi Montalcini and her group discovered that some endocannabinoid-related NAEs were able to control mast cell behavior (Aloe et al. 1993), PEA was recognized as the parent molecule of this new class of naturally occurring and semi-synthetic NAEs, collectively termed ALIAmides, i.e. amide molecules able to perform an Autacoid Local Injury Antagonism (Levi-Montalcini et al. 1996). Many research findings have been obtained since then, all confirming that PEA and related ALIAmides are able to down-modulate mast cell responses (Cantarella et al. 2011; De Filippis et al. 2013; Esposito et al. 2011; Facci et al. 1995; Mazzari et al. 1996; Skaper and Facci 2012; Skaper et al. 2013; Vannacci et al. 2002). Notably, PEA can decrease immunologically induced degranulation from canine mast cells freshly isolated from skin biopsies (Cerrato et al. 2010) and oral administration of PEA to allergic cats results in increased intracellular granular content (i.e. reduced degranulation) of skin mast cells (Scarampella et al. 2001). The ability of PEA to down-modulate mast cell degranulation was recently shown in ex vivo conditions. In a recent study, a canine skin model was used to investigate the ability of PEA to counteract changes induced by compound 48/80, a well-known secretagogue that causes mast cell degranulation. Cultured skin biopsies were challenged

with 10 and 100μg/mL compound 48/80, without or with 30μM PEA-um. The exposure of skin biopsies to PEA-um 24 h before and 72 h after compound 48/80 was able to decrease the number of degranulating mast cells and to decrease both the content of histamine quantified within the medium and the diameter of epidermal blood capillaries (Abramo et al. 2017).

Finally, other ALIAmides like Adelmidrol® (azeloyldiethanolamide) and N-palmitoyl-D-glucosamine (or Glupamid) were shown to control skin mast cell degranulation thus limiting hyperactive responses (Abramo et al. 2008; Cerrato et al. 2012b; Miolo et al. 2007; Miolo et al. 2006). In conclusion, mast cells and the endocannabinoidome are key elements in skin pathophysiology and ALIAmides can help restore skin homeostasis through the down-modulation of mast cells and other skin cell types.

9.4 Therapeutic Opportunities Offered by the C(ut)annabinoid System

Several preclinical studies have largely demonstrated the effects of endocannabinoids and related NAEs in skin diseases (Milando and Friedman 2019). Generally, one can assume that cannabinoid receptor antagonists exacerbate skin inflammation, whereas receptor agonists attenuate it. The assumption mainly comes from the elegant experiment by Karsak et al. (2007) who showed that mice lacking the cannabinoid CB1 and CB2 receptors had increased allergic response, while those lacking the endocannabinoid degradative enzyme (Fatty Acid Amide Hydrolase, FAAH) and thus having augmented endocannabinoid levels, had a significantly lower allergic response (Karsak et al. 2007). Few years later, the German dermatologist Ralph Paus and his colleagues found that treatment with AEA suppresses keratinocyte proliferation and induces cell death, probably through the sequential activation of CB1 and TRPV1 both in vitro and ex vivo (Toth et al. 2011). The findings were considered relevant for the treatment of hyperproliferative dermatoses (Toth et al. 2011). The effect of the endocannabinoid system on itch (pruritus) was also addressed and interesting findings were collected in mice (Tosun et al. 2015). In fact, augmenting the endocannabinoid tonus by inhibition of degradative enzymes (i.e. FAAH and monoacylglycerol lipase, MAGL) reduced scratching behaviour (Tosun et al. 2015). In the veterinary dermatology field, most research has been done on the activity of ALIAmides with special attention to PEA. In the following paragraphs, we will limit our focus to these molecules, with reference to dogs and cats and some of their dermatological conditions (excluding tumors), given that the more general field of endocannabinoids in skin diseases has been extensively reviewed in recent years (Milando and Friedman 2019; Toth et al. 2019; Eagleston et al. 2018; Trusler et al. 2017; Tüting and Gaffal 2017).

9.4.1 Skin Inflammation

Peripheral nerves play a key role in skin inflammation; several skin diseases, such as contact dermatitis and atopic dermatitis, depend on neurogenic inflammation (Choi and Di Nardo 2018; Gouin et al. 2015). Rita Levi Montalcini's group first showed that PEA significantly reduced neurogenic inflammation in the ear pinna (Aloe et al. 1993), the effect mainly dependent upon mast cell down-modulation (Mazzari et al. 1996). It is now increasingly acknowledged that several cell types beside mast cells are targets of PEA; the pro-inflammatory responses of activated keratinocytes, macrophages, and T cells are indeed down modulated by this ALIAmide (Chiurchiu et al. 2018; Petrosino et al. 2010; Gabrielsson et al. 2017). In this regard, it is also noteworthy that PEA is able to elevate the levels of the anti-inflammatory cannabinoid 2-AG in keratinocytes and enhance the activation and desensitization of TRPV1 by 2-AG (Petrosino et al. 2016b). In addition, after a single oral administration, PEA elevates plasma 2-AG levels in hypersensitive dogs (Petrosino et al. 2016b). These in vitro and in vivo observations strengthen the so-called "entourage hypothesis", i.e. that PEA could produce not only direct but also indirect effects, by (1) elevating the levels, (2) reducing the degradation, or (3) increasing the affinity of endocannabinoids for their receptor(s) (Petrosino and Di Marzo 2017). Challengingly, PEA not only acts as an entourage compound on skin endocannabinoids but is itself the subject of an entourage effect in the skin (Petrosino et al. 2016b). In fact, Adelmidrol® (a PEA analogue) increases the endogenous levels of PEA in canine keratinocytes, suggesting that the anti-inflammatory effect of Adelmidrol might partially depend on the increase of endogenous concentrations of PEA (Petrosino et al. 2016a). Several different models of skin inflammation (either acute or chronic) have shown to benefit from PEA administration. Carrageenan-induced acute inflammation, for example, is significantly and dose-dependently inhibited by PEA (Conti et al. 2002; Costa et al. 2002; Mazzari et al. 1996; D'Agostino et al. 2007; Loverme et al. 2005). Notably, in this model PEA behaves differently from other NAEs (i.e., anandamide), being the only one able to reduce skin inflammation by pretreatment regimen (Wise et al. 2008). Moreover, the effect of PEA is also evident when the compound is administered after inflammation is established, i.e., curative efficacy (Costa et al. 2002). Notably, the effective dose of PEA (12.5 mg/kg) is similar to those of the non-selective cyclooxygenase inhibitor diclofenac (5 mg/kg) and the synthetic glucocorticoid dexamethasone (10 mg/kg) (Wise et al. 2008). Different ALIAmides, such as N-palmitoyl-D-glucosamine and Adelmidrol, also exert a remarkable protective effect against carrageenan-induced acute skin inflammation (Impellizzeri et al. 2016). Formalin-, compound 48/80- and dextran-induced edema are also significantly reduced by PEA (Jonsson et al. 2006; Mazzari et al. 1996). Similarly, the topical application of PEA decreases the phorbol ester-induced ear edema (Loverme et al. 2005). Moreover, the ALIAmides PEA and Adelmidrol also inhibit chronic skin inflammation, as shown by the considerable body of literature on the reduction of (1) subcutaneous granuloma formation, (2) angiogenesis, (3) leukocyte infiltration, (4) and release of pro-inflammatory mediators in chronic

inflammation models (De Filippis et al. 2009; De Filippis et al. 2010; Solorzano et al. 2009). Interestingly, down-modulation of mast cell degranulation emerged to be the main mechanism of action (De Filippis et al. 2013, 2014).

Awaiting publication, a randomized, double-blinded, placebo controlled clinical study led by Dr. Margaret Curtis in Australia, found in 13 dogs the use of a multi-phytocannabinoid product (DermaCann®) substantially reduced Canine Atopic Dermatitis Extent and Severity Index (CADESI-4) scores by an average of 51% after 56 days on treatment. Plasma skin inflammatory biomarkers, Chemokine Ligand 1 (CXCL1) and Chemokine Ligand 2 (CCL2), we also markedly reduced. No significant adverse events were reported for this study. (Personal communication with Layton Mills, Managing Director at CannPal, 7/19/20)

9.4.2 Wound Healing

Skin mast cells are recognized to play a crucial role in the key events of wound healing (Noli and Miolo 2001; Noli and Miolo 2010; Wulff and Wilgus 2013) and are recognized as one of the most intriguing cellular targets of the endocannabinoidome system (Sugawara et al. 2012; Aloe et al. 1993). Some evidence exists that cannabinoid receptor agonism regulates keratin expression and is relevant to wound healing (Ramot et al. 2013). The ALIAmide (and PEA congener) Adelmidrol has been studied in different models of wounds and found to promote skin wound healing. Topically applied on canine experimental skin wounds (punch biopsies), Adelmidrol down-modulated mast cell granule release (without affecting mast cell number) (Abramo et al. 2008). The effect was paralleled by a decrease in wound volume and improved healing over time (Mantis et al. 2007). More recently, Adelmidrol was confirmed to exert important effects on the healing and closure of skin wounds in a diabetic mouse model (Siracusa et al. 2018). PPARs are possible targets in the wound healing field since it was shown that PPAR-α and PPARβ mutant mice display impaired repair; PPAR-α and β are important for the rapid epithelialization of a skin wound, each of them playing a specific role: the first is mainly involved in the early inflammation phase of the healing, whereas the second is implicated in the control of keratinocyte proliferation (Michalik et al. 2001).

9.4.3 Skin Allergy

The pioneer study by Petrosino et al. (2010) was the first to demonstrate that PEA exerts an anti-allergic effect; they found that PEA (5–10 mg/kg) attenuated skin inflammation in contact allergic dermatitis, in a way considered to be mediated by TRPV1 receptors (Petrosino et al. 2010). Moreover, they found that PEA treatment reduced inflammatory chemokine release by challenging keratinocytes (i.e. in vitro model of allergic contact dermatitis) through a TRPV1-mediated mechanism

(Petrosino et al. 2010). Similar results were later obtained in the same model with cannabidiol (Petrosino et al. 2018). Surprisingly, the effective concentration of the phytocannabinoid turned out to be twofold higher than the efficacious concentration of PEA (Petrosino et al. 2018). Interesting results were also found in canine models of skin allergy: a single oral administration of PEA in the ultramicronized form (dose range 3–30 mg/kg) to hypersensitive Beagle dogs was shown to significantly reduce the antigen-induced wheal area, with no significant differences between the two higher doses, suggesting that 10 mg/kg is sufficient to exert the maximum inhibitory effect (Cerrato et al. 2012a). This study determined an excellent bioavailability of PEA in dogs; it is rapidly absorbed after oral administration and reaches plasma peak level after 1–2 h, and then returns to basal within 4 h. When blood levels of PEA were compared with the clinical effects at different times, an evident correlation was obtained, with anti-inflammatory effects of PEA being more long-lasting (up to 8 h) than plasma concentrations. The hypothesis of a secondary pathway, i.e. the ability of PEA to up-regulate other endogenous bioactive compounds of the endocannabinoid family was thus put forward (Cerrato et al. 2012b). Indeed, it was later found that a single oral dose of ultramicronized PEA to hypersensitive Beagle dogs resulted in a 20-fold increase in 2-AG blood levels, confirming the original hypothesis (Petrosino et al. 2016b). Finally, PEA administration was shown to delay development of clinical signs in a double blinded placebo-controlled crossover study performed on dogs with experimental allergic dermatitis (i.e., high-IgE Beagles epicutaneously sensitized to house dust mites, HDM) (Marsella et al. 2005). In particular, the dietetic supplementation with PEA at 15 mg/kg/day for 7 days delayed clinical signs after daily challenges with HDM; within the placebo-treated group, clinical scores (pruritus and skin lesions) increased significantly at 24 h (two challenges), while in PEA supplemented-group scores increased at 48 h (after the third challenge), indicating that PEA was able to delay the development of clinical signs in this model of canine atopic dermatitis (Marsella et al. 2005). An inhibitory effect on canine allergic skin wheals was also observed after topical application of the ALIAmide Adelmidrol for 3 and 6 consecutive days (Cerrato et al. 2012b). The ability to significantly reduce skin wheals in spontaneous hypersensitive Beagle dogs also demonstrates that Adelmidrol decreases both early- and late-phase reactions. Interestingly, the effect of topically applied Adelmidrol on allergic wheal inhibition is the same order of magnitude as that of topical corticosteroids (Cerrato et al. 2012b). Finally, Adelmidrol has recently been proven to reduce chemokine production in the skin following allergic stimulation (Petrosino et al. 2016a).

9.4.4 Pruritus

Pruritus (or itch) is the hallmark symptom of different skin diseases, especially of allergic nature. It is perhaps the most promising target for endocannabinoid treatment in the field of dermatology. The pathophysiological mechanisms of itch

encompass a complex interplay among skin elements (keratinocytes, mast cells, and sensory nerves) known to be regulated by endocannabinoids (Ikoma et al. 2006; Metz and Stander 2010). Nearly 20 years ago it was shown that peripheral administration (i.e. skin patch) of a synthetic cannabinoid receptor agonist attenuated histamine-induced itch in humans (Dvorak et al. 2003). Since then, several research groups have focused on the antipruritic effects of endocannabinoids and confirmed the benefits of topical and systemic treatments on clinical and experimentally induced itch (Eagleston et al. 2018; Trusler et al. 2017; Mounessa et al. 2017).

Except for the aforementioned study by Dvorak and collaborators (2003) and one small trial with Dronabinol, a synthetic THC, all the papers published so far on cannabinoids in pruritus deal with ALIAmides (Adelmidrol®) with PEA being considered one of the most promising compounds in this respect. (Abramovits and Perlmutter 2006; Neff et al. 2002; Avila et al. 2020). On the human side, PEA proved to be equally effective to hydrocortisone (1%) in reducing atopic dermatitis-associated pruritus (Kemeny 2005). In haemodialysis patients, a 3-week therapy with PEA completely eliminated pruritus in nearly 40% of trial subjects (Szepietowski et al. 2005). The reduction of itch in patients with prurigo, lichen simplex, and pruritus were reported to average 86.4% (Stander et al. 2006). Interestingly, it has recently been suggested that the effect on itch could rely—instead—on the ability of PEA to restore skin barrier properties (Yuan et al. 2014). In line with this hypothesis, PEA was previously found to decrease transepidermal water loss (TEWL) to a significantly greater extent compared to commonly used dermatologic preparations, i.e. triamcinolone cream 0.05% and pimecrolimus 1% cream (Zerweck et al. 2006). Based on all the aforementioned findings, PEA is currently listed among the common treatments for pruritus in humans (Leslie et al. 2015). The PEA congener Adelmidrol also showed promising results in reducing pruritus; in a pilot study on twenty pediatric patients with atopic dermatitis, a 4-week treatment with Adelmidrol resulted in 80% complete remission of pruritus (Pulvirenti et al. 2007). Finally, an elegant study by some members of the Endocannabinoid Research Group found that PEA remarkably reduced itch in a rodent model of allergic dermatitis, and this effect was reversed by both CB2 and PPAR-α antagonists, suggesting the involvement of both receptors (Vaia et al. 2016).

9.5 N-Acylethanolamines in Skin Diseases: Clinical Data in Dogs and Cats

In clinical dermatology ALIAmides have given promising results in the treatment of spontaneous allergic and inflammatory skin diseases. The first study on the use of NAEs in veterinary dermatology dates back a couple of decades, when a clinical evaluation of PEA in allergic cats was conducted (Scarampella et al. 2001). Fifteen privately owned cats with eosinophilic plaque and eosinophilic granuloma were orally given micronized PEA at the dosage of 10 mg/kg daily for 1 month, with no

other drugs permitted. Ten of 15 (67%) cats showed clinical improvement of pruritus, erythema, alopecia, and eosinophilic lesions. Improvement was observed as soon as 15 days after treatment initiation. Interestingly, in cats with eosinophilic plaque, the clinical improvement was directly and significantly correlated to the increase in mast cell densitometry. No side effects or adverse reactions were observed (Scarampella et al. 2001). Recently a double-blinded placebo-controlled study was conducted in allergic cats with the aim of evaluating the efficacy of PEA-um in delaying relapses of clinical signs after steroid withdrawal (Noli et al. 2019). Sixty cats with non-flea hypersensitivity dermatitis were treated with methylprednisolone for 1 month and until resolution of signs, and with concurrent oral daily PEA-um at 15 mg/kg (30 cats) or placebo (30 cats). PEA-um or placebo were continued after steroid withdrawal and until relapse of clinical signs, and "time to flare" was compared between the two groups. Thirteen cats in the PEA group and 12 in the placebo could be followed until relapse, with a significant difference in mean time to flare between the two groups (40.5 days in the PEA group vs. 22.2 days in the placebo group), suggesting that PEA exerts an excellent proactive activity in preventing feline allergic flares. Interestingly, during concomitant methylprednisolone treatment, the severity of pruritus was significantly lower in the PEA-um treated cats compared to placebo, suggesting a possible additional steroid sparing effect, which is worth future studies (Noli et al. 2019).

A double-blinded randomized placebo-controlled cross-over study was conducted using PEA-um on 20 client-owned dogs with clinical signs of canine atopic dermatitis (CAD) (Waisglass et al. 2009; Waisglass 2012). The dogs did not undergo a hypoallergenic diet trial so it is possible that they could have been affected by FIAD (food induced atopic dermatitis) or NFIAD (non-food induced AD) or both. Dogs were orally administered either placebo or PEA at 15 mg/kg daily for 45 days, then a wash-out period of at least 4 weeks was observed before switching to the other treatment for further 45 days. Severity of the clinical signs was assessed by CADESI (Canine Atopic Dermatitis Extension and Severity Index), evaluating erythema, lichenification, and excoriation on 40 body areas. Erythema significantly decreased while on PEA treatment compared to placebo, as well as the total CADESI score in the second arm of the study (i.e., between the third and fourth visit). Interestingly, dogs on PEA appeared to continue to improve for a period after discontinuation of the treatment. Tolerability was excellent with no difference seen between the two treatments (Waisglass et al. 2009; Waisglass 2012).

Finally, an open multicentric study was recently performed in 160 dogs with non-seasonal atopic dermatitis to evaluate the efficacy of oral administration of ultramicronized PEA (Noli et al. 2015). PEA-um was administered daily at 10 mg/kg for 56 days, and parameters evaluated were pruritus (Visual Analogue Scale range 0-10), skin lesions (Canine Atopic Dermatitis Lesion Index CADLI, range 0-50), and quality of life (QoL, range 0–45). All parameters decreased significantly during the treatment period; by the end of the study 30% of dogs had absent or very mild (<2) pruritis, 62% had normal lesional values (<5), and 45% had reached QoL values compatible with healthy animals. Tolerability was good to excellent with only

four dogs reporting mild adverse events associated with the treatment. (Noli et al. 2015).

Finally, an open-label observational study was conducted to evaluate the efficacy of a topical formulation containing Adelmidrol against allergic pruritus in dogs. Privately-owned dogs with atopic dermatitis and pruritus lasting longer than 4 weeks were included and treated twice daily for 30 days. In the thirteen dogs completing the study there was a statistically significant decrease in pruritus and erythema (both on owner and veterinarian assessment). Body odor and quality of life significantly improved at the end of the treatment (Fabbrini and Leone 2013). Similarly, to dietary supplementation of PEA, topical treatment with Adelmidrol may thus be a useful tool in the management of allergic skin diseases in dogs.

9.6 Conclusion

The endocannabinoid system of the skin, also known as "c(ut)annabinoid" system, has recently emerged as a key pro-homeostatic signalling system. The biosynthetic and catabolic pathways of endocannabinoids are shared with other bioactive lipid mediators—the so called endocannabinoid-like mediators—giving rise to an extended endocannabinoid system or endocannabinoidome. PEA is a natural-occurring N-acyethanolamine, the parent molecule of ALIAmides, and one of the most studied components of this system. It is synthesized "on demand" for protective purposes, in response to actual or potential injury. Dermal mast cells together with several other cell types, including keratinocytes, are target of PEA. Once excessively activated during inflammatory or allergic conditions, these cells are controlled by locally produced PEA and their responses down modulated and kept within the physiological threshold. One might argue that pathological situations may arise in which endogenous PEA levels in the skin are inadequate to deal with the ensuing insult. In these cases, exogenous administration may be a viable and "according-to-Nature" approach. A great deal of evidence exists showing consistent improvement of skin health following dietary supplementation or topical use of PEA and congeners in either experimental or companion animals. In the future, veterinary interest and use of products acting through the endocannabinoidome, including phytocannabinoids, will continue to rise. The literature warrants further investigation on the dermatological effect of ALIAmides and practitioners should be knowledgeable about their use as single or add-on intervention for managing skin disorders in canine and feline patients.

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Chapter 10
Cannabinoids in Oncology and Immune Response

Louis-Philippe de Lorimier, Trina Hazzah, Erik Amazonas, and Stephen Cital

10.1 Introduction

Cannabinoid therapy has been important to human cancer patients for many years due to its ability to improve quality of life (QoL) and its potential to treat or palliate cancer. It was only a matter of time for this interest to cross over and enter the veterinary oncology arena. There are numerous in vitro and in vivo studies in various animal cancer models evaluating the anticancer effects of cannabinoids. The initial report on the antiproliferative properties of cannabinoids was published over 45 years ago when Munson and colleagues demonstrated that orally administered Δ9-tetrahydrocannabinol (THC) inhibited lung adenocarcinoma tumor growth in vivo with a murine model and improved survival time of treated mice when compared to controls (Munson et al. 1975). Although no published clinical study has critically evaluated the effects of cannabinoid therapy in pets with naturally occurring tumors as of yet, there are countless anecdotal reports that describe an apparent safe use in this population with resulting improved QoL and measurable tumor responses, even when used as monotherapy and, in some cases, after failing conventional therapeutic approaches. The intent of this chapter is to elucidate the current understanding of how phytochemicals from the cannabis plant can be used to

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mitigate adverse effects secondary to cancer therapy or the disease itself and discuss the postulated anticancer effects of these plant-based compounds in the small animal patient. Contemporaneously to the manuscript preparation for this chapter, the authors are aware of multiple ongoing and upcoming in vivo clinical trials with naturally occurring cancers in pets; we hope future results will provide strong supportive evidence for practical applications in veterinary oncology patients. Many clinicians are already incorporating these compounds into their therapeutic approaches with variable apparent success.

10.2 The Endocannabinoid System and Cancer

As previously discussed in Chaps. 1 and 2, the endocannabinoid system (ECS) is a complex and dynamic series of receptors, signaling pathways, endogenous molecules, and enzymes striving to maintain homeostasis in mammalian physiology. The physiological impacts include regulation of cell division, differentiation, migration, and cell death in both normal and diseased states. Alterations in the ECS have been demonstrated with various cancers but it remains unclear if these changes play a role in the malignant transformation of the cancer cells or are, rather, a consequence of the cancer being present. While the exact mechanisms leading to the development and progression of most types of cancers (and the best ways to treat and obtain remission) have yet to be fully elucidated, important findings are constantly made allowing us to better understand the complex processes at play. Despite an incomplete understanding of the exact role exogenous cannabinoids play in the progression of cancer, evidence in both human and animal models have shown them to be safe and effective for the symptomatic relief of disease, treatment related complications, and adverse effects. There are exceptions, however, with occasional clinical studies showing lower response rates to certain anticancer treatments in cannabis users, and in vitro studies showing beneficial effects of THC on certain cancer types but detrimental effects with other cancer cell lines, such as human papilloma virus related head and neck squamous cell carcinoma. There is also nominal evidence suggesting a serious risk of interactions between exogenous cannabinoids and many standard chemotherapy agents. As usual, further studies are needed (Taha et al. 2019; McKallip et al. 2005; Liu et al. 2020).

Endocannabinoid receptors, CB1 and CB2, with variants in expression, are documented to affect angiogenesis and cancer cell motility, invasion, proliferation, adhesion, and apoptosis. As G protein-coupled receptors (GPCR), CB1 and CB2 receptors can interact dynamically with other GPCRs and also non-GPCRs (delte: GPCR's and non-GPCR's), forming homodimers, heterodimers, and higher order oligomers. Several CB1 and CB2 heteromers have been described in cancer cell forms, such as CB2 with CXCR55, GPR55, and the tyrosine kinase receptor HER2, among others. A common heteromer is the association of the CB2 receptor with the GPR55 orphan receptor. Several cancer types, specifically bone and liver neoplasia, show overexpression of this heteromer. The expression of the GPR55-CB2

receptor heteromers influences cannabinoid signaling in such a way that direct targeting with THC may lead to a reduction of tumor growth (Moreno et al. 2019).

Each of these protein complexes appear to possess unique pharmacological and signaling properties where modulation via negative crosstalk between receptors or positive crosstalk and cross-antagonism may result in antitumoral activity through the ECS (Figs. 10.1 and 10.2).

In human hepatocellular carcinoma and prostate carcinoma, upregulated expression of CB1 and CB2 receptors was demonstrated compared to normal liver and prostate. It is postulated that this upregulation may, in fact, be beneficial and associated with a better prognosis. Also of interest, this study found that the components of the ECS in tumors may alter the response to the tumor microenvironment or changes in niche signaling (Chakravarti et al. 2014).

It is possible that increased expression of ECS receptors by tumor cells may lead to improved tumor response to treatment, yet individual cancer cell types may respond differently to specific ECS modulations. This altered expression may help explain the anticancer mechanisms of phytocannabinoids.

As discussed earlier, the tumor ECS receptor density and profile may be a prognostic factor for patients with certain cancer types and may predict response to treatment. It has been demonstrated that under-expression of ECS receptors in certain cancer types, such as breast cancer, was associated with increased tumor growth and metastasis when THC was administered to mice in an in vivo study (McKallip et al. 2005). This discovery demonstrates the dynamic interaction between phytocannabinoids and the ECS but, more importantly, conflicts the anecdotal perceived benefits of using phytocannabinoids, especially THC, to suppress all types of cancer progression.

Endocannabinoid-degrading enzymes fatty acid amide hydrolase (FAAH), monoacylglycerol lipase (MAGL), and N-arachidonoyl glycine (NAGly, an anandamide metabolite) have also been implicated as possible strategies to combat cancer progression. Other endocannabinoid-related fatty acids, such as 2-AG ether, *O*-arachidonoylethanolamine, *N*-arachidonoyldopamine, and oleic acid amide, have all been shown to affect cannabinoid receptors and are under investigation for their potential roles and effects in cancer therapy (Hamtiaux et al. 2012).

Human cancer cell line research has found the upregulation of endocannabinoid degrading enzymes can increase binding affinity for the CB1 and CB2 receptor. Why the increase in these types of enzymes allows for CB affinity is still unclear. The depletion of endocannabinoids along with possible competitive receptor binding affinity can contribute to the under-expression of CB1 receptors, which has shown to result in the growth of intestinal adenoma in one study (Wang et al. 2008; Nomura et al. 2010).

Consistency in data has been troublesome in this specific area of research but many cancer biologists would agree: the main beneficial effects of cannabinoids (endogenous or exogenous) against tumor cells involve the inhibition of cell proliferation and induction of cancer cell death by apoptosis and autophagy. Agonists of CB1 and CB2 receptors appear to promote apoptotic cell death in glioma cells by

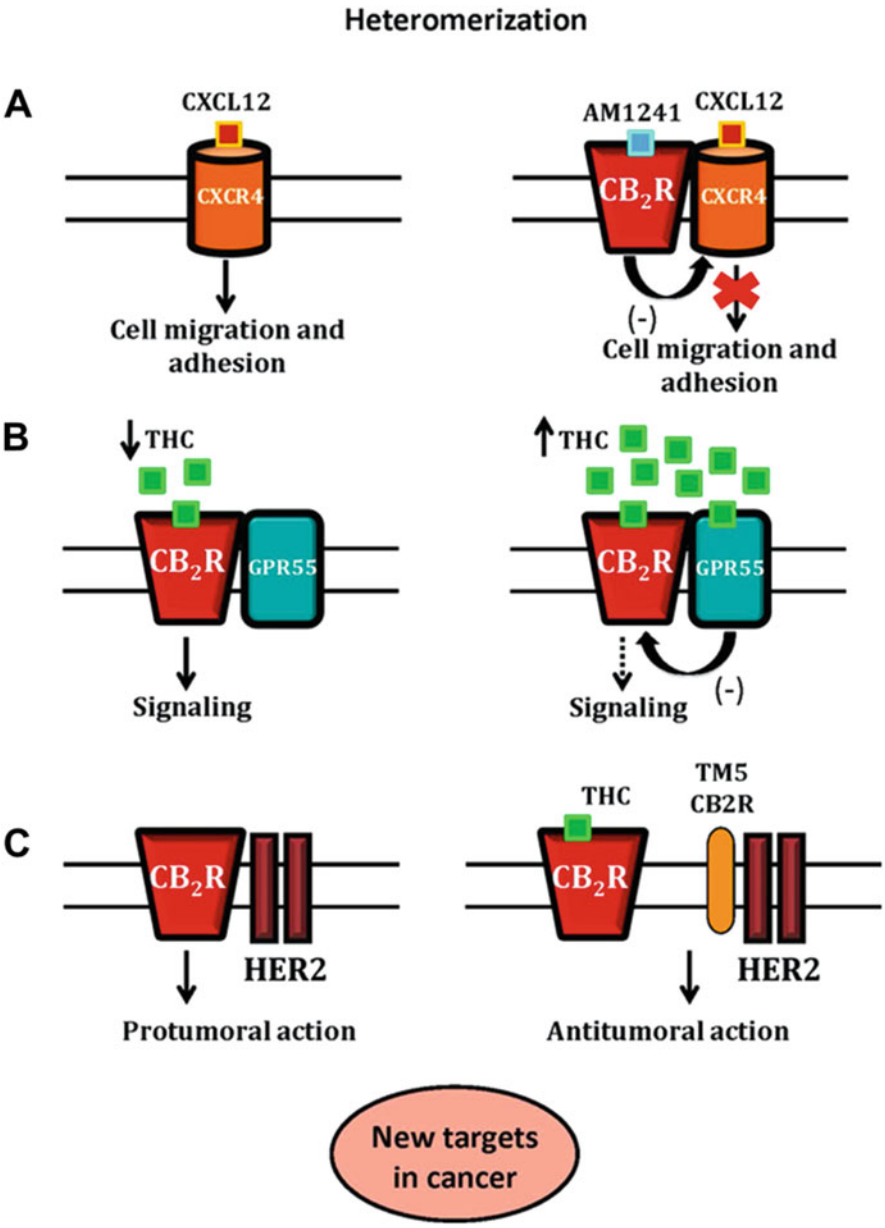

Fig. 10.1 A schematic representation of CB2-CXCR4, CB2-GPR55, and CB2-HER2 heteromers and their role as new targets in cancer. In panel (**a**), the activation of CB2-CXCR4 heteromers inhibits prostate cancer cell migration and adhesion. The binding of CXCL12 to its receptor, CXCR4, induces CXCR4-mediated cell migration and adhesion. The application of both CXCR4 and CB2 agonists inhibits the effect of the CXCR4 agonist, due to the presence of functional CB2R-CXCR4 heteromers. In panel (**b**), the hypothetical effect of THC on the CB2R-GPR55 heteromer. At low concentrations, THC acts as a CB2R agonist promoting signaling. At higher concentrations, THC targets GPR55, acting as an antagonist, and by cross-antagonism, inhibits CB2 signaling. (**c**) Proposed mechanism of control of the CB2-HERs heteromer in human breast cancer. THC creates

induction of de novo synthesis of ceramide, a sphingolipid with proapoptotic activity (Galve-Roperh et al. 2000).

Moreover, a recent study with human and canine glioma cells showed that CBD appears to be itself cytotoxic, anti-proliferative, and anti-migratory. However, the high concentrations required for these effects may not be pharmacologically attainable in living organisms. Regardless, the researchers, using attainable concentrations, did find an increase in cell line sensitivity to CBD via distressed mitochondrial function. Finally, the researchers found that combining CBD with the autophagy inhibitor hydroxychloroquine (HCQ) resulted in increased human and canine glioma cell sensitivity to CBD, suggesting autophagy as a pathway for mediating CBD-induced cell death by a non-canonical function of receptor-interacting serine/threonine-protein kinase 3 (RIPK3) (Gross et al. 2020).

These examples, along with several other studies, stress the delicate balancing act in which ECS receptor expression, endocannabinoids, and ECS related enzymes are involved, affecting the overall health of our patients as it relates to cancer and many other disease states (Miller and Devi 2011).

10.3 Inhibition of Angiogenesis and Metastasis

In addition to apoptosis and autophagy, the anticancer effects of cannabinoids include other mechanisms as well. Certain cannabinoids can inhibit angiogenesis by blocking activation of the vascular endothelial growth factor (VEGF) pathway. Downregulation of VEGF itself, or inhibition of its receptors (VEGFR1 and VEGFR2), has been demonstrated in various cancers including glioma, skin cancer, and thyroid carcinoma. Cannabinoid involvement with blockade of ceramide biosynthesis leads not only to apoptotic cell death by induction of de novo synthesis of ceramide, but also tumor starvation via inhibition of VEGF production and VEGFR2 activation with consequently reduced neo-angiogenic abilities (Casanova et al. 2003; Blázquez et al. 2004; Portella et al. 2003).

Antimetastatic effects of CB receptor agonists, such as CBD, echo antiproliferative effects in that the mechanism appears to rely on ceramide biosynthesis and a critical modulatory effect involving p8 protein. Tumor development, specifically transcriptional regulators as well as its downstream targets, activate transcription factor 4 (ATF4), C/EBP homologous protein (CHOP), and Tribbles homolog 3 (TRIB3). Oxidative stress, calcium depletion, viral infection, or some anticancer agents may also trigger endoplasmic reticulum (ER) stress. This elegant response results in translation arrest, degradation of misfolded proteins, and

Fig. 10.1 (continued) disruption triggering the inactivation of HER2 and producing antitumor response (permission to use with full citation. Moreno, E., Cavic, M., Krivokuca, A., Casadó, V., & Canela, E. (2019). The Endocannabinoid System as a Target in Cancer Diseases: Are We There Yet? *Frontiers in Pharmacology, 10*. doi: 10.3389/fphar.2019.00339)

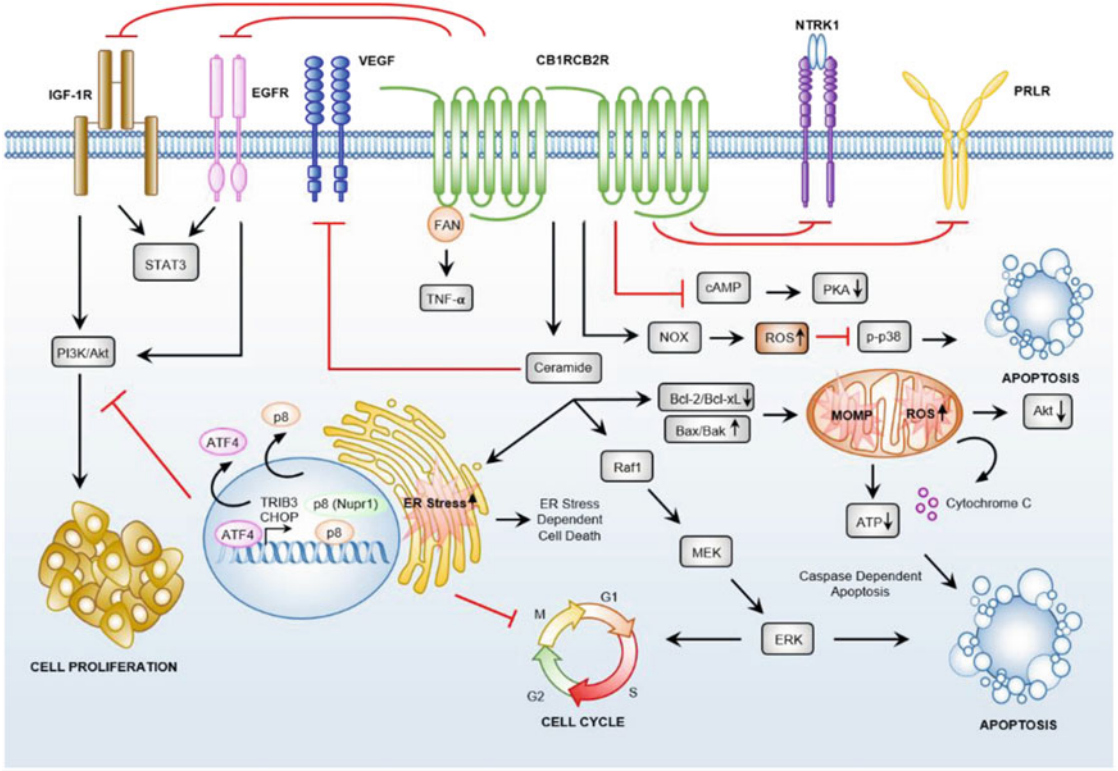

Fig. 10.2 Cannabinoid induced anti-cancer crosstalk between G-protein-coupled receptor (GPCR) and non-GPCR signaling pathways. Cannabinoid interaction with CB1 and CB2 and GPCRs to induce cancer cell death by promotion of cellular stress response pathways, increased ceramide synthesis, and reduced oncogenic growth and proliferation signaling (permission to use with full citation Afrin, F., Chi, M., Eamens, A. L., Duchatel, R. J., Douglas, A. M., Schneider, J., et al. (2020). Can Hemp Help? Low-THC Cannabis and Non-THC Cannabinoids for the Treatment of Cancer. *Cancers, 12*(4), 1033. doi: 10.3390/cancers12041033)

restoration of ER protein-folding capacity. However, response failure may lead to ER stress and activation of an intrinsic apoptosis pathway. The activation of p8 pathways causes inhibition of pro-survival protein kinase B (Akt) by TRIB3, which in turn leads to an inhibition of rapamycin complex 1 (mTORC1) and eventually to autophagy-mediated cell death (Schröder and Kaufman 2005; Ivanov et al. 2020; Salazar et al. 2009, 2013) (Fig. 10.3).

CBD also appears to downregulate expression of Id-1, which is an inhibitor of basic helix–loop–helix transcription factors in breast cancer, and decreases lung tumor invasion and metastasis by upregulation of intercellular adhesion molecule 1 (ICAM-1) which is linked to tissue inhibitor of matrix metalloproteinases-1 (TIMP-1) (Śledziński et al. 2018; McAllister et al. 2010).

Inhibitors of fatty acid amide hydrolase (FAAH), specifically arachidonoyl serotonin, have been shown to exhibit TIMP-1-dependent anti-invasive properties (Winkler et al. 2016).

Several other receptors, endocannabinoids, and endocannabinoid derivative-like compounds have been shown to have anticancer effects, yet direct or conclusive actions remain to be elucidated. There are several classes of receptors associated with cancer disease states that appear amenable to modulation by cannabinoids. This includes such receptor types as the transient receptor potential channel class [including TRPV, vanilloid TRP, TRP ankyrin (TRPA), TRP melastatin (TRPM)], nuclear receptors, peroxisome proliferator-activated receptor gamma (PPARy), and orphan receptor types such as GPR18 and GPR19, along with many others.

With the above-mentioned mechanisms described as having potentially beneficial effects against various tumor types, there is hope that exogenous cannabinoids may play a distinct role in the armamentarium against veterinary cancers, alone or in combination with other therapies. Additional in vitro studies are ongoing, prospective clinical studies are accruing cases, and future editions of this textbook will hopefully provide valuable and applicable data supporting the use of cannabinoids for veterinary patients with cancer.

10.4 Cannabinoids and the Immune System, Cell Proliferation and Signaling Pathways Related to Cancer

Erik Amazonas

One of the most complex physiological systems in vertebrates is the immune system. Since this biological system is responsible for every aspect of survival, the accurate regulation of its cells and components is critical. Immune cells are capable of detecting, pursuing, and eliminating foreign material such as bacteria, protozoa, fungi, viruses, or the cells infected by these organisms. In order to accomplish these functions, the immune system is equipped with a vast array of immunological cells and an unparalleled number of molecules such as cytokines and chemokines.

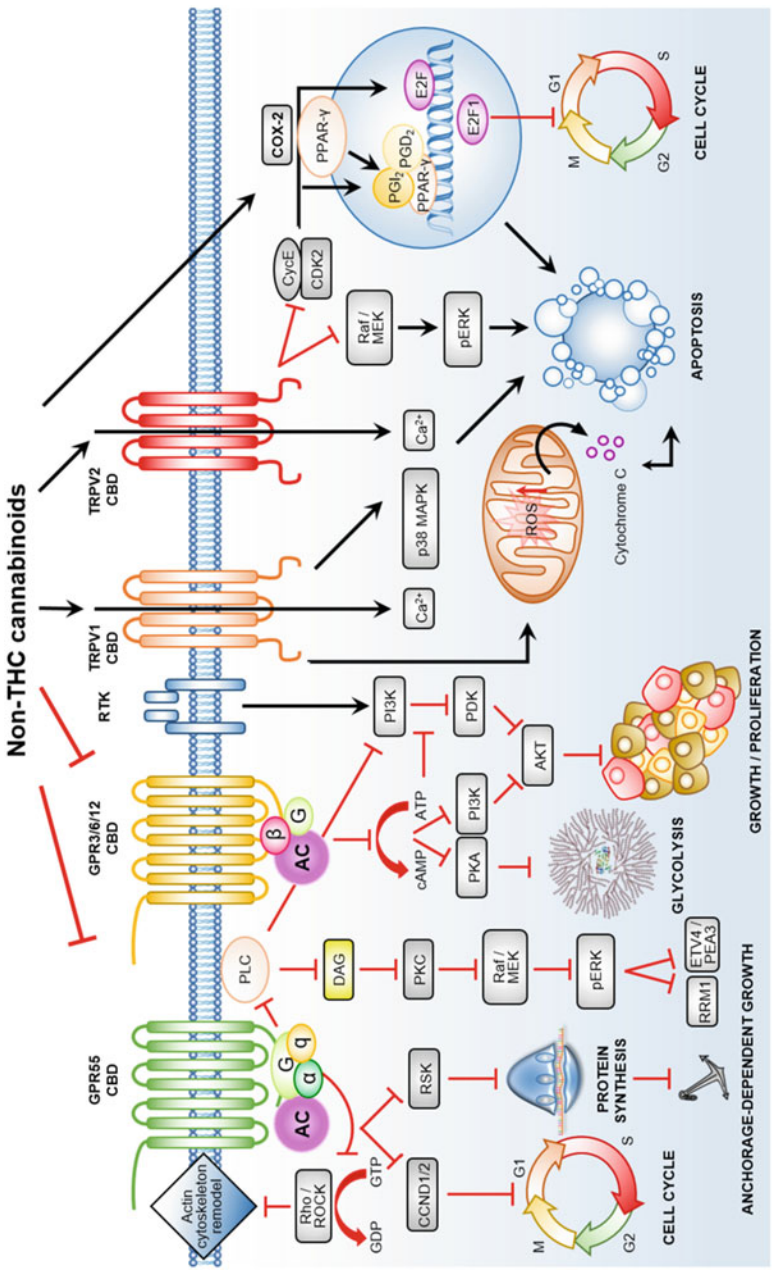

Fig. 10.3 Some of the signaling pathways understood in regard to anti-cancer effects of cannabinoids (permission to use with full citation Afrin, F., Chi, M., Eamens, A. L., Duchatel, R. J., Douglas, A. M., Schneider, J., et al. (2020). Can Hemp Help? Low-THC Cannabis and Non-THC Cannabinoids for the Treatment of Cancer. *Cancers, 12*(4), 1033. doi: 10.3390/cancers12041033)

The control of all cellular and molecular interactions is intrinsic and exceedingly complex. The ECS plays an important role in modulating the extensive signaling within the immune system (Turcotte et al. 2016).

The simple homeostatic balance within immunological cells is critical for maintaining health-disease balance. *Homeostasis* comes from the Greek terms "*homeo*" (similar, equal) and "*stasis*" (stable) and refers to physiological mechanisms involved in ensuring suitable conditions of the internal cellular environment in order for cell functions to function properly. When a given cell is under complete homeostasis, the cell is physiologically healthy. Thus, maintenance of cellular homeostasis is a key feature in any healthy biological system. The almost exclusive action of the ECS is the maintenance of homeostatic conditions within the cells of all body systems. Endocannabinoids, such as anandamide (AEA) and 2-arachidonoylglycerol (2-AG), regulate homeostasis by interaction, not only through the cannabinoid receptors (CB1 and CB2), but also through a number of non-cannabinoid receptors, expressed on both the cell membrane surface and the nuclear membrane. The non-cannabinoid receptors include: G protein-coupled receptors (GPCRs) 55 (GPR55), 18 (GPR18) and 119 (GPR119); nuclear peroxisome proliferator-activated receptors (PPARs); transient receptor potential (TRPs) vanilloid (TRPVs), ankyrin (TRPAs), melastatin (TRPMs) types; serotonin receptors (5-HT), N-methyl-D-aspartate receptor (NDMA, a glutamate receptor), glycine receptors (GLR or GlyR), nicotinic acetylcholine receptors (nAChRs), voltage-gated calcium channels (VGCCs), and voltage-gated potassium channels (VGKCs) (Pertwee 2015; Galiazzo et al. 2018). Understanding the action of endogenous and exogenous cannabinoids on such a variety of receptors in different cells and tissues clears the way to comprehending how cannabinoid therapy could benefit many different pathological conditions and why it is the most important homeostatic system of the body.

A brief review of ECS regulation follows. When calcium-channels are activated, membrane depolarization drives calcium (Ca^{+2}) into the cell inducing a series of events depending on the cell type's normal function (such as neurotransmitter release, transcription up-regulation, and/or hormonal release). Potassium-channels are crucial for returning the cell back to its resting state, counteracting the depolarized state. TRPs also modulate the Ca^{+2} and magnesium (Mg^{+2}) influx and are found in almost all cells, including on organelle membranes. 5-HT receptors modulate neurotransmitter and hormone release, affecting various physiological processes. The nAChRs are also linked to ion channels and enhance neurotransmission. The GlyR is an ionotropic receptor with inhibitory effects on neurotransmission. PPARs are nuclear receptors that serve as transcriptional factors for the expression of several genes. GPR55 seems to be involved in modulation of cytoskeleton regulation, cell cycle, and ion-channel control (Lauckner et al. 2008; Moriconi et al. 2010). The GPR18 evokes direct migration and proliferation of microglia (McHugh et al. 2011), the CNS' first active immune defense. Taking all of these diverse interactions of the ECS, endogenous cannabinoids, and multiple receptors into account, and also that any given cell expresses at least one set of such

receptors, it can be assumed that virtually every cell within a vertebrate's body will be affected by cannabinoid activity.

Generally speaking, and taking into account the known effects of each unique exogenous cannabinoid alone, it could be said that "cannabinoids are immunosuppressive" in their nature. We can, at the very least, affirm that cannabinoids are potent anti-inflammatory agents. However, there is a lot more to this process, and the actual mechanisms at play become quite complicated with a more in-depth and rigorous evaluation. Even a single cannabinoid can inhibit or induce a given immunological pathway based on dosage. In vitro studies have shown inhibitory effects when administered in micromolar concentrations, whilst at nanomolar ranges stimulatory effects are perceived (Croxford and Yamamura 2005). The pro-inflammatory cytokine triad, IL-1, IL-6 and TNF-α, is shown to be inhibited by cannabinoids in a consistent fashion (Maresz et al. 2007), although there are also findings showing the stimulation of IL-2 and IL-3 cytokines by anandamide via non-CB receptors. Aspects of the ECS and cannabinoid actions on the immune system, and its specific cells and products, will be discussed through the sections below.

10.5 Cannabinoid Receptors in the Immune System

Most of the aforementioned receptors are widespread and highly expressed within the immune system with well elucidated mechanisms and pathways. Therefore, this chapter will focus on the cannabinoid receptors (GPCRs) and the overall effects of cannabinoids at each receptor. It is well established that CB1 receptors have the highest expression within the central nervous system (CNS) while CB2 receptors are most abundant within the immune system (although CB1 and CB2 receptors are expressed and possess biological relevance at both sites).

The presence of CB1 receptors was reported in higher levels in B cells than natural killer cells, followed by polymorphonuclear neutrophils, $CD8^+$ T cells, monocytes, and $CD4^+$ T cells (Massi et al. 2006). CB2 receptor expression in immune cells is much higher than CB1 and has been described in the following decreasing order: B cells > natural killer cells > monocytes > neutrophils > $CD8^+$ T lymphocytes > $CD4^+$ T lymphocytes (reviewed by Rom and Persidsky 2013). CB2 receptors are expressed in such an extensive manner on immune cells that the ECS has been considered a key modulator of the immune system (Turcotte et al. 2016; Navarini et al. 2019). Naïve lymphocytes express higher levels of CB2 receptors as compared to activated lymphocytes (Rom and Persidsky 2013), suggesting an important role in immune cell activation, proliferation, differentiation, and effector functions (Basu et al. 2011). CB2 receptor expression on immune cells varies according to how and which different stimuli activates the immune cell. For instance, splenocytes stimulated by bacterial lipopolysaccharide (LPS) down-regulate CB2 receptors while co-stimulation of clusters of CD40 up-regulate CB2 receptors (Lee et al. 2001).

10.5.1 Cascade Signaling

Immunity relates to the maintenance of adequate levels of both endogenous, self-produced compounds, and foreign material that could eventually be pathogenic invaders against whom the immune system must be prepared to defend. Any given cell can enter a pathological state even with no pathogen involvement. Cellular homeostasis is a critical feature for healthy cells to sustain and unhealthy cells to pursue. The following sections discuss some important signaling pathways within the immune system that are intimately controlled by the ECS. Many of them are not immune-specific signaling pathways, yet the immune effects will be highlighted. The distribution of cannabinoid receptors, action of cannabinoids on several other non-cannabinoid receptors, and activation/inhibition of the diverse pathways below grant the ECS the status of, perhaps, the most important immunomodulatory system.

10.5.2 cAMP Signaling

CB1 and CB2 receptors are $G_{i/o}$ protein coupled receptors known for their inhibitory effects on adenylate cyclase (AC) and consequent inhibition of the ubiquitous second messenger cyclic adenosine monophosphate (cAMP) levels within the cell (Duggirala et al. 2015). Low levels of intracellular cAMP open rectifying K^+ channels, halt the excitatory membrane depolarization, and lead the cell back to its resting state (Howlett and Shim 2000–2013). cAMP is also involved in cytoskeleton-remodelling (Duggirala et al. 2015) and CB2 receptor-mediated inhibition of cAMP leads to reduced mobility in leukocytes (Andrade-Silva et al. 2016).

The protein kinase A (PKA, or cAMP-dependent protein kinase)-mediated signaling cascade is crucial for gene expression in immune cells and is completely dependent on intracellular cAMP levels (Massi et al. 2006). PKA regulates the nuclear factor of activated T-cells (NFAT) family known to be key transcription factors for interleukin 2 expression. Via CB2 receptor agonism, cannabinol (CBN) and THC inhibited NFAT activation by lowering cAMP levels and down-regulation of the AP-1 DNA-binding protein necessary for NFAT DNA-binding in EL4-IL 2 tumorigenic cells (Massi et al. 2006). Cannabinoid inhibition of cAMP also reduced binding of the PKA-dependent transcription factor to the cAMP-response element (CRE) in the DNA. CRE and κB are both DNA motifs to which PKA-dependent transcription factors may bind. Binding activity of both transcription factors were reduced in immune cells (Massi et al. 2006). cAMP signaling cascade is crucial for active immune response and cannabinoid inhibition of this signaling pathway antagonizes cAMP immunostimulation (Massi et al. 2006).

10.5.3 MAPK/ERK/JNK/p38 Pathway

As $G_{i/o}$ receptors, CB1 and CB2 receptors regulate the mitogen-activated protein kinase (MAPK or MAP Kinase) signaling pathway (Souza and Rosa 2019). This pathway involves the extracellular signal regulated kinase (ERK, also named MAPK), c-Jun N-terminal kinases (JNKs) and p38, p42/p44 mitogen-activated protein kinases (p38, p42/p44), and tightly regulates several transcription factors for genes controlling cellular proliferation, transformation, and apoptosis (Howlett and Shim 2000–2013). The MAPK pathway is activated by a series of phosphorylation events following intracellular activation of G-protein Ras and leads to activation of the serine/threonine kinase Raf, which in turn activates MEK and later ERK. The latter can phosphorylate several other proteins in the cytoplasm and nucleus (Demuth and Molleman 2006) and serves as or induces transcription factor for several genes. Activation of the MAPK signaling pathway stimulates cell proliferation and is essential for immune cells' expansion under inflammatory or infectious processes. Excess MAPK/ERK signaling has been associated with cancer-related cell survival. CB1 and CB2 receptors trigger different actions upon the MAPK pathway (activation/inhibition) and, more interestingly, different agonists (endo-, phyto- or synthetic cannabinoids) for CB1 or CB2 receptors also have different effects (Massi et al. 2006). CB1 receptor agonism by THC activates MAPK (Demuth and Molleman 2006), whilst CB2 receptor agonism with the synthetic CB2 agonist JWH-133 reduces intracellular G-protein phosphorylation and ultimately inhibits ERK1/2. Activation of CB2 receptors is sufficient to inhibit CXCR4-mediated signaling pathways (Costantino et al. 2012). 2-AG, CP-55,940 (a synthetic cannabinoid full CB1/CB2 receptor agonist), and to a lesser extent AEA, activated p42/p44 MAPK (Kobayashi et al. 2001). As they should, most studies on cannabinoid effects address the effects of a single cannabinoid or, when achievable, two or three cannabinoids at once. The totality of effects on the MAPK signaling pathway by the different cannabinoids is still not entirely clear. However, several studies and case-reports have suggested that actual modulation rather than suppression of the MAPK pathway occurs in an orchestrated way (Russo 2011; Gallily et al. 2014; Blasco-Benito et al. 2018).

10.5.4 NF-κB Pathway

The NF-κB pathway is critical for the immune system and several immunological events trigger this pathway. This pathway induces the expression of hundreds of different genes regulating the complex aspects of immunological response. The transcription factor NF-κB in non-activated cells is associated with IκB, which inhibits NF-κB activity, not allowing the later to move to the nucleus and perform transcriptional induction. Classical triggers for this pathway involve proinflammatory signaling by IL-1, TNF-α, TLRs, and antigen receptors. These activate IκB kinase (IKK) which phosphorylates IκB. Once phosphorylated, IκB

dissociates from NF-κB and it can now enter the nucleus and bind to specific gene promoters on the DNA. Among those NF-κB-induced genes are IL-1β, IL-6, IL-18, TNF-α, GM-CSF, IL-4, and several chemokines, adhesion molecules, inducible nitric oxide synthase (iNOS, a potent producer of nitric oxide, an oxidative element with high damage potential on cells), and cyclooxygenase-2 (COX-2, important in arachidonic acid metabolism and further inflammatory processes). NF-κB also promotes the expression of IκB which can then suppress NF-κB activity. Control of the NF-κB pathway and activity is crucial in immunity. Anandamide interferes with the phosphorylation of IκB, inhibiting NF-κB activity. An alternative pathway of NF-κB activation is triggered by TNF receptors and plays a special role in the NF-κB transduction pathway for the activation and development of lymphocytes. A third NF-κB pathway not involving IKK is activated by DNA-damaging drugs and UV-light.

10.5.5 Cytokine/Chemokine Profile

The immune response is rigorously controlled by interactions of a large number of cell types and their metabolites. One of the most extensive classes of such metabolites are the cytokines. These are signaling molecules which bind to specific receptors on many different cell types, including the cell from which it was secreted, in response to varied conditions. Cytokines control the immune response by intercellular communication and constitute an extensive and diverse class of short-lived proteins which include: interleukins (IL, signaling for leukocytes), chemokines (CC, chemotaxis of leukocytes), interferons (INF, response to viral infections and immune stimulation), colony-stimulating factors (CSF, stimulate cell proliferation), transforming growth factor (TGF, stimulates differentiation), and tumor necrosis factor (TNF, promotes cell death). Cytokines may act in a synergistic or antagonistic manner and regulate every aspect of the immune response (Tizard 2013).

Cytokines can be functionally divided into two groups: pro-inflammatory and anti-inflammatory (Berger 2000), based on the cytokine phenotype profile of specific T lymphocytes present and their physiological actions. This type of cytokine grouping and organization allows for a more comprehensive review of the cannabinoid effects on cytokine action.

T cells are subdivided into two subsets according to the kind of receptors they present on their surface: $CD4^+$, known as helper T cells, and $CD8^+$, known as the cytotoxic or "killer" T cells. $CD4^+$ are prominent cytokine producers, can give rise to a number of different cytokines, and thus are subdivided into the subgroups T_h1 and T_h2 according to their cytokine profile (Berger 2000). The typical immune response can also be divided in steps or categories along the inflammation process and is entirely mediated by the cytokine profile. The first and immediate response is the acute phase; cytokines present in this stage are rapidly triggered to induce the next set of mediators in order to restrain possible pathogens and eventually resolve the

inflammation process.

In ongoing inflammation, a second set of cytokines are induced by the first wave of acute phase mediators. These promote a very active inflammatory (delete: inflammation response and dramatically regulate cell-mediated immune reactions. Such cytokines belong to the Th1 response type. Acute phase and T_h1 cytokines are generally referred to as pro-inflammatory mediators (IL-1, IL-2, IL-6, IL-12, IL-17, TNF-α, IFN-α, IFN-β, IFN-γ). As inflammation progresses, there is an increased need to resolve the inflammatory response and effectively eliminate the cause of injury. T_h1 response is very aggressive at the cellular and tissue levels and cannot, or should not, persist for too long. A shift must occur towards a cytokine profile that causes less aggressive cellular damage and is involved in the ultimate recruitment of lymphocytes, such as B cells. Cytokines of the latter stage are the T_h2 type cytokines (IL-4, IL-5). Last, but definitely not least, every immune response should eventually cease. Resolution of inflammation involves a dramatic shift from pro-inflammatory mediators towards anti-inflammatory and suppressive ones. This last process involves regulatory T cells (T_{reg}) and other cytokines such as IL-10 and TGF-β. The pro-inflammatory T_h1 is often referred to as T_h1/T_h17, due to another prominent $CD4^+$ T cell subpopulation expressing high levels of IL-17 expression (T_h17), that triggers a strong inflammatory response as phagocytic and toxic as T_h1. It is important to note that every inflammatory process and cytokine profile has damage potential and it must never last for long. T_h1/T_h17 cytokines promote aggressive phagocytosis and tissue damage, whilst T_h2 cytokines, although less aggressive and phagocytic, can elicit hypersensitivity and allergies especially if prolonged (Tizard 2013).

The predominant ways cytokine signaling is regulated include:

1. Changes in receptor expression.
2. Specific binding proteins.
3. Cytokines that exert opposite effects (antagonism) (Tizard 2013)

Regulated gene expression of cytokines is crucial for accurate immune response. Several immune-related genes are regulated by transcription factors that are themselves regulated by the cAMP and MAPK signaling pathways (Massi et al. 2006). Cytokine production by macrophages and activation of lymphocytes also involve the cAMP signaling cascade (Tizard 2013). The ECS is involved, therefore, in the regulation of several transcription factors within immune cells, influencing the expression of several immune-related proteins including surface receptors (Massi et al. 2006), and as the overall modulator of the immune response (Tanasescu and Constantinescu 2010).

10.5.6 The T_h1/T_h17 and the T_h2 Inflammatory Responses

T lymphocytes (T cells) play a very important role in immunity as they participate in several types of immune response and intimately regulate the mechanisms of the

inflammatory process; they are the first and most powerful immune response after the physical barriers. As stated previously, $CD4^+$ T cells are subdivided into T_h1 and T_h2 cells by means of their cytokine repertoire. The ECS fine-tunes the Th1/T_h2 responses and should never be neglected in anti-inflammatory therapies.

10.5.7 T_h1/T_h17

The T_h1/T_h17 inflammatory response is characterized by an accumulation of pro-inflammatory cytokines (IL-1, IL-2, IL-6, IL-12, IL-17, TNF-α, IFN-α, IFN-β, and IFN-γ subtype) and results in powerful cellular inflammation with high phagocytic activity (Romagnani 2000). These T cells are mainly produced by macrophages, dendritic cells, B cells, and neutrophils. In order to comprehend the ECS and cannabinoid action on this population, it is important to be aware of some aspects of how T_h1/T_h2 polarization of naive T_h cells occurs. When IL-12 is present, differentiation of T_h1 cells is triggered and robust IFN-γ, TNF-α, IL-1, IL-6, IL-12, and IL-18 expression promotes further differentiation. Otherwise, naïve T_h cells undergo Th2 polarization characterized and driven by IL-4 and IL-13 (Tizard 2013). IL-12 rapidly induces activation of signal transducer transcription 4 (STAT4), crucial for transcription of a number of cytokine genes, including IFN-γ itself (Romagnani 2000). IL-18 signals via JNK and NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) and also contributes to augment IFN-γ expression and T_h1 differentiation. While IL-12, IL-18, and IFN-γ are the main drivers of T_h1 profile, T_h23 triggers the T_h17 profile which has similar cellular-mediated effects. CB2 receptor agonists reduce IL-12 production in the CNS immune cells (microglia) (Basu et al. 2011). Maresz et al. (2007) demonstrated that 10 mg/kg of THC was able to reduce experimental autoimmune encephalomyelitis (EAE) in a model for multiple sclerosis, acting via CB1 recptors on neurons rather than T cells, which responded via CB2 receptor agonism and reduced about 40% T cell proliferation and 60% INF-γ and IL-2 production. JWH-133-stimulated CB2 receptor enhanced activation of ERK1/2 in murine macrophages, decreasing IL-12 production and stimulating anti-inflammatory IL-10 cytokine production, which dramatically disrupts IL-12 production by itself (Correa et al. 2009). THC also suppresses IL-12 and IFN-γ contributing to reduced T cell proliferation (Jean-Gilles et al. 2010).

Interleukin 1β (IL-1β) is a powerful T_h1 proinflammatory cytokine and is involved in neurodegeneration, not only by means of the inflammatory cytokine network it stimulates, but also by increasing glutamate-mediated excitatory synaptic transmission whilst inhibiting GABA-mediated inhibitory stimulus (Rossi et al. 2015). In a similar manner, TNF released from activated microglia increased glutamatergic synapses. Both glutamate and GABA-mediated neurotransmission are effectively regulated by the ECS, as discussed in the chapters on cannabinoid action in nervous system conditions (Chap. 6). It is worth noting that IL-1β is capable of abrogating the inhibitory effects on GABA and glutamate

neurotransmission (De Chiara et al. 2013). THC and WIN-55,212-2 have been shown to reduce TNF-α production by alveolar macrophages (Sherman et al. 1991).

A recent study demonstrated enhanced neutrophil and monocyte recruitment in B6.129P2-Cnr2tm1Dgen/J mice (which will be referred to as CB2−/− mice moving forward) (Kapellos et al. 2019).The study also addressed gene expression profile of mice neutrophils finding that, compared to wild-type, CB2 receptor-deficient neutrophils overexpressed genes for: chemokine and chemokine receptors (CCR7, CCL-22, CXCL-10, CXCL-l3, and C-X-C motif chemokine receptor 1), cytokines (IL-5, IL-23a, IL-1α, and IL1-β), T-cell co-stimulatory molecules (CD40, MHC-II, class II antigen Eb, and CD86), inflammasome activation (NLR family pyrin domain containing 3—NLRP3), complement component (complement component 1q B chain), prostaglandin pathways (post-transcriptional gene silencing 2 and prostaglandin I2 receptor), and signaling pathways (PKC-b and TNF-α-induced protein 3). Genes related to pathogen recognition receptors (Tlr9), lectins (chitinase 3-like protein 3), and nitrite production (nitric oxide synthase 2—NOS2) were all downregulated in $CB2^{-\backslash-}$ neutrophils. These findings demonstrate that the lack of CB2 receptors in neutrophils induces them to overexpress chemotactic and inflammatory cell recruitment genes, thus altering the neutrophil signaling profile in the acute inflammatory response. Similarly, increased $CD4^+$ T cell proliferation, IL-2, and IFN-γ occurred in the central nervous system, and not in the spleen, of CB2 receptor-deficient mice (Maresz et al. 2007). Activation of the CB1 receptor does not seem to mediate this regulation on CNS T-cells since the CB2 receptor agonist JWH-133 was able to recover the cannabinoid anti-proliferative effects on T cells in CB1 receptor-defective mice, denoting evidence for CB2 receptor mediation. The $CB2^{-/-}$ T cells (CNS) showed a 50% increase in proliferation and 50% decrease in apoptotic cells. In contrast, such a difference was not observed in spleen T cells suggesting that T cell function within the CNS is controlled through the CB2 receptor. The authors pointed out that there is differential activation of CB1 and CB2 receptors in the spleen and CNS because of lower endocannabinoid concentrations in the spleen. Since exogenous CB1 receptor agonist administration was needed in order to perceive CB1 receptor-mediated effects, whilst lack of CB2 receptors alone was enough, the authors suggested that endogenous endocannabinoid levels are sufficient for CB2 receptor agonism, but not for activation of CB1 receptors. Such evidence paves the way for CB2 receptor-mediated immune suppression by raising endocannabinoid levels in the CNS and/or upregulating CB2 receptor expression on T cells (Maresz et al. 2007). Lee et al. (1995) found missed effects of 2-AG on T cell proliferation; at high cell density proliferation was enhanced, as opposed to marked inhibition at low cell density. This might be due to greater metabolization of 2-AG into arachidonic acid (because of the high number of active cells) that can be a source of prostaglandin E_2 (PGE_2). This prostaglandin activates adenylate cyclase which raises cAMP levels and leads to induction of the MAPK/ERK/JNK/p38 pathway, leading us back to the cAMP – MAPK controlling pathway discussed above. In keratinocytes, CB1 receptor agonism inhibits the activation of Th1 and Th17 T cell responses, by IL-12 and IL-23 inhibition by anandamide, limited CXCL10 and CCL8 pro-inflammatory chemokines and thus overall attenuates T cell inflammation

and negatively regulates Th1/Th17 polarization in the skin (Chiurchiù et al. 2016). This also attenuates some cases of allergies since CCL8 promotes Th2 allergic inflammation. Dendritic cells, the main producers of IL-12 and IL-23 in the skin, also showed depletion of cytokine levels on CB1 stimulation, endorsing the role in regulating Th1/Th17 polarization by CB1 receptor agonism (Chiurchiù et al. 2016).

10.5.8 Th2

Dendritic cells that do not secrete IL-12 promote IL-4, IL-5, IL-9, IL-10, and IL-13 expression; this leads to a phagocytic-independent inflammation and shifts towards a powerful antibody-driven immune response (Tizard 2013; Romagnani 2000). These T cells and the profiled cytokines are defined as the Th2 subset. The polarization of Th2 response within T cells is mediated by IL-4 early expression and binding to TCR in naive Th cells during an immune response (Romagnani 2000). Concentration of IL-4 increases as other lymphocytes are activated and Th2 differentiation occurs. Rather than STAT4, as in Th1/Th17 polarization, STAT6 transcription factor signaling pathway is activated promoting Th2-inducing cytokines and activating the transcription factor GATA3. The latter promotes IL-4, IL-5, and IL-13 expression and drives most of the Th2 polarization forces. STAT6 knockdown mice showed deficient Th2 response. IL-10 is a potent inhibitor of IL-12 Th1 (Peritt et al. 1996) and IL-23 Th17 polarization in its own way. Even Th1 T cells, Natural Killer (NK) cells (Peritt et al. 1996), and microglia (Correa et al. 2010) expressing IL-12 induce IL-10 expression as a negative feedback loop. Thus, IL-10 acts as a potent Th2-promoter cytokine and increases IL-4 expression, further hampering Th1/Th17 polarization while promoting Th2 differentiation.

Anandamide (AEA) strongly upregulates IL-10 production via CB2 receptors by different pathways. It activates ERK1/2 and JNK MAPK signaling pathways, which leads to enhanced IL-10 expression, and downregulates IκB phosphorylation which in turn halts NF-κB ability to reach the transcription sites in the DNA. It has also not been ruled out that AEA-activated CB2 receptor agonism raises IL-10 levels by an independent, unknown mechanism (Correa et al. 2010). These authors argue that either sustained levels of AEA, by inhibition of its hydrolysis by FAAH and/or CB2 receptor agonist stimulation, could pave the way to therapeutic procedures that balance the IL-12/IL-23/IL-10 profile and thus provoke accumulation of anti-inflammatory cells at the injury site. This rationale makes perfect sense if one considers that the very definition of Th1/Th17/Th2 may be not discrete subsets but rather a continuous spectrum that drives diverse leukocytes' immunological states towards one extreme phenotype (e.g. T_h1) or to the other (T_h2) by means of a relative abundance of polarization-inducing cytokines and chemokines. This rationale is supported by the known interchangeable phenotype seen on some T_h1 lymphocytes that shifts to T_h2 phenotype after IL-4 stimulation or T_h2 T cells shifting towards T_h1 phenotype as consequence of IL-12 exposure (Tizard 2013). Also, a single T cell can present both T_h1 and T_h2 cytokine profiles and are so-called T_h0 T cells. These are

more commonly observed in the initial stages after immune response initiation and are decreased as the immunological response extends and enters either cytokine profile (Tizard 2013), supporting the fact that T_h0 cell may be a precursor to T_h1, T_h17, and T_h2 and that the ECS can be a major player in regulating these polarizations.

10.6 Phytocannabinoids' Effects on Immune-Related Mechanisms

Cannabinoids, such as THC and CBD, act by the same mechanisms (and others) described above. There is quite enough evidence on the potent anti-inflammatory properties of THC and CBD. Little is known about the other 100+ known cannabinoids and even less so for the uncountable terpenes.

10.6.1 Δ9-Tetrahydrocannabinol (THC)

THC has potent anti-inflammatory activity. It interacts with several cannabinoid and non-cannabinoid receptors such as CB1, CB2, GPR55, PPARγ, TRPV1, and others. Reviews from 2016 and 2017 (Turcotte et al. 2016; Oláh et al. 2017) summarize the known effects, thus far, of cannabinoids within the immune system. THC inhibits activation of $CD4^+$ T cells and inhibits IL-12 expression by CB1 receptor interaction while upregulating GATA-3 via CB2 receptor agonism, inducing a $T_h1 \rightarrow T_h2$ shift. It was also shown to: decrease IL-2 and IFN-γ secretion, macrophage migration, monocyte recruitment, and splenocyte proliferation; diminish antibody formation and T_h2 cytokines in T cells; impair inflammatory response; and induce apoptosis in macrophages and T cells via Bcl-2, caspase and NF-κB-dependent apoptosis. The acid form of THC, THCA, also possesses anti-inflammatory, immunomodulatory, neuroprotective, and anti-tumor effects but more research is needed to determine if THCA works by the same mechanisms as THC.

10.6.2 Cannabidiol (CBD)

CBD has been the most commonly accepted cannabinoid for medical purposes since it, along with THC, is one of the most abundant cannabinoids in *Cannabis* and, most importantly, lacks the strong intoxicating effects of THC. Like THC, the pharmacological properties of CBD are widespread through diverse pathological conditions and many of them involve immune regulation. Although CBD is a weak agonist at CB1 and CB2 receptors, it interacts with many receptor-independent mechanisms and can act as a CB1 receptor antagonist (reviewed in Russo and Marcu 2017).

CBD inhibits IL-6, TNFα, IL-8, and MIP-1α and MIP-1β chemokines. It also suppresses IL-2 and IFN-γ and their critical transcription factors (AP-1, NFAT) by CB1 and CB2 receptor-independent mechanisms, while promoting IL-10 expression. This leads to stimulatory effects on T regulatory cells (T_{reg}) while exerting antiproliferative effects in $CD4^+$ T cells and thus attenuating the response, antigen presentation, and leukocyte transmigration. CBD also promotes antioxidant effects by inhibition of nitric oxide production.

The cytokine profile promoted under CBD regulation was reported to be beneficial in Type I diabetes, which is in congruence with the therapeutic intentions for this disease: lower T_h1 and raise T_h2 cytokines levels (Weiss et al. 2008). In this particular case, IL-6 inhibition is of great importance.

10.7 Immunological Entourage Effect

Although little is known about the pharmacological benefits of THC and CBD, and the other *Cannabis* spp. components (including terpenes and flavonoids), we do have enough certainty that, in cannabinoid therapy, and concerning which cannabinoid(s) to use, that "more is better". As cited previously, the entourage effect that occurs with whole cannabis flower-derived products can deliver much better results as each cannabinoid individually acts on different pathways synergistically, some of them activating, others inhibiting, allowing the net result to fall somewhere in or near homeostatic range. Virtually all immune-related influences of the ECS, and therefore cannabinoids, are accomplished via different signaling pathways shared among several biological routes. Control of adenylate cyclase gives power of the cAMP signaling cascade which regulates MAPK/ERK/JNK/p38 and NF-κB signaling. Control over these signaling cascades affects many orders of gene expression of straightforward immune-related genes (e.g. cytokines and chemokines) or indirect immune-related genes (such as growth factors, cyclins, transcription factors, cytoskeleton and extracellular matrix proteins) that regulate cell cycle and migration processes, which are so crucial to leukocyte proliferation and recruitment. The ECS tightly controls ion channels and therefore thousands of different results can be achieved, from inhibiting to stimulating metabolic pathways. The ECS controls the cytokine and chemokine repertoire in possibly all immune cells and thus could be the main force driving T_h1, T_h17 or T_h2 polarization. Cannabinoid actions, regulating the very early stages of cell proliferation, differentiation, recruitment, mobility, and the overall cytokine network, promise great future opportunities for fine-tuning immune responses as we advance our knowledge of the ECS and its regulation by endo-, phyto-, or synthetic cannabinoids.

10.8 Clinical Use of Cannabinoids in the Cancer Patient

Cannabinoids have been used as medicine for almost 5000 years. There is evidence that the ancient Chinese, Egyptians, and Southeast Asians used cannabis to treat a variety of conditions including inflammation, GI disorders, glaucoma, and others. During the last decade, numerous studies have been published suggesting that endocannabinoids, phytocannabinoids, and synthetic cannabinoids have anti-neoplastic activity. There have been several preclinical studies that reveal evidence of the ECS being involved in the process of cancer formation and metastasis.

Below is a detailed summary of the current knowledge of cannabinoids and cancer based on preclinical and clinical research. It is organized based on individual tumor type. In addition, veterinary academic laboratories are currently examining cell-based studies surrounding cancer cell survival. Interactions with chemotherapy and mechanisms surrounding cell death will be discussed based on available preliminary results.

10.9 Brain Tumors

Glioblastoma multiforme (GBM) is the most common malignant brain tumor and, biologically, is one of the most aggressive cancers that occur in people. Due to its invasive and resistant properties, the median survival time is typically 12–15 months with resection, surgery and chemotherapy (Likar and Nahler 2017). Interestingly, when normal cells transition to malignancy, they tend to express more cannabinoid receptors, which potentially makes them more sensitive to cannabinoid therapy. In most brain tumors the ECS is unregulated and appears to be under epigenetic control (Likar and Nahler 2017; Sánchez et al. 2001; Cudaback et al. 2010).

As reviewed in other sections of this chapter, cannabinoids have multiple anti-neoplastic mechanisms. One of these involves inhibition of tumor cell migration via inhibition of matrix metalloproteinase (MMP) expression (specifically MM-2 and MMP-9). This was proven in cell cultures, in mice bearing gliomas, and in two human patients with glioblastoma multiforme. THC induces apoptotic death in glioma cells mostly via CB1 and CB2 receptor-dependent stimulation. Although CBD has weak affinity for both CB1 and CB2 receptors, it kills glioma cells via other pathways, including: anti-inflammatory effects (inhibits both COX and LOX), enhancement of reactive oxygen species, action on TRPV2 channel receptors, transcription inhibition of tumor-related genes such as Midkine (MDK), and DNA-binding protein inhibitor ID-1 (an inhibitor of basic helix-loop-helix transcription factors) (McAllister et al. 2007). The ID-1 gene is overexpressed in multiple different tumor types, such as gliomas, and plays a role in tumor invasiveness (Likar and Nahler 2017). In addition to the anti-neoplastic effects of CBD and THC, both cannabinoids play a role in palliative treatment for brain tumors, acting specifically against nausea, vomiting, pain, anxiety, and sleep disturbances (Likar and Nahler

2017). In glial tumors, initial in vitro studies show synergy of both CBD and THC via multiple anti-neoplastic mechanisms including: inhibition of cell proliferation, modulation of the cell cycle, induction of reactive oxygen species (ROS), promotion of apoptosis, and modulation of extracellular signal-regulated kinase (ERK) and caspase activities. Interestingly, these effects were not observed with either compound individually (Marcu et al. 2010).

Based on a 2000 publication showing antineoplastic effect with intratumoral administration of THC in malignant gliomas in rats, a similar trial was conducted in humans (Galve-Roperh et al. 2000). A clinical phase 1 pilot study evaluated nine patients with recurrent glioblastoma multiforme receiving THC intratumorally. All patients in the study failed previous treatments including surgery and radiation therapy. The delivery method was safe without intoxicating effects. Steroid administration was used to help control cerebral edema, and there was no apparent drug interaction between THC and steroids. Median survival time from the beginning of cannabinoid administration was 24 weeks. Two of the patients survived for approximately 1 year (Guzmán et al. 2006). Due to the small sample size, the effect of THC on survival time is unclear. Although intratumoral delivery of THC may allow for high concentrations of localized therapy in the brain, it is extremely invasive and therefore, is not recommended in veterinary patients.

Historically, temozolomide has shown promising results as a treatment for malignant refractory gliomas (Stupp et al. 2005). Treatment regimens combining CBD and DNA-damaging agents (temozolomide, carmustine, or cisplatin) produced synergistic antiproliferative and cell-killing effects in both human GBM and mouse GBM cell lines (Stupp et al. 2005; Deng et al. 2017). GW Pharmaceuticals set out to evaluate the combination of cannabinoids along with temozolomide therapy in a proof of concept study. It was a phase II, randomized, placebo-controlled clinical trial evaluating 21 patients with recurrent GBM. Twelve patients received dose-intense temozolomide along with Nabiximols (1:1 THC:CBD oral spray - Sativex®) while nine patients were randomized to receive placebo plus standard of care. The results from this exploratory study showed that patients treated with combination therapy had an 83% 1-year survival rate compared with 53% for patients in the placebo group. The median survival time for the combination group was 550 days (vs. 369 days for the placebo group). Nabiximols was generally well tolerated (GW Pharmaceuticals 2017).

A subsequent study revealed that CBD and THC prime glioma cells to respond better to ionizing radiation therapy (RT). Cells pre-treated with a combination of THC and CBD for four hours prior to irradiation showed increased radiosensitivity compared to pre-treated cells exposed to either cannabinoid individually. The additive effect of cannabinoids to radiation therapy was associated with increased markers of autophagy and apoptosis (Scott et al. 2014). This was supported in vivo as well, showing that *combining* cannabinoids and radiation therapy in glioma implanted mice was a successful treatment strategy. When CBD and THC (at a 1:1 ratio) was given intra-peritoneally 16 days post implantation, along with

radiation therapy, there was a >85% decrease in tumor volume. This was noted 21 days post therapy. There was little response to RT alone (Scott et al. 2014).

10.10 Prostate Cancer

Prostate cancer is the second most common cancer found in men in the United States and is the second leading cause of cancer related deaths (Ramos and Bianco 2012). Prostate glandular epithelium expresses multiple components of the ECS; overexpression of many ECS components correlates with both grade and progression of this cancer (Díaz-Laviada 2011). Activation of CB1 and CB2 receptors in prostate carcinoma cells results in apoptosis and cell cycle arrest via activation of PI3K/Akt pathway with subsequent triggering of RAF1/ERK1/2 and nerve growth factor induction. Also, cannabinoid agonists produce a reduction in both androgen receptor expression as well as prostate specific antigen expression (and secretion) (Nithipatikom et al. 2004; Sarfaraz et al. 2006).

THC induces apoptosis in androgen refractory, metastatic human prostate cell lines in a dose dependent fashion, but completely independent of cannabinoid receptors (Ruiz et al. 1999). A 2013 study (in vitro and in vivo) evaluated multiple non-THC cannabinoids; CBD was the most potent cannabinoid to delay prostate carcinoma cell proliferation and trigger apoptosis. This process was partially mediated by antagonism of TRPM8 as well as the production of reactive oxygen species (ROS). It was also suggested that CBD might help reverse the conversion of the androgen-dependent phenotype to the more resistant androgen-independent form. Lastly, the same study proved CBD potentiated the anti-cancer effect of some common drugs used to treat prostate cancer (chemotherapy drug docetaxel and anti-androgen drug bicalutamide) (De Petrocellis et al. 2012).

10.11 Melanoma

Melanoma is the cause of the largest number of skin cancer related deaths in the US as well as internationally (Blazquez et al. 2006). Cannabinoid receptors (CB1 and CB2) are present in both normal skin as well as in skin tumors (Blazquez et al. 2006). A 2015 study revealed that THC administration resulted in activation of autophagy and apoptosis in melanoma cells (Armstrong et al. 2015). This same study administered equal parts THC and CBD to mice with BRAF wild-type melanoma xenografts and there was a substantial inhibition of melanoma viability, proliferation, and tumor growth.

An earlier study showed that, when these cannabinoid receptors were activated, apoptotic death of only the tumor epidermal cells occured (sparing the normal skin cells) and tumor proliferation was reduced (via cell cycle arrest at G1-S secondary to inhibition of pro-survival protein Akt) (Blazquez et al. 2006). During the in vivo portion of the study, cannabinoid administration inhibited melanoma progression,

angiogenesis, and metastasis. When a selective CB2 receptor agonist was administered malignant tumor growth was inhibited in nude mice (Blazquez et al. 2006). It is important to note that β-caryophyllene is a potent CB2 receptor agonist; therefore, finding chemovars with high levels of this particular terpene may potentially add beneficial effect in melanoma cases. β-caryophyllene was found to have anti-tumor effects in mice with melanoma. This is further discussed, below, in the terpene section (10.19.).

10.12 Pancreatic Cancer

Pancreatic cancer is one of the most aggressive cancers that exist and represents the fourth major cause of cancer related death in the United States (Sharafi et al. 2019). It is often times referred to as a "silent killer" because symptoms do not show up until the late stages of disease and the prognosis is grave at that point. Cannabinoid receptors are highly expressed in pancreatic cancer tissue versus the normal pancreas. Both THC and CBD appear to produce antiproliferative, anti-invasion, proapoptotic, and anti-angiogenic effects via cannabinoid receptors (CB1, CB2 and GP55) as well as receptor-independent pathways (Sharafi et al. 2019). Gemcitabine and cannabinoid receptor agonists trigger autophagy of pancreatic cancer cells via increased production of ROS and inhibition of GP55, resulting in antiproliferative effects (Donadelli et al. 2011; Ferro et al. 2018). This combination therapy opposed the mechanisms that lead to drug resistance to gemcitabine. Gemcitabine induced both cannabinoid receptors (CB1 and CB2) by nuclear factor-kappaB (NF-κB) dependent mechanisms (Donadelli et al. 2011). Cannabinoids, specifically CBD, substantially increased apoptotic effects and improved survival time when combined with gemcitabine (Sharafi et al. 2019). Cannabinoid receptor activation decreases inflammatory mediators (such as IL-6, IL-10 and TNF-a) which are responsible for the formation of inflammation and fibrosis associated with chronic pancreatitis. There is some evidence that there is a correlation between chronic pancreatitis and the development of pancreatic cancer (Dhar et al. 2015).

10.13 Lung Cancer

Lung cancer is one of the most common cancers in the world and is the leading cause of cancer related death in the United States. Cigarette smoking, high levels of pollution, as well as exposure to asbestos or radiation increase the risk. The first report of antiproliferative effects of cannabinoids came from Munson et al. in 1975 where it was shown that oral administered Δ9THC, Δ8THC, and CBN inhibited Lewis lung adenocarcinoma cell growth in vitro cell lines and in a murine model. CBD did not demonstrate any growth inhibition effect in this study (Munson et al. 1975). Lung tumors that overexpress epidermal growth factor receptor (EGFR) tend

to be biologically more aggressive and chemo-resistant. A 2007 study confirmed that cell lines from primary lung tumors express CB1 and CB2 receptors and that THC inhibited EGF-induced growth in these cell lines. In addition, THC reduced the EGF-stimulated cancer cell migration and invasion.

The next portion of the study evaluated SCID mice that had metastatic lung cancer implanted and were then administered THC (5 mg/kg) once daily, intraperitoneally for 21 days. The tumors decreased in weight and volume by 50% and had reduction in macroscopic lung surface lesions by 60% in THC treated mice compared to the non-treated mice. Immunohistochemical analysis of the tumor samples from the THC treated mice revealed anti-proliferative and anti-angiogenic effects of THC by demonstrating significant reduction in staining patterns for Ki67 (a proliferative marker) and CD31 (an endothelial marker associated with angiogenesis). THC inhibited EGF-induced AKT phosphorylation, which regulates a multitude of cellular functions such as proliferation, angiogenesis, invasion and apoptosis (Preet et al. 2007).

CBD also plays a role by inhibiting cell invasion and metastasis via increasing levels of tissue inhibitor matrix metalloproteinases-1 (TIMP-1) (Ramer et al. 2012). In addition, a 2013 study established CBD had proapoptotic effects on human lung tumor cell lines via modulation of COX-2 and PPAR-$_{\gamma}$ and caused tumor regression in vivo (Ramer et al. 2013).

10.14 Lymphoma and Leukemia

Previous studies demonstrate that THC and other cannabinoids induce apoptosis in murine and human leukemia and lymphoma cell lines, as well as primary acute lymphoblastic leukemia (ALL) cells, as early as four hours following exposure. This differs from what was found in non-lymphoid tumors as previous studies found it took 2 or more days for cannabinoids to induce apoptosis. THC is effective in vivo to induce apoptosis, decrease tumor load, increase survival time, and in curing a significant proportion of LSA tumor bearing mice (McKallip et al. 2002).

Non-Hodgkin's lymphoma is the most common hematological malignancy found in humans, accounting for 85% of all lymphoma cases. Gene expression data revealed that B-cell lymphoma is one of the top three cancers (along with glioma and gastric tumors) that exhibit high expression of CB1 and CB2 receptors (Pham et al. 2016). A 2016 study showed that CB1 receptors are highly expressed in non Hodgkin's-B cell LSAs (including mantel cell lymphoma (MCL) and diffuse large B-cell lymphoma) and that targeting CB1 receptors leds to cell growth inhibition. A specific study found that MCL tumors express high levels of CB1 receptors and moderate levels of CB2 receptors. It also concluded that cannabinoid receptor ligands induce decreased viability, growth suppression, and cell death by apoptosis and should be considered an anti-neoplastic agent in the treatment of MCL (Flygare et al. 2005).

A previous study that evaluated the effects of an endocannabinoid (anandamide) analog on certain lymphoid malignancies revealed that it induced cell death in MCL and chronic lymphocytic leukemia (CLL) but not in Burkitt lymphoma cell lines. The in vivo portion of the study showed the anandamide analog caused significant reduction of tumor size and mitotic index in mice xenografted with human MCL. These results suggest that therapies using cannabinoid receptor ligands (such as certain phytocannabinoids or endocannabinoid analogs) have efficacy in reducing tumor burden in malignant lymphoma overexpressing CB1 and CB2 receptors (Gustafsson et al. 2008).

A 2017 published study demonstrated that CBD reduced cell viability in human leukemic cell lines (although incompletely) and impeded cell cycle activity by inhibiting cells from entering the G2/M phase; CBD also reduced cell size (a reduction which persisted post treatment) (Kalenderoglou et al. 2017). A case study published described a 14-year old girl diagnosed with an aggressive form of ALL (positive for the Philadelphia chromosome mutation). She failed standard chemotherapy, radiation therapy, and a bone marrow transplant after 3 years of therapy and, without any other available conventional therapy, the family started to administer cannabinoid extracts. Cannabinoid extracts have been shown to be effective in the treatment for ALL that are Philadelphia chromosome mutation positive. Her blast count peaked at 374,000 (4 days after starting cannabinoid resin) and on day 5 of therapy her blast count started to decrease. She started with once a day dosing and slowly increased the dose up to a total of three times a day. Her blast levels continued to wax and wane due to variance in the potencies of cannabinoids in different strains (as they were unable to get the same product each time). After 27 days of treatment, she had a significant decrease in blast count which caused her to develop tumor lysis syndrome and require allopurinol administration. Day 78 after starting cannabinoid therapy, she passed away from neutropenic colitis with perforation. Her total white blood cell count (WBC) was 1.4 at that time. The factors that were associated with increased leukemic blast counts in this case were dosing intervals >8 hours, exposure to less potent strains, or suboptimal therapeutic dosing. More research needs to be performed to determine the chemovar and ratios needed to provide a consistent anti-leukemic therapeutic profile (Singh and Bali 2013).

10.15 Breast Cancer

Breast cancer is the second most commonly diagnosed cancer among women in the United States (Blasco-Benito et al. 2018). Although early detection and the development of newer therapies have improved patient outcomes, many patients develop resistance or are unresponsive to conventional therapies.

Pre-clinical research demonstrated that cannabinoids produce an antitumor response in breast cancer. Dr. Cristina Sanchez in Madrid compared THC isolates to THC rich plant extract in vitro. Both formulations had anti-tumor properties, but THC rich plant extract was more potent than the THC isolate for the three different

breast cancer subtypes (ER+/PR+, HER2+, and triple negative breast cancer lines) (Blasco-Benito et al. 2018).

Specifically for hormone sensitive breast cancer, THC's anti-tumor effect in HER2-positive breast cancer was mediated by the CB2 receptor. CB2 receptor activation results in dimerization with HER2; that bond has been associated with poor treatment outcome in breast cancer. When THC binds to the CB2 receptor, it breaks the CB2-HER2 dimer, which results in downstream signaling, causing tumor regression (Blasco-Benito et al. 2019).

The combination of cannabinoids with estrogen receptor therapy, HER2 targeted therapy, or with chemotherapy (cisplatin) produced an additive antiproliferative response in cell culture, with up to 25% more anti-tumor effect. However, the same did not hold true in vivo. This study emphasized the importance of the entourage effect by demonstrating that the THC rich extract cannabis oil with multiple compounds produced a more efficacious therapeutic response than did pure THC (Blasco-Benito et al. 2018).

A previous study unexpectedly found that CBD (followed by cannabigerol (CBG) and cannabichromene (CBC)) acted as a more potent inhibitor of cancer growth than THC. Both pure CBD and CBD-rich extract were confirmed to have anti-tumor effects in vivo in mice bearing highly invasive breast cancer xenografts. Furthermore, both compounds inhibited the formation of lung metastasis after inoculation with breast cancer cells (Ligresti et al. 2006). The mechanism behind CBD's inhibition of growth, invasion, and metastasis of breast cancer cells is due to induction of ROS as well as downregulation of Id-1.

10.16 Colorectal Cancer

Colorectal cancer is one of the major causes of cancer related death in the world and is the third most commonly diagnosed cancer in both men and women in the United States. Since the mid 1980s, survival rates have improved which is mostly attributed to increased awareness and improved screening. Due to the high incidence of colorectal cancer, it is essential to develop more protective strategies and novel treatment options for this disease.

Previous data demonstrates endocannabinoids inhibit the proliferation of colorectal cancer cell lines via CB1 receptor activation (Ligresti et al. 2003). Anandamide and other endocannabinoids are present throughout the gastrointestinal tract and have the ability to control cell proliferation. These endocannabinoids are transported into the cell and can be metabolized by cyclooxygenase 2 (COX-2). COX-2 is overexpressed in the majority of colorectal cancers which is associated with the promotion of tumorigenesis, angiogenesis, and immune modulation (Eberhart et al. 1994; Patsos et al. 2005). Anandamide significantly inhibited tumor cell growth and induced cell death (in a non-apoptotic fashion) in COX-2 expressing colorectal cell lines, and had no effect on the low COX-2 expressing cell lines (Patsos et al. 2005). Anandamide (or potentially a similar acting

phytocannabinoid) may prove to be beneficial in treating tumors that have developed a resistance to apoptotic death.

Both CB1 and CB2 receptors are expressed in human colorectal adenoma and carcinoma cells and THC induces apoptosis of colorectal cancer cells (Greenhough et al. 2007). A subsequent study confirmed CB2 receptors have stronger expression in colon cancer tissue (compared to CB1 receptors which are mostly found in normal colonic epithelium). However, the activation of both receptors induced apoptosis via TNF-α production in colon cancer cell lines. This study proved that TNF-α acts as a link between cannabinoid receptor activation and ceramide production (which is a proapoptotic lipid) (Cianchi et al. 2008).

10.17 Liver Cancer

The two most common malignant hepatic tumors found in people are hepatocellular carcinoma (HCC) and cholangiocarcinoma. In dogs, the most common form of hepatic cancer is HCC. A 2006 study revealed the expression of both CB1 and CB2 receptors was increased in HCC tissues and that was associated with improved prognosis and survival rates (Xu et al. 2006). THC and a synthetic CB2 receptor agonist reduced tumor growth and eliminated ascites in two different HCC derived tumor xenografts. The anti-tumor effect was confirmed to be mediated by activation of the CB2 receptors followed by increased ceramide levels and PPAR-γ activity causing induction of autophagy (Vara et al. 2011; Vara et al. 2013). Anandamide reduced growth in cholangiocarcinoma xenografts and inhibited angiogenesis (Ladin et al. 2016).

10.18 Thyroid Cancer

Thyroid cancer is the most commonly diagnosed endocrine malignancy in the United States. The few studies evaluating cannabinoids in thyroid cancer confirmed that the ECS was expressed within the thyroid gland and that stimulation of CB1 receptors inhibited thyroid cancer cell growth and angiogenesis (via inhibition of vascular endothelial growth factor) (Portella et al. 2003).

10.19 Terpenoids and Cancer

Terpenes exert anti-tumor effects in vitro as well as in vivo against a variety of tumors. Some examples are described below:

d-Limonene (Miller et al. 2011; Sobral et al. 2014; Cho et al. 2017; Lu et al. 2004; Chen et al. 2015)
This monoterpene has immunomodulatory and anti-proliferative effects as well as protective effects against chemical-induced tumors in a variety of tissue types such as breast, pancreas, intestine, liver, and colon. Lu et al. revealed d-Limonene inhibited the proliferation of human gastric cancer cells by inducing apoptosis (via activation of caspases and suppression of the PI3K/Akt pathway).

Pinenes (Cho et al. 2017; Jin et al. 2010; Kusuhara et al. 2012; Li et al. 2009)
α-pinene, a monoterpene, has been shown to have proapoptotic and anti-metastatic action in a melanoma model. When investigated in human hepatoma cell lines, it demonstrated anti-proliferative actions via induction of G2/M cell cycle arrest. α-pinene can also trigger oxidative stress signaling pathways. Mice that were kept in an environment enriched with α-pinene showed reduction in melanoma tumor size. Evaluation of β-pinene also revealed moderate cytotoxicity against cancer cell lines.

Myrcene (Cho et al. 2017; Saleh et al. 1998)
Myrcene is another monoterpene that exhibits significant antiproliferative and cytotoxic effects against multiple tumor cell lines such as breast cancer, human cervical carcinoma, human lung carcinoma, human colon adenocarcinoma, and leukemia.

Caryophyllenes (Cho et al. 2017; Dahham et al. 2015; Jung et al. 2015)
α-caryophyllene, a sesquiterpene, also known as humulene, has anti-proliferative effects in solid tumor cell lines. It has also been shown to inhibit lymph node metastasis in obese mice fed a high fat diet.

β-Caryophyllene exhibited potent anti-proliferative, anti-migratory, anti-invasive, and pro-apoptotic effects against colorectal cancer cells. Similar to its sister compound, humulene, it also inhibited solid tumor growth and lymph node metastasis of melanoma cells in obese mice.

p-Cymene (Cho et al. 2017; Li et al. 2016)
This monoterpene demonstrates inhibition of tumor invasion and metastasis via mechanisms that include the inhibition of MMP-9 expression and the augmentation of TIMP-1 production along with the suppression of ERK1/2 and p38 MAPK signal pathways in tumor cells.

10.20 Combination of Cannabinoids and Chemotherapy

Both CBD and THC enhance the cytotoxic effect of several chemotherapeutics including some vinca alkaloids (vinblastine, vincristine, and paclitaxel), anthracyclines (doxorubicin, mitoxantrone, irinotecan), antimetabolites (cytarabine, gemcitabine), and alkylating agents (carmustine, temozolomide, and cisplatin) (Ramer and Hinz 2017). Both THC and CBD increase cytotoxic effect of vinblastine in resistant leukemia cells via downregulation of p-glycoprotein (Holland et al.

2006). Anandamide enhances paclitaxel induced apoptosis effect in gastric carcinoma cell lines (Miyato et al. 2009). THC enhances cytostatic properties of cytarabine, doxorubicin, and vincristine in leukemia cells (Liu et al. 2008).

10.21 Veterinary Cell Culture Studies

There is a paucity of data in preclinical and clinical disease for cannabinoid use and activity, with the primary evidence coming from cell culture systems of specific cancer types and preclinical tumor induction or xenograft models. Additionally, many of the preclinical models suggest very high concentrations of cannabinoids may be necessary for anti-tumor activity. In veterinary oncology the most prevalent tumors may not mimic their human counterparts in incidence and biological activity, making it imperative to study veterinary species from the "ground up", starting with cell culture studies trying to understand preliminary dose required, chemotherapeutic interactions, and mechanisms of action.

Unlike human medicine, where significant work is being done on THC, CBD, and other cannabinoids, the legal landscape in veterinary medicine is lagging behind human use so there is little research being done on THC in the veterinary arena, with more work currently being done with CBD derivatives. Although much of the research is being done on inflammation and pain, there is growing interest in CBD for cancer and potentially cancer-related side effects including nausea, cancer pain, and appetite stimulation.

Current cell culture studies being done at Cornell University (Wakshlag personal communication) have provided a preliminary view into CBD's potency as an apoptotic agent, and its use with standard chemotherapies including doxorubicin and vincristine. The first pertinent finding was that CBD could induce cell death at concentrations between 1 and 5 ug/mL in 48-h growth and proliferative assays, while cannabidiolic acid (CBDA) could not induce apoptosis, suggesting that there were targets for CBD that a simple carboxylic acid moiety on CBD could hinder. More interestingly, when similar concentrations of CBD and CBDA were used in tandem from a whole plant extract, the dose for effective inhibition of cell growth could be decreased by approximately half. Whether this was due to the addition of CBDA with CBD in tandem or the modest terpene levels in the plant extract could not be fully elucidated. Regardless, this study suggests some synergy between molecules in the whole hemp extract.

Of the five cell lines studied in some of this work (3 osteosarcoma's, 1 mammary carcinoma, and 1 lymphoma) the effects of CBD were rather universal across cell lines. When using 10 ug/mL or more of CBD, cell death could be achieved through apoptotic mechanisms. However, autophagy induction appears to precede apoptosis in the three cell lines examined. Further studies to examine whether this pre-apoptotic effect was due to mitochondrial damage/mitophagy and apoptosis are being studied, but alterations in proapoptotic and apoptotic mitochondrial

proteins, such as Bcl-2 and Bax, do not appear to be influenced. It is well recognized that cell signaling may be part of the events surrounding apoptosis and, like other cell culture studies, all three of the cell lines showed an increase in MAP kinase signaling. Exactly how these are activated (likely through GP55 or other orphan g-protein receptors) is currently being examined. The most robust signaling appears to be through sustained extracellular regulated kinase (ERK) and c-jun N-terminal kinase (JNK) activation.

Of particular note are the preliminary results surrounding the interaction between common chemotherapies in the three canine cell lines examined (osteosarcoma, mammary carcinoma, and lymphoma). Doxorubicin and CBD treatment across all cell lines appears to show a mild synergistic to additive effect when both are at higher concentrations; when both are at lower concentrations there may be some mild antagonism. When treating cells with CBD and vincristine there is a clear synergistic effect, at doses that hindered cell proliferation or induced cell death for all cell lines. These findings need to be expanded across the wide array of chemotherapeutics and canine and feline cancer cell types to better understand if and how CBD can be used. As of now, the concentrations in cell culture that are necessary to induce cell death or hinder cell proliferation appear to be between 1 and 10 ug/mL, with the highest serum concentrations documented are around 0.5–1 ug/mL, using oral dosing between 2 and 10 mg/kg body weight (Bartner et al. 2018; McGrath et al. 2019). This leaves a slight gap between what appears to be effective in vitro and what can be reasonably delivered in vivo based on simple pharmacokinetics, further suggesting the need for a better understanding of dosing and preclinical xenograft modeling as a next step to potential in vivo aspects for treating canine and feline cancer with CBD, hemp extracts, and other cannabinoids.

10.22 Conclusion and Perspective

The above review of the human literature and veterinary cell culture studies has demonstrated that cannabinoids exert an extensive array of anticarcinogenic properties such as antiproliferative, induction of apoptosis and autophagy, anti-invasive, anti-metastatic, immunomodulatory effects, and synergy with conventional therapies. THC has also been shown to inhibit epithelial to mesenchymal transition in endometrial cancer (Zhang et al. 2018).

Cannabinoids may play an integral role in treating veterinary cancer patients in both a definitive and palliative setting. Incorporating cannabis products can help palliatively by reducing adverse effects seen with chemotherapy including nausea, vomiting, inappetence, and depression but it can also be used to work synergistically with conventional therapies in an effort to increase the anti-tumor therapeutic potential. It is the author's experience that epithelial tumors tend to respond best to cannabinoid therapy. Interestingly, the majority of the tumors that were presented above are carcinomas. Some potential theories include:

1. Perhaps epithelial tumors tend to have altered levels of cannabinoid receptors, endocannabinoids, or degrading enzymes compared to other tumor types; therefore the addition of phytocannabinoids can help overall to balance the system.
2. Overexpression of COX-2 is common in carcinomas and COX-2 is a target in which endocannabinoids can induce apoptotic cell death. This mechanism was supported when selective inhibition of COX-2 in colon cancer cell lines protected against cell death induced by anandamide (Patsos et al. 2005). If this particular mechanism contributes significantly to the anti-tumor effect of cannabinoids then perhaps using a COX-2 inhibitor simultaneously with treatment protocols may indeed be detrimental, as it may interfere with the anti-tumor effect of the cannabinoids.
3. Many epithelial tumors overexpress ID1 which results in tumor proliferation and progression. CBD is a potent inhibitor of ID1.

There are currently multiple in vivo studies evaluating the use of phytocannabinoid therapy in canines with lymphoma and transitional cell carcinoma. As cannabis is becoming legal in many states, pet owners are looking to their veterinarians for advice and guidance on how to effectively and safely treat their pet.

Dogs, having a much higher density of CB1 receptors in the hindbrain compared to other species, experience a higher sensitivity to the side effects of THC including inebriation, urinary incontinence, incoordination, and static ataxia—commonly known as intoxication (Freundt-Revilla et al. 2017). However, if THC is introduced in small amounts, and the dose is titrated slowly, tolerance to the negative effects is achieved and dogs can handle extremely high doses of THC without intoxication. The key to dosing dogs with THC is patience and careful monitoring. The combination of both THC and CBD in formulations can enhance the antineoplastic effect due to the synergistic action, but CBD also neutralizes the adverse effects seen with the THC. The author feels strongly about the importance of creating custom formulas for each individual patient based on the specific disease and the formula; custom formulas should be adjusted for the presence of comorbidities, breed, age, drug interaction, pet owner's tolerability for side effects, and ultimate goals (palliative vs. definitive intent). The formula should contain as many cannabinoids as possible along with an anti-neoplastic profile of terpenes. If multiple products are being combined, then it is recommended to mix and match from different manufacturers to allow for a wider spectrum of terpenes, flavonoids, and other cannabinoids. As there is still so much information we do not know regarding which exact compounds of the plant are required to provide anti-tumor effect, it is essential to expose the cancer patient to as many full spectrum products as possible. The concept is reinforced by studies demonstrating that cannabis full plant extracts that contain multiple compounds had superior antineoplastic effect compared to pure phytocannabinoid isolates (Blasco-Benito et al. 2018; Baram et al. 2019). It should also be noted, due to the immunosuppressive effects of cannabis, it is recommended to avoid giving any cannabinoid product to a patient while undergoing anti-tumor immunotherapy as there is evidence to support that cannabis can negatively affect response rates when used simultaneously with immunotherapy (Taha et al. 2019; Zhang et al. 2018).

Although cannabinoids have shown antitumor activity in multiple cell lines, rodent cancer models, and scant human clinical trials, there is still not enough data to confirm which specific chemovars, doses, ratios, or even extraction methods are required for effective clinical incorporation for the cancer patient (human or veterinary). Despite the work that still needs to be done, there is sufficient evidence to support the use of cannabinoids in the veterinary cancer patient both for definitive and palliative treatment. Once the legal landscape is less restrictive, it is imperative that we collaborate to collect data and perform well-designed clinical trials to further establish the details involved in the antitumor activity of cannabinoids in veterinary medicine.

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Chapter 11
Nutritional Analysis of Cannabis

Robert Silver, Joseph Wakshalg, Susan Wynn, and Katherine Kramer

11.1 Introduction

As the hemp cultivar of *cannabis* becomes more widely legalized around the globe, its economic value as an agricultural commodity is developing into a multi-billion dollar industry. Hemp has been used in a wide spectrum of products as diverse as paper, cloth, building materials, biofuels, livestock bedding, nutraceutical and medicinal products, and as a food source for both humans and animals.

In 2004 the Hemp Industries Association sued the US Drug Enforcement Agency (DEA) in the Ninth Circuit Court over the scheduling of hemp seed and hemp seed oil as Schedule One Controlled Substances. As a result of this ruling, these items are now exempt from the Controlled Substance Act and can be sold as food for humans. However, the Food and Drug Administration's Center for Veterinary Medicine (FDA-CVM) has not yet endorsed hemp seed or hemp seed oil as an approved ingredient for animal feed. Until the appropriate Feed Additive Petitions (FAP) are submitted to the FDA and approved, the presence of any part of the hemp plant in animal feed will cause that feed to be considered adulterated, leading to the FDA

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issuing a stop sale order for that food product; it may also remove that product from retail shelves in every state. The Association of American Feed Control Officials (AAFCO) will not approve animal feed containing any part of the hemp plant until the FDA-CVM receives and approves the FAP for the use of a specific hemp plant part as a specific feed ingredient application for a specific species of animal. For food animal species the approval process will be much more detailed and prolonged to ensure the safety of human consumption of meat, milk, or eggs that may contain trace amounts of phytocannabinoids.

Currently, there is an effort by a group of hemp industry stakeholders and regulatory experts to submit FAPs for hemp seed in dogs, cats, and horses (https://thecohia.org). This is a multi-year effort to fund research studies supporting the safety of hemp in each of the target species for this FAP. The FDA-CVM has guaranteed this group of stakeholders that approval of these FAPs will be fast-tracked, once all of the necessary studies have been completed and appropriate documents submitted.

Research has continued to unveil the diverse components of the cannabis plant and that it richly deserves the nickname of 'the plant of a thousand and one molecules' (Andre et al. 2016). With much attention recently being devoted to the study of the plant's distinct phytocannabinoids, it is important that the nutrient composition not be overlooked as it is well known that nutrition has a significant impact on health. Cannabis, specifically hemp, has proven to have multiple health benefits for people and animals due to its nutritional profile.

This chapter will provide a short review of

- *Cannabis* botany
- The historical and present day uses of the hemp plant in animals
- Industrial processing of hemp and resulting byproducts
- The nutritional composition of hemp
- Current research evaluating the safety and nutritional value of hemp in food production animals

To clarify, the cultivar of the cannabis plant that contains THC in amounts greater than 0.3%, measured at harvest on a dry matter basis ("marijuana"), provides minimal nutritional value for humans or animals. It is low in fiber, has poor quality seed oil, and contains the intoxication potential of high levels of THC. The cannabis cultivar that contains less than 0.3% THC on a dry matter basis at harvest is called "hemp" and is the appropriate cultivar to use routinely in animals. For the purposes of this chapter, the term "hemp" will be used, whether referring to the fiber fraction of the plant, the seed oil and protein fraction of the plant, or the resin oil producing fractions. If identification of one specific cultivar is needed for continuity of content, then the intention of the product will be noted at that time. The medicinal resins or "phytocannabinoids", produced primarily in the flowers and leaves of the mature cannabis plant, will not be a subject of discussion in this chapter, as the safety and value of feeding phytocannabinoids to agricultural and companion animals has yet to be studied thoroughly.

11.2 Cannabis Botany

The *Cannabis sativa* L. plant has been used by humans since the dawn of prehistory, with anthropological evidence reaching back to the pre-agrarian period over 10,000–25,000 years ago. It is thought that the plant originally grew in the Kush mountains of Afghanistan but, over these many millennia, has spanned the entire globe. Cannabis, a very hardy plant, has been able to adapt and grow under many diverse environments and climates.

The early cultivars of the cannabis plant are thought to have contained very low levels of THC, perhaps similar to the current levels of THC found in contemporary hemp cultivars. With the increased recognition of the value of THC for spiritual, medicinal, and recreational uses, plant cultivators have worked to increase the THC content of the cannabis plant; currently cannabis cultivars exist which contain as much as 35% THC naturally occurring in the flowers and leaves. The plant itself has diverse phenotypic expressions that result in multiple cultivars, each one of which has resulted in specific agricultural and medicinal products (Upton et al. 2014).

A member of the Family *Cannabaceae*, which it shares with hops (*Humulus lupus*) and hackberry (*Celtis occidentalis*) plants, cannabis has such diverse phenotypic manifestations that there is ongoing controversy amongst botanists regarding its taxonomic classification into sub-species, cultivars, and chemovars. *Cannabis* plants are annually wind pollinated and can be either dioecious, containing both male and female reproductive parts on the same plant, or monoecious, where the entire plant is either male or female. The plants are branched and have woody interiors and hollow internodes.

The hemp chemovar contains several cultivars, depending upon the main agricultural product of the plant. The hemp plant's morphology will determine the production type of cultivar. Low THC hemp has four production types: fiber, seed oil, grain, and narcotic resin strains. Seed oil, grain, and narcotic resin production types typically are found on the female plants that are more branched and moderate in height (Lente et al. unpublished). Fiber-based cultivars can grow quite tall and are relatively unbranched, as the internodes will disrupt fiber bundles. Plants grown for fiber can be dioecious, yet male characteristics are generally favored for fiber, as the males usually are very tall, with minimal branching, and can be densely grown together (Lente et al. unpublished).

The cannabis plant has the ability to purify the soil of contaminants such as heavy metals and hydrocarbons, a property known as "bioremediation". Hemp was planted around the Chernobyl nuclear reactor accident site in Ukraine for this purpose (Meers et al. 2005; Vandenhove and Van Hees 2005). Once the contaminants are removed from the soil, they are sequestered in the hemp plant. The fibers are free of the contaminants and can be used for industrial purposes, but the seeds and seed oil remain contaminated and will need to be discarded. Bioremediation is an important reason to analyze each harvest for heavy metals and hydrocarbons to ensure that the plant has not become contaminated and thus made unfit for either food or medicine.

11.3 A Brief History

Hemp originated in Eurasia millennia ago and is believed to have been introduced to North America in 1606 by the British who settled in Jamestown, Virginia. Hemp was grown primarily for fiber in European settlements in Canada and the British colonies for several hundred years, thriving in Kentucky, Missouri, and Illinois. During World War I the hemp industry expanded to twelve US states, and in Canada there was a small commercial hemp fiber industry in the 1920s–1930s, mostly in the western provinces. In the 1920s Agriculture Canada and the United States Department of Agriculture (USDA) conducted hemp fiber research. In the twentieth century hemp fiber became less competitive for use as rope, clothing, and paper, and eventually became obsolete (to a large extent due to the rise of the plastics industry).

In 1938, the Canadian Opium and Narcotics Act made cultivation of any cannabis illegal in Canada. The US Marihuana Tax Act of 1938 placed 'marihuana' under the control of the US Treasury Department, requiring permission from the DEA to grow cannabis. In 1970, the US Congress repealed The Marihuana Tax Act and replaced it with the Comprehensive Drug Abuse Prevention and Control Act which effectively eliminated the hemp industry in North America.

In 1994, Canada began to allow research on hemp and in 1998 new regulations allowed the commercial cultivation of hemp under licensing from Health Canada. Although the United States passed the US Agricultural Act of 2014 and section 7606 authorized state departments of agriculture to permit pilot programs for hemp research, hemp remained classified as a Schedule 1 controlled substance under the Controlled Substance Act. With the passage of the Agricultural Act of 2018, including the Hemp Farming Bill amendment, hemp was de-scheduled and cultivation and commercialization of hemp in the United States began in earnest (Cherney and Small 2016).

Hemp seed and hemp seed oil have been used as food for at least 3000 years for both humans and animals. Currently, the major animal feed market for hemp seed is as a constituent of wild bird seed. European farmers, who also grow hemp as a commercial crop, have reportedly fed the plant material to their livestock to good effect. However, a few adverse events have been reported when feeding the whole hemp plant (with or without flowers and leaves) to beef and dairy cattle, sheep, and poultry (Cherney and Small 2016; Small and Marcus 2002).

11.4 Hemp as a Global Commodity

The global market for hemp consists of more than 25,000 products in nine submarkets: agriculture, textiles, recycling, automotive, furniture, food and beverages, paper, construction materials, and personal care. Hemp is grown for its fiber, its seed or can be grown as a dual-purpose crop with the stalk and the seed being the harvested products. The stalk interior has short woody fibers that are called

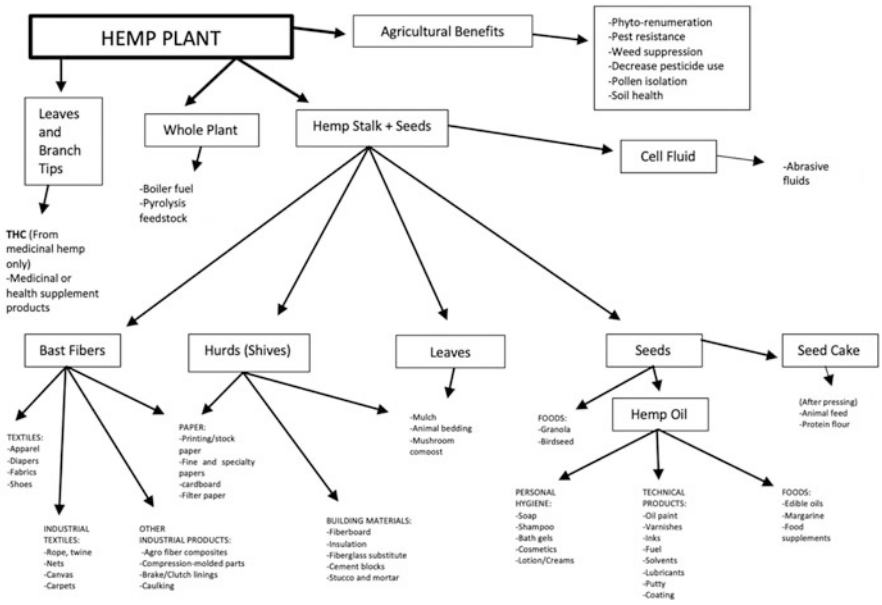

Fig. 11.1 Modern uses for industrial hemp

"hurds". The external portion of the stalk has long "bast" fibers. Hemp seed or "grain" is smooth and about 1/8–1/4 inch long.

The hemp bast fibers are used in fabrics and textiles, yarns and spun fibers, paper, carpeting, home furnishings, construction and insulation materials, auto parts, and composites. The shorter hurd fibers are used in animal bedding, papermaking, and oil absorbents. Hemp seed and oilcake are used in a range of foods and beverages and as an animal feed protein source. Hemp is promoted as a rotational crop for use as an animal feed supplement alternating with corn and other crops. Cold-pressed and extracted oil from crushed hemp seed is used in soap, shampoo, lotions, bath gels, and cosmetics.

Hemp is also finding use as a composite product. "Hempcrete" is a mixture of hemp hurd and lime products that can be used as a building material. Additionally, hemp is used by the automotive and aviation sectors as a lightweight insulating material, in hemp plastics and in related composites as a fiberglass alternative. Hemp is also being promoted for use as a potential biodiesel feedstock and cover crop (Johnson 2017) (Fig. 11.1).

11.5 Individual Components of Hemp; Analysis and Industrial Processing

11.5.1 Seeds

In terms of nutrient content, the seeds (also called "grain") have the richest content of nutrients and the lowest content of cannabinoids, making them the most valuable part of the cannabis plant as a dietary component for humans and animals. A detailed analysis of the nutrient content of the hemp seed is discussed in the next section. Hemp seeds are rich in both omega-3 (ω-3) and -6 (ω-6) fatty acids, as well as the anti-inflammatory fatty acid gamma linolenic acid (GLA), and are a source of high-quality protein. Historically, pressed hemp seed cake has been used to feed livestock, but there is limited scientific literature of controlled studies evaluating the benefits and risks of feeding seed oil and protein cake containing very low levels of phytocannabinoids.

The seed or grain from the hemp plant can be used whole, or the hulls can be removed and used as a fiber source. The remaining dehulled hemp seed is used for its protein and oil content, although the oil is most commonly derived from the whole hemp seed. Once the oil is removed the remaining material is termed "hemp seed meal". Pressing this material results in "hemp seed cake" which is rich in high quality protein.

Dehulled hemp seeds are a good source of protein and are available as a human food product in health food stores where they are often called "hemp hearts". The "fines" are a mixture of broken dehulled seeds and hulls, resulting in a blend that is finer in size than the seeds; this is sold for human consumption as well.

Hemp seed has been used as a feed concentrate in poultry and, to lesser extent, in cattle and sheep. Hemp seed is a common ingredient in bird feed mixtures. The fiber and protein portions of the hemp seed may be better suited for livestock. The dehulled seed and oil products, as well as the protein component of the seed, are appropriate for monogastric feeding.

The cannabinoid content of the seeds is quite low although seeds are located in the bracts of the flowers which have the highest cannabinoid content. The seeds will garner cannabinoids from contact and harvesting. Most seed growers wash the seeds after harvest to remove the cannabinoids (WSDA Report 2017).

The European Food Safety Authority Panel on Additives and Products or Substances used in Animal Feed (EFSA FEEDAP) has established a provisional maximum tolerable daily intake level (PMTDI) for THC of 0.0004 mg/kg of body weight for humans (EFSA Journal 2011). The European Industrial Hemp Association proposes that the average daily intake of THC be 0.035 mg/kg of body weight, which is significantly higher than the EFSAP determination. Other countries have established their own upper limits on THC content from hemp seeds; for example, New Zealand's upper limit is 0.006 mg/kg body weight (EFSA Journal 2011). Canada has placed a THC limitation of 10 ppm (0.001%) on raw and semi-finished hemp seed products such as hemp seed oil (Industrial Hemp Regulations 2020).

These values are set for human safety in an effort to avoid the adverse effects of ingesting hemp seed that may contain low levels of THC. There are no studies that parallel this work in animals to determine safe ingestion levels for THC, and there are no studies to determine the limits of CBD in hemp seed for animals as of yet.

11.5.2 Flowers and Leaves

The flowers and the leaves contain the "trichomes", glandular structures that synthesize cannabinoids, terpenes, and flavonoids. For the manufacture of resin-based products that are used in both nutraceutical and pharmaceutical products, the flowers and leaves are first harvested, and their resinous oils are extracted. Flowers and leaves contain quite a bit of nutrient value based on nutritional analysis of the post-extraction biomass (Lente et al. unpublished). During the process in which the resins and polyphenols are removed by CO_2 or ethanolic extraction, there remains a significant amount of protein, carbohydrate, and fiber, which is very clearly found yet rarely utilized for feeds (Silver 2016). Many tons of this post-extraction biomass material are being produced on a daily basis by the medical hemp industry. As such, it is a potentially valuable "waste product" of the exponentially growing cannabidiol (CBD) industry. Post-extraction biomass often contains a small amount of both CBD and THC.

Due to high cannabinoid content, cannabis flowers and leaves are not suitable as sources of daily nutrition until the phytocannabinoids have been extracted with high efficiency. With modern day extraction technology, as much as 90% of the cannabinoids are removed. This allows the remaining plant material to be considered an animal feed ingredient, as the remaining cannabinoid levels are below the accepted limit and are incapable of contaminating food products rendered from that animal. Long-term feeding studies will need to be conducted to determine the safety of feeding small amounts of cannabinoids to agricultural and companion animals. Studies will also be needed to measure the cannabinoid content of the food products of animals fed this post-extraction biomass in order to determine how much cannabinoid may be transferred to food products, and whether those products will be safe for human consumption.

11.5.3 Stalks

Hemp stalks are an important product for fiber content but there is very little evidence that hemp stalk fiber has been ever used for animal feed. The fiber itself has been used extensively in Europe for animal bedding. Hemp fiber, and in fact the entire cannabis plant, has antimicrobial qualities that show efficacy against *Staphylococcus aureus* and other pathogens, making it a superior material for animal bedding (Appendino et al. 2008; Ali et al. 2012).

In order for the fiber in the stalks to be utilized it must be extracted through several different processes. The primary bast fibers, located just under the bark in the phloem-associated tissues of the stem, are 5–40 mm long and will consolidate into fiber bundles that are 1–5 m in length. The long bast fibers are the strongest fiber material found in the hemp stem. Bast fibers traditionally have been used in paper making for over 2000 years. Traditionally the bast fibers are separated from the bark by "retting", a form of microbial degradation that allows the outer parts of the stem to slough off and loosens the inner woody core from the phloem fibers.

The woody material around the pith of the stem is called hemp "shives" or "hurds". They are very low density with good sound and thermal insulating properties. They are also used in the production of Hempcrete. The shives/hurds have been used in Europe as a high-quality bedding for small pets and horses, especially for those with allergies to other types of straw. Hurds are valuable for poultry bedding or cat litter, due to their high absorption capacity which can reduce manure and litter box odor. Hurds are also useful as garden ground cover.

Shives/hurds are shorter than the bast fibers (0.55 mm long) and are cemented together with lignans. Some applications for the short bulky fibers of the hurds include the manufacture of cheaper grades of paper and use in wood composites (e.g. fiberboard). Hemp hurds are high in silica which, when mixed with lime, undergoes mineralization to produce a stone-like material, which can be "blown" or poured into wall cavities and attics for insulation (Small and Marcus 2002).

11.5.4 Roots

Hemp roots have historically been used for traditional medicines and are not used as a food source or for industrial purposes. Although the roots are the least used part of the hemp plant, they contain a number of phytochemicals, such as alkaloids and terpenes, that appear to have medicinal benefits. Roots are typically dried and then steeped to make teas or ground into powder to be ingested or applied topically. The literature discusses a number of medical applications, including gout, inflammation, rheumatism, degenerative arthritis, fever, skin burns, tumors, childbirth, postpartum hemorrhage, gonorrhea, emesis, gastritis, erysipelas, detoxifications, infections, and parasites (Ryz et al. 2017).

11.6 The Nutritional Components of Hemp Seeds

The hemp plant can yield six types of feed materials: *unhulled hemp seed* (26–37.5% crude fat, 25% crude protein, 28% crude fiber); *dehulled hemp seed* (32% crude protein, 44% crude fat); and *hemp seed meal or cake* (about 11% lipids, 33% crude protein, 43% fiber). The seed protein can be further processed to yield a *protein isolate*; *hemp seed oil* (about 56% linoleic, 22% alpha-linolenic acid), *hemp seed*

Table 11.1 Summary of hemp seed ingredients

Whole hemp seed	Approx. 24% protein, 30% oil, 35% fiber
Dehulled hemp seed (AKA: Hemp hearts)	Approx. 32% protein and 44% oil
Hemp seed oil	Omega 3 [20%] & Omega 6 [55%] fatty acids: Ratio = 1:2–3
Hemp seed meal or cake from oil pressing	Approx. 32% protein, 11% oil, 39% fiber
Hemp seed hulls	65% fiber primarily with lesser amounts of oil and protein
Hemp seed fines	Similar to whole hemp seed profile

Table 11.2 Proximate analysis of hemp seed and its derivatives

	Hemp seed	Dehulled hemp seed	Hemp seed meal/cake w/hull	Hemp hulls
Dry matter (%)	94.1	95.1	95.1	94.9
Crude protein (%)	24.0	35.9	40.7	12.7
Crude fiber (%)	30.4	46.7	10.2	10.3
Acid detergent fiber (%)	23.5	3.3	21.5	50.2
Neutral detergent fiber (%)	32.1	7.8	30.5	64.9
Ash (%)	4.8	6.4	6.7	3.9
Gross Energy (kcal/kg)	5783	6620	4875	4828

hulls (65% fiber, lesser amounts of protein and oil); and *hemp seed fines* (similar to the whole hemp seed profile) (EFSA Journal 2011) (Table 11.1).

Proximate analysis of seeds from four hemp cultivars was recently reported. Table 11.2 displays mean values for various fractions of hemp seed (House et al. 2010). As with any food, it should be noted that these values change with locale, cultivation and processing techniques.

The USDA Food and Nutrient Database for Dietary Studies (FNDDS) contains nutrient profiles for various forms of hemp, including hemp oil, hemp seed and hemp hearts. These profiles entered by USDA are fairly comprehensive and are updated every 2 years. Individual companies will often provide profiles for their hemp products, but these are often incomplete. Nutrient profiles should contain the concentrations of energy, moisture, macronutrients, and micronutrients. The nutrient profile for hulled hemp seeds can be found in Table 11.3.

Table 11.3 Nutrient profile—hulled hemp seed

Nutrient	Unit	Value per 100 g	3.0 tbsp = 30.0 g
Proximates			
Water	g	4.96	1.49
Energy	kcal	553	166
Energy	kJ	2313	694
Protein	g	31.56	9.47
Total lipid (fat)	g	48.75	14.62
Ash	g	6.06	1.82
Carbohydrate, by difference	g	8.67	2.6
Fiber, total dietary	g	4	1.2
Sugars, total	g	1.5	0.45
Sucrose	g	0.85	0.26
Glucose (dextrose)	g	0.2	0.06
Fructose	g	0.31	0.09
Lactose	g	0.07	0.02
Maltose	g	0.07	0.02
Minerals			
Calcium, Ca	mg	70	21
Iron, Fe	mg	7.95	2.38
Magnesium, Mg	mg	700	210
Phosphorus, P	mg	1650	495
Potassium, K	mg	1200	360
Sodium, Na	mg	5	2
Zinc, Zn	mg	9.9	2.97
Copper, Cu	mg	1.6	0.48
Manganese, Mn	mg	7.6	2.28
Vitamins			
Vitamin C, total ascorbic acid	mg	0.5	0.1
Thiamin	mg	1.275	0.383
Riboflavin	mg	0.285	0.085
Niacin	mg	9.2	2.76
Vitamin B-6	mg	0.6	0.18
Folate, total	µg	110	33
Folic acid	µg	0	0
Folate, food	µg	110	33
Folate, DFE	µg	110	33
Vitamin A, RAE	µg	1	0
Carotene, beta	µg	7	2
Vitamin A, IU	IU	11	3
Vitamin E (alpha-tocopherol)	mg	0.8	0.24
Lipids			
Fatty acids, total saturated	g	4.6	1.38
16:00	g	2.866	0.86

(continued)

Table 11.3 (continued)

Nutrient	Unit	Value per 100 g	3.0 tbsp = 30.0 g
18:00	g	1.244	0.373
20:00	g	0.312	0.094
22:00	g	0.121	0.036
24:00:00	g	0.056	0.017
Fatty acids, total monounsaturated	g	5.4	1.62
18:1 undifferentiated	g	5.276	1.583
18:1 c	g	5.023	1.507
20:01	g	0.124	0.037
Fatty acids, total polyunsaturated	g	38.1	11.43
18:2 undifferentiated	g	27.459	8.238
18:2 n-6 c,c	g	27.358	8.207
18:2 CLAs	g	0.202	0.061
18:3 undifferentiated	g	10.024	3.007
18:3 n-3 c,c,c (ALA)	g	8.684	2.605
18:3 n-6 c,c,c	g	1.34	0.402
18:04	g	0.617	0.185
Fatty acids, total trans	g	0	0
Cholesterol	mg	0	0
Amino acids			
Tryptophan	g	0.369	0.111
Threonine	g	1.269	0.381
Isoleucine	g	1.286	0.386
Leucine	g	2.163	0.649
Lysine	g	1.276	0.383
Methionine	g	0.933	0.28
Cystine	g	0.672	0.202
Phenylalanine	g	1.447	0.434
Tyrosine	g	1.263	0.379
Valine	g	1.777	0.533
Arginine	g	4.55	1.365
Histidine	g	0.969	0.291
Alanine	g	1.528	0.458
Aspartic acid	g	3.662	1.099
Glutamic acid	g	6.269	1.881
Glycine	g	1.611	0.483
Proline	g	1.597	0.479
Serine	g	1.713	0.514

Reference: USDA National Nutrient Database for Standard Reference 1 April 2018 Software v.3.9.5.1_2019-01-29

11.7 Protein

As previously stated, hemp seed meal is produced from the whole hemp seed (with or without the hull). Once oil is pressed from the seeds, the remaining seed cake contains approximately 30–40% protein (Callaway 2004; Silverside and Lefrancois 2005; Mustafa et al. 1999). Hemp seed cake is the common form of hemp fed to livestock; it is among the highest seed sources of protein, usually containing about 32–33% protein and 9–10% fat. In contrast to buckwheat, where amino acids are concentrated in the bran, the hemp hull contains fewer total amino acids than the whole hemp seed (Mattila et al. 2018).

All nine essential amino acids required by humans (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) are found in hemp seed. Hemp seed also contains high levels of arginine and glutamic acid, with moderate levels of alanine, asparagine, and glycine.

An animal's protein requirement is the requirement for individual amino acids and protein quality is a measure of how the amino acid profile of a specific food fulfills the amino acid requirements of a given species. High biologic value proteins contain higher concentrations of the amino acids considered essential for the species in question; proteins that are lower in biologic value have "limiting" amino acids, i.e. certain essential amino acids are limited in concentration or availability. House et al. examined protein quality of hemp seed and seed products by analyzing amino acid content. On a percent, as-fed basis, whole hemp seeds had the lowest levels of methionine, cysteine, tryptophan, and isoleucine while hemp seed meal was low in methionine, cysteine, and tryptophan (House et al. 2010).

The Amino Acid Score (AAS) of a protein indicates the extent to which a dietary protein meets the needs of an individual (or in the case of animals, the species) for a particular amino acid. The AAS is determined by comparing the test protein to a standard reference (casein). A score greater than 1.0 suggests that there are no limiting amino acids, while a score less than 1.0 indicates that an amino acid deficiency may result. The numerical score reflects the lowest score over the range of amino acids tested. The amino acid scores for hemp seed, dehulled hemp seed, hemp seed meal, and hemp hulls are 0.62, 0.61, 0.58, and 0.50, respectively. This does not take into account the bioavailability of these amino acids which may differ dramatically when comparing the dehulled seed to the hull. Lysine is the first limiting amino acid, with either tryptophan or leucine as the second and third limiting amino acids, depending on the product.

The AAS provides one measure of protein quality but protein digestibility is considered a better measure of the quality of a dietary protein. The Protein Digestibility-Corrected Amino Acid Score (PDCAAS) incorporates the AAS factoring in protein digestibility. The PDCAAS is expressed as a percent; casein has a score of 100% and all other protein sources are compared to that standard. A comparison of protein feeds and their PDCAAS is found in Table 11.4. It is important to note that these tests involve only rats and humans. Each species has

Table 11.4 Protein digestibility compared among protein sources

Protein source	Protein digestibility (PDCAAS) %
Casein	100
Egg white	100
Beef	92
Soy protein isolate	92
Chickpeas (canned)	71
Pea flower	69
Kidney beans (canned)	68
De-hulled hemp seed	61
Pinto beans (canned)	57
Rolled oats	57
Lentils (canned)	52
Hemp seed	51
Hemp seed meal	48
Whole wheat	40
Almond	23

House et al. (2010)

unique amino acid requirements, requiring arginine and taurine (for cats and sometimes dogs), which were not assessed in this study.

Hemp seed protein is found to be similar to nut proteins and contains relatively high levels of arginine (94–128 mg/g protein) (House et al. 2010). Despite the high concentrations of arginine, and a sulfur-rich protein fraction, the protein quality of hemp seed is similar to other vegetarian proteins, such as soybean or egg white. Vegetarian diets using hemp as a main source of protein will require supplementation of lysine and other amino acids to be complete.

Hemp seed does provide a complete protein for herbivores and poultry. Currently, inclusion rates have been established in Europe with the maximum incorporation rate in complete feed being 3 % in poultry for growth, 5–7% in laying poultry and 2–5% in pigs for hemp seed and hemp seed cake, 5% in ruminants for hemp seed cake and 5% in fish for hemp seed (EFSA Journal 2011).

11.8 Fats and Oils

Hemp seeds contain approximately 30–35% oil (Leonard et al. 2019). About 80% of the oil is composed of polyunsaturated fatty acids (PUFA), rich in the essential fatty acids linoleic acid (54% 18:2 ω-6) and alpha-linolenic acid (approximately 20% 18:3 ω-3). The ω-6: ω-3 ratio in hemp seeds is normally 2.5–3.5:1, which is the recommended ratio for humans. As seen in Table 11.5, the proportion of PUFA compared to other fatty acids is significantly higher in hemp oil compared to other common plant sources. Diets high in PUFAs have been associated with multiple

Table 11.5 Comparison of hemp seed oil to other popular seed oils

	Type	Hemp oil	Flaxseed oil	Olive oil	Sunflower oil	Borage oil	Corn oil	Coconut oil	Fish oil
Linoleic acid (%)	18:2 (n-6)	54	15	7–10	65–68	38	58	1.6	0.7
Alpha-linolenic(%)	18:3 (n-3)	17–22	41–47	<1	0.2	18–26	1	0	0.05–0.35
Gamma-linolenic(%)	18:3 (n-6)	3–4	0	0	0	20		0	0.035
Eicosapentaenoic(%)	20:5 (n-3)	0	0	0	0	0	0	0	8–14
Docosahexaenoic(%)	22:6 (n-3)	0	0	0	0	0	0	0	5–24
Arachidonic (%)	20:4 (n-6)	0	0	0	0	0	0	0	0.1–2

Callaway (2004), USDA database

health benefits including a diminished risk of cancer, cardiovascular disease, and autoimmune and inflammatory disease (Leonard et al. 2019).

11.9 Vitamins and Minerals

The USDA Nutrient Database lists hemp seeds as containing vitamins A, C, E, and multiple B vitamins (folate, niacin, pyridoxine, riboflavin, and thiamine). Hemp seeds are especially rich in the antioxidant vitamin E and contain primarily gamma-tocopherol with smaller concentrations of alpha and delta-tocopherols. In a study of several hemp genotypes, the average tocopherol concentration was found to be 0.88 g/kg in hemp seed oil (Galasso et al. 2016).

Hemp seeds naturally contain calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc. They are an exceptional source of iron, magnesium, and potassium. Due to its bioremediation capabilities, hemp is known to extract minerals from the soil with ease and thus can contain many other minerals. Hemp can often glean cadmium, phosphorus, and potassium from fertilizers (Mihoc et al. 2012). Due to this effect it is extremely important to test the mineral content of the hemp product being considered (Table 11.6).

11.10 Carbohydrates and Fiber

Hemp seed contains low carbohydrate levels, compared to levels of protein and fat. The carbohydrate profile consists of mostly fiber with very low levels of sucrose, dextrose, fructose, and lactose.

Although there is some fiber found in the seed cotyledon, the majority of fiber is found in the hull. It contains an approximate 20:80 ratio of water-soluble and

Table 11.6 Vitamin and mineral content (mg/100 g) of hemp seed

Vitamin E	90.0
Thiamin (Vitamin B1)	0.4
Riboflavin (Vitamin B2)	0.1
Phosphorus	1160
Potassium	859
Magnesium	483
Calcium	145
Iron	14
Sodium	12
Manganese	7
Zinc	7
Copper	2

Callaway (2004)

water-insoluble fiber. This fiber content ratio is similar to both flaxseed and lupin seed. The insoluble fiber consists of cellulose, lignin, and hemicellulose. (Leonard et al. 2019) To date there is limited analysis on the functionality of this fiber.

11.11 Phenylpropanoids

Also known as phenolic compounds, phenylpropanoids are a class of secondary plant metabolites synthesized from either phenylalanine or tyrosine. Distributed throughout the entire plant, this large group is responsible for several important plant functions, including growth, development, color pigment, and defense against pests, pathogens, and UV radiation (Kumar and Goel 2019). They also provide a major source of dietary antioxidants and are currently being researched for their nutritional benefits. Phenylpropanoids include phenolic acids and flavonoids.

Flavonoids, or bioflavonoids, consist of a 15-carbon structure with two phenyl rings and can be further classified into flavonols, flavones, stilbenes, and lignans. Over 20 flavonoids have been found in the cannabis plant, mostly in the leaves and flowers with very low levels in the seeds and almost none found in the roots. Compared to flaxseed, which is well known for having an abundant amount of phenols and lignans, hemp seed contains approximately 20-times less total lignans. A full discussion of the flavonoids in cannabis can be found in Chap. 4.

Although hemp seeds appear to have low levels of flavonoids, compared to the leaves and flowers, the overall phenolic content appears to be substantial, most likely due to phenolic amides. In one study hemp seed had an overall phenolic content higher than flaxseed (Galasso et al. 2016). The combinations of phenols with tocopherols is thought to confer significant antioxidant properties to the hemp seed.

11.12 Anti-nutritional Factors

Like many whole seeds, hemp contains anti-nutritional factors, including: phytic acid, trypsin inhibitors, condensed tannins, saponin, and cyanogenic glycosides. Phytic acid is the main organic form of phosphorus. Phytate is formed when phytic acid binds to a mineral and is present at high levels in hemp seed (up to 7% in one cultivar) (Galasso et al. 2016). This high level of phytate may significantly limit the digestibility of protein in hemp seed rations fed to monogastric animals. Trypsin inhibitors may also limit digestibility by impeding protein metabolism. More research is needed to assess how and to what extent these anti-nutritional factors may impact the use of hemp seed in the diets of food production animals.

11.13 Agricultural and Livestock Nutritional Applications for Hemp Seed and Its Derivatives

There are several factors influencing the growing research into the use of hemp seed in production animal diets. Hemp and its by-products are relatively inexpensive and becoming more available. Another factor involves the trend of using therapeutic plants to replace the use of antibiotics in feed as many countries are banning this practice. Overall, there appears to be a move towards diet compositions that can provide better health for the animal while still meeting consumer demand. The major impediment to the development of hemp as a source of animal feed is the presence of cannabinoids in the resin glands of the flowers and leaves and trace amounts in the seeds and stalk, which may carry over into meat, milk, or egg products thereby entering the human food chain. There are insufficient studies, as yet, investigating the potential risks or safety of feeding phytocannabinoid-rich plant material to either humans or animals.

As previously discussed, the use of hemp has been limited to livestock and poultry primarily in Europe and Canada, where there may be some geographical advantages as the more temperate climates appear to be ideal for hemp production due to enzymatic activity: CBD synthase predominates in milder temperatures while THC synthase appears to be upregulated in warmer, tropical climates. The average production of seed per hectare in these regions appears to be between 4000 and 4500 lb/year which is far lower than other seed crops (Callaway 2004); however, the current practices of hemp harvesting may be hindered to some degree since equipment is typically designed for harvest of other crops. This causes time and product losses in the field that may be remedied by investment into specialized equipment for such endeavors (Small and Marcus 2002). A full understanding of the plant's utility from an agricultural perspective is still in its infancy but a considerable amount of research has been devoted to the hemp seed and hemp seed cake by-products with good reason. These by-products are relatively inexpensive and will be even more plentiful as the hemp industry continues to grow.

11.14 Poultry

The effects of ω-3 and ω-6 fatty acids in poultry are well studied for their roles in metabolism, growth and development, productive performance, immune response, reproduction, and egg and meat quality (Alagawany et al. 2019). Diets high in PUFAs are of special interest in the search to produce healthier products (containing higher ratios of fatty acids) for human consumption. Ruminants' ability to ferment and alter fatty acids through ruminal flora has led to less interest due to their subsequent inability to incorporate the ingested fatty acids into meat or milk. Therefore, enriched omega fatty acid diets are less attractive to the beef, lamb, and

pork industries. The poultry industry has embraced the investigation of hemp seed and hemp seed cake use in egg and meat production.

11.15 Egg Production

Hemp seed feed, as either the whole seed, hemp seed cake, or hemp seed oil, has been a focus of study due to the similarities in the fatty acid profiles of hemp and canola. Hemp seed feed has added benefits due to its GLA and ALA content, both of which can be converted to DHA in poultry to some degree. The benefits of producing eggs with higher levels of beneficial ω-3 fatty acids has tremendous appeal to the egg industry. Obtaining a better understanding of how this can affect egg quantity, quality, consumer perception, and biochemistry is extremely important in understanding inclusion rates for ideal production scenarios in laying hens. Initial studies examining adult egg producing birds fed dehulled hemp seed at up to 30% of the diet demonstrated negative repercussions on egg weight of 30%. This was presumed to be due to the higher neutral detergent fiber. However, hens consuming either a 4.5 or 9% hemp seed oil (maximum 11% fat across diets) or a 10–20% hemp seed inclusion showed no negative impact on egg production (Neijat et al. 2014; Goldberg et al. 2012). Interestingly, when assessing the influence of heated hemp seed meal it was shown that laying hens fed 15% of raw dehulled hemp seed versus a similar quantity of hemp seed heat treated for 60 min at 170°C showed improved egg weights and increased PUFA (LA, ALA and DHA) concentrations in eggs with no decrease in sensory evaluation during human consumption (Konca et al. 2019).

To date, research evaluating the fatty acid profile of eggs from hens fed diets containing up to 20% hemp seed or 9% hemp seed oil indicates that these diets can be used safely and effectively to increase the ω-3 content of eggs. Five different studies reached similar conclusions that using hemp seed oil or de-hulled hemp seed at higher feed inclusions resulted in eggs with increased levels of ALA, GLA, and DHA when compared to other non-ALA or GLA enriched oils. Increased levels ranged from two- to fourfold with higher inclusions being more effective. Maximal inclusion rates appear to be approximately 12% hemp seed oil or up to 30% dehulled hemp seed (Silverside and Lefrancois 2005; Neijat et al. 2014; Goldberg et al. 2012; Konca et al. 2019; Park et al. 2014). Although these eggs contain a higher fraction of beneficial ω-3 fatty acids, they still do not fulfill the recommended daily human consumption levels of ALA, EPA, and DHA (Konca et al. 2019).

11.16 Broiler Growth

Studies have also investigated using hemp seed as a means to improve broiler growth and increase ω-3 levels in meat. Research examining growth and hemp seed inclusions (oil, ground dehulled hemp seed, or hemp seed cake) found feed conversion

rates for growth and meat quality for inclusions ranging from 2.5% up to 20% in typical rations (Stastnik et al. 2016; Jing and House 2017). The importance of dehulled ground hemp seed or hemp cake may be important concepts as prior studies have suggested that upper limits of 10% inclusion, using whole seed as compared to dehulled whole seed, may be limiting factors in maximal consumption rate for broiler growth for optimal feed conversion (Stastnik et al. 2016).

Similar to the egg research findings, adding hemp seed or hemp seed oil to feed appears to increase the ω-3 level in broiler meat. Studies indicate that the benefits of hemp seed inclusions may apply to many farm raised birds including ducks, pheasant, and quail. A recent study examining the use of hemp seed meal in Japanese Quail showed similarities to poultry in which egg quality and production were not compromised and that fatty acid composition of meat improved. Increases of 3–4-fold in ALA concentrations and decreases in palmitic and oleic acid were noted with the use of the highest quantity of hemp seed meal, with no negative repercussions regarding quality, meat coloring or quantity (Yalcin et al. 2018).

11.17 Livestock

Although there is limited research, there is an interest in the use of hemp seed and hemp seed oil for livestock, mainly for meat production (beef and lamb primarily) and dairy milk production. Unlike poultry, ruminant species undergo modification of fatty acids and proteins in the rumen making the overall health benefits of ω-3 fatty acid consumption in ruminants less translatable to nutritional benefits in human consumption of meat or milk. The idea of feeding whole plant (hemp meals or flours) may be more attractive to producers, but this results in an exceedingly high neutral detergent fiber (NDF) rate of approximately 67%, which can negatively affect rumen health (Toonen et al. 2004). Most dehulled hemp seed or hemp seed cake preparations have modest NDF rates (35–45%) which translates to modest fiber for rumen fermentation without excessive rumen fill. Hemp seed cake appears to be more desirable due to a lower overall fat content than hemp seed (10–11% fat in hemp seed cake compared to 23% in hemp seed) since fat can also hinder rumen function.

11.18 Milk Production

There have been no long-term studies examining the effects of hemp feed on milk production or in-depth analyses of its effect on the fat, protein, or individual fatty acid content of milk. Studies have been performed examining possible exposure to THC through hemp feed. These studies have been the basis for the feed incorporation rates of hemp seed or hemp seed cake established for the European Food Safety Authority (EFSA) Guidelines. The limit of consumption for THC, based on European standards, is approximately 0.0004 mg/kg, and includes a 100-fold safety

factor with the assumption of 1.5–2 liters of daily milk consumption. This restriction is also based on the assumption that the hemp feed contains no more than 0.2% THC. At this level, it is predicted that 0.15% THC will be found in the milk from a cow that has consumed 0.5 kg of hemp daily (EFSA Journal 2011). However, the average hemp plant produces only 0.08% THC (not 0.2%), leading to the conclusion that milk consumption is safe, even with the average cow consuming 0.5–1 kg of whole hemp plant. This low inclusion rate makes hemp a poor substitute for corn silage/haylage and an impractical feed alternative.

Hemp seed feed (meal or cake) may be a good alternative to soybean meal, to some degree, in light of its very low cannabinoid content. One would expect that adding substrates of this nature may have a positive impact on milk protein and fat concentrations. One study, using forty Red Swedish dairy cows, investigated the effects of feeding increasing proportions of hemp seed cake on milk production and consumption. The proportion of hemp seed cake added to the diet began at zero and was increased to 318 g/kg over the course of 5 weeks. The inclusion diets had significant effects on not only milk yield but milk protein, fat, and lactose content. The maximum milk yield was achieved with the 143 g/kg diet as higher proportions resulted in decreases in both milk protein and fat concentration (Karlsson et al. 2010). Clearly, more studies are needed to assess the ideal hemp inclusion rate for benefits in milk production.

11.19 Meat Production

Using hemp seeds and their derivatives showed no benefits in dry matter intake and growth in steers fed up to 14%, and lambs fed up to 20%, of their diet as hemp seed meal (Mustafa et al. 1999; Gibb et al. 2005). The dietary level of starch in hemp seeds is very low, while fermentable fiber (as NDF) is relatively high. This leads researchers to theorize that higher levels of hemp seed in the diet will have negative repercussions. However, this theory has not been well tested and, to date, these levels do not appear to affect growth or meat quantity. Equally important is meat quality which has been examined in only one study. The results of consumption of nearly 90% of hemp seed meal on meat quality proved to be similar to typical soybean and barley diets (Hessle et al. 2008). A more recent examination of lamb longissimus dorsi fat content showed no changes in overall fat content and only a modest increase in DHA (0.06–0.14%) when lambs were fed 16% hemp seed meal compared to a variety of other meals (Turner et al. 2012). Surprisingly, there has been very little swine research using hemp seed meal, with only digestibility and fiber fermentation being examined. These studies showed that up to 28% of the ration can include hemp seed without affecting digestibility of protein, fat, and organic matter (Presto et al. 2011; Kim and Nyachoti 2017). Further swine growth and carcass characterization is needed to better appreciate this potential feed ingredient. Overall, there is a paucity of data on meat production, carcass quality, and

sensory perception work that is sorely needed due to this burgeoning new market for dehulled hemp seed, hemp seed cake, and even hemp seed hulls.

11.20 Prospects for Future Use of Hemp for Nutrition in Animals

The acceptance of hemp as an agricultural commodity in the United States has been rapid following the passage of the 2018 US Agricultural Act. There is still significant research that needs to be done to document this plant's safety and value for industrial, nutritional, medical, and bedding applications for animals.

In order for hemp to be fully accepted as an animal feed ingredient the following requirements will need to be met:

- Approved Food Additive Petitions for all species and all nutritional ingredients derived from hemp.
- Increased responsible labeling and marketing of hemp herbal extracts containing CBD by vendors.
- More pharmacokinetic and pharmacodynamics studies in each species to determine optimal dosing amounts and frequencies.
- Feeding trials examining improvement in lean muscle mass, quality of meat, losses to shipping or feeding.
- Meat, milk, and egg analyses to ensure no contamination of these food products with phytocannabinoids above a certain threshold level.

11.21 Conclusion

Hemp seed has multiple nutritional benefits for both people and humans. It is an excellent source of protein and fiber (digestible and non-digestible) with substantial antioxidant properties. It has immense potential to become an acceptable feed additive in many of our veterinary species due to these qualities. As our knowledge of nutrition as medicine grows, more research into the benefits of hemp is certainly warranted.

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Chapter 12
Cannabinoids in Equine Medicine

Chelsea Luedke and Trish Wilhelm

12.1 Introduction

Changes in culture along with scientific advancements surrounding cannabinoids have opened up a new avenue for hemp and cannabis product usage in the horse industry. At the present time, owners are very interested in and open to using cannabis products for horses. Horse professionals (especially veterinarians) must keep current on the safety and therapeutic value of cannabis, as the landscape is quickly evolving. This chapter will discuss what is known to date about efficacy and dosing in horses. There is a paucity of university-led research on cannabis therapy in equines; however, new changes in legalization of hemp federally in the United States are expected to open up opportunities for research groups to start investigating this topic.

12.2 Cannabis History in Relation to Horses

The use of cannabis in horses is not novel. Ancient Greeks were reported to have used cannabis plants for colic and wound care, as well as using crushed cannabis leaves as a poultice bandage to be applied directly to wounds (Butrica 2002). Varieties of *Cannabis indica* have been administered to horses in the US dating back to the early 1900s, with a prominent cavalry manual citing doses of one-half to one dram of *Cannabis indica* as a therapeutic for spasmodic colic, acute indigestion,

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and impactions (Carter 2003; Plummer et al. 1909). The cavalry had a list of common veterinary medications the medical staff should have in the unit; included in the list of medications was *Cannabis indica* and the appropriate number of doses based on how many hundreds of horses were in the unit.

Interestingly, the American Veterinary Medical Association's Annual Proceedings published as early as 1913 reported use of *Cannabis indica* in horses with colic and for use as an anesthetic (AVMA 1913). At this time, extracts from the plant *Cannabis sativa* were referred to as *Cannabis indica*. Dr. Herbert F. Palmer presented material describing the preferred method of extraction to be alcohol; furthermore, he highly recommended testing of the fluid extract for potency due to variability in plants. Dr. Palmer stated that using fluid extract as the main anodyne (therapeutic) had become commonplace for many veterinarians treating colic with oral fluids. He strongly advised against the use of IV forms of extract as there had been numerous reports of delayed severe thrombus formation in horses. Also notable in these AVMA Proceedings is the sensitivity of horses to cannabis as compared to dogs. Dr. Palmer did a small study with dogs using cannabis fluid extract and observed behavior changes in dogs at 0.2 g/kg body weight versus horses being given 0.02 g/kg having similar effects. The Proceedings further state that the University of Pennsylvania had used *Cannabis indica* fluid extract with success in gastrointestinal disturbances as well as during surgical procedures for analgesia using three ounces of fluid extract. Doses of 0.5–4 ounces of *Cannabis* fluid extract being given orally were noted in the meeting notes as being therapeutic for colic and analgesia in horses. Mentions of *Cannabis* being preferred over opium for pain relief without causing constipation are numerous in the AVMA Proceedings and cavalry manual. The concentrations of various cannabinoids and other phytochemicals in these fluids were not described, nor was the technology available at the time to quantify the various molecules present in the fluid. It is likely the plants used to make these fluids were much less potent compared to present day *Cannabis* plants.

Although multiple cultures used cannabis preparations on their horses, unfortunately no scientific documentation occurred after the early 1900s, likely because hemp farming was deterred by the Marihuana Tax Act of 1937. Until 2014, legislation in the United States did not allow university-led research to be performed on any cannabis products. There have been very few equine studies completed in a controlled setting at the time of publication; this chapter will discuss one case study of use for a horse with allodynia, an ECS receptor identification study using horse tissues, and a pharmacokinetic study examining the anti-inflammatory and behavioral effects of CBD in exercising thoroughbreds. During the writing of this chapter there were two active clinical studies at universities evaluating cannabinoid use in horses for anxiety and pain.

12.3 Routes of Administration

Numerous preparations are available to horse owners: paste, powder, pellets, oils, ground *Cannabis* flower, and salves are a few of the options on the market. Horse owners are accustomed to dosing horses by utilizing feed additives or oral syringe delivery. Although oil is difficult to deliver to a horse without substantial product loss, oral transmucosal (OTM) absorption of cannabinoids is preferred over oral administration to avoid the first pass effect within the hepatic system, though little no research has been published on what percentage of cannabinoids from OTM administration is absorbed via this method. Additionally, of the monogastric species, horses have notoriously low absorption of many classes of medications. Variables including low gastric pH, slow gastrointestinal transit times, and binding of medications to digesta have been linked to poor bioavailability in horses (Welsh et al. 1992; Lees et al. 2004). Additionally, oil-based preparations of ketoprofen have been studied in horses and have significantly lower absorption rates than administration of the drug in hard gelatin capsules (Landoni and Lees 1996). Most cannabinoid formulations are oil based, thus potentially presenting another barrier to gastric absorption, if OTM administration is not used.

Reports of farmers feeding cattle and horses raw *Cannabis* plant material or biomass from processing plants have been documented, with anecdotally positive feedback. Controlled studies on dairy cow milk production and milk fat content have been supportive of the use of hemp seed cakes as additives to regular feed (Karlsson et al. 2010). Increased embryo quality has been reported after using exogenous cannabinoids during in vitro maturation (Lopez-Cardona et al. 2016).

12.4 Safety in Horses

The authors have vast experience in administering hemp derived products to horses, ranging from 10 mg twice a day up to 3000 mg/day. No adverse effects were observed in horses that had hourly monitoring for behavior, appetite, fecal output, normal vital parameters, and exercise tolerance. The use of high doses of THC in horses has not been pursued due to possible unwanted side effects (e.g. ataxia, neurological abnormalities, increased anxiety, etc.) and legal concerns. In deciding on what CBD:THC ratio to utilize, it may be more therapeutic in severely painful horses to incorporate THC. However, the starting THC dose should be low, and gradually increased to the desired therapeutic effect. It should also be noted that animals, like humans, can develop a tolerance to THC and further THC dose escalation may be needed. CBD therapy at certain doses for a long duration of time may become cost prohibitive for owners, so this could pose another reason to add small amounts of THC into the cannabis protocol if the owners are willing, and the horse's condition and affect permits.

When selecting a product, it is imperative to perform due diligence regarding product quality from any suppliers or manufacturers. Because this is a new and emerging market, navigating the landscape can be difficult with inconsistent labeling and marketing terms. Product traceability is necessary to ensure sufficient quality of plants based on location of farming. Full chain of custody documentation from farm to sale of product is ideal. Third party analytical testing should be routine for every batch and available at all times to the public. Recommended final product certificates of analysis (COA's) include cannabinoid profile (potency), bacterial/microbial/mycotoxin evaluation, elemental analysis, terpene/flavonoid profile, and presence of residual solvents, heavy metals, and pesticides/fungicides/herbicides. These guidelines are necessary when searching for a safe and effective product for horses. For more on product selection see Chap. 13.

12.5 Pharmacokinetic Data

Intravenous or injectable methods of dosing are not common at this time, making bioavailability studies difficult. The authors have unpublished data using a hemp extract paste (Fig. 12.1) with a high concentration of CBD to perform pharmacokinetic curves. Two healthy horses were given doses of 0.7 mg/kg (Horse 1) and 0.845 mg/kg (Horse 2) of CBD orally (food and water not accessible for 30 min before and after dosing) in a small volume (4.8 ml) to allow for mucosal absorption. Plasma samples were collected at 0, 2, 6, 12, 24, and 36 h. The horses were monitored for behavior, appetite, normal vital parameters and fecal output. No adverse side effects were observed. The graph below highlights the curve obtained after CBD extraction and liquid chromatography-mass spectrometry using Colorado State University's Pharmacology Core Research Lab. This data set allows for

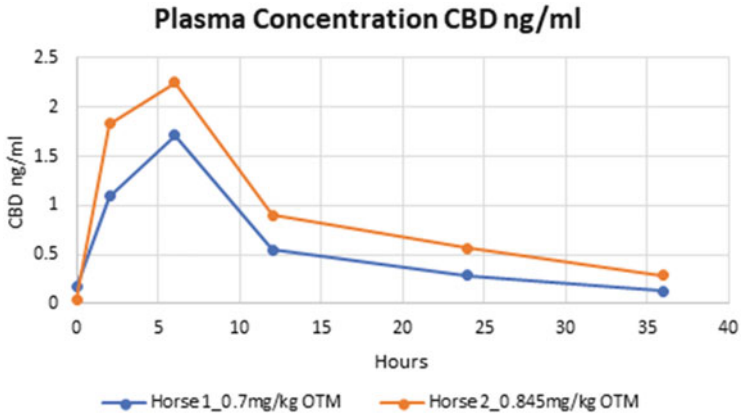

Fig. 12.1 Plasma concentrations of CBD in 2 healthy horses over 36 h after sublingual administration (VetCS, Centennial, CO, 2019)

calculation of a half-life of 9.05 h, which is significantly longer than has been previously reported in canine, murine, and human studies. Clearly a larger sample size and varying doses of CBD are indicated to further understand the difference in elimination rates between species, as well as any additional accumulation of cannabinoids. Standardized safety and efficacy research will be crucial to understanding any effects of cannabinoid dosing on hematologic properties in horses.

In a poster prepared by K. Jones et al from Murray State University we saw similar PK dose responses. In the first part of the three-part pilot series the researchers dosed the horses with either 50 mg of CBD (product profile was not provided) in an oil form or pelleted form. Serum samples were collected at two times points: 60 and 120 min. The oil, given orally, had significantly lower ng/ml concentration compared to the CBD in the pelleted form which further supports canine studies suggesting better bioavailability when given with food. Both serum concentration levels are considered sub therapeutic at ~0.02–0.17 ng/ml, compared to canine and feline studies targeting serum concentrations >50–100 ng/ml. The second portion of the study dosed six horses each with three different dosages of an undescribed CBD product.

In a dissertation project out of Auburn University in December of 2019, Heather Ayn Davis performed a pilot efficacy study and PK study in equines to describe the disposition and time course of drug concentrations of CBD and THC when administered OTM to healthy horses. In the pilot study three adult horses (528–578 kg) diagnosed with navicular syndrome by gait analysis via a Lameness Locator using diagnostic blocking (nerve and/or intra-articular anesthesia) and confirmed through radiographs, were used. Horses had not been treated with any medications for six months prior to study start, or any other medications for 2 weeks prior to study start. The day before drug administration and at the close of the study (Day 7) a physical examination accompanied by a CBC and chemistry profile were performed. Each participant was administered a cookie product containing 360 mg of CBD every 12 h for 7 days (Canna-Biscuits for Dogs, Maple Bacon MaxCBD, Canna-Pet, Seattle, WA). Blood samples were collected at two and 12 h after each dose. It was not noted if a full Certificate of Analysis was evaluated for this product to verify cannabinoid concentrations. The response to therapy in this pilot study was evaluated using the Lameness Locator inertial sensor system (which is body mounted) that analyzes horse gait at a trot on a flat surface. Evaluations were performed at baseline and daily during the course of treatment.

Neither THC nor CBD were detected at any time point in any horse during the 7-day period. The authors used this pilot study to develop a foundation for selecting a formulation, as well as a dose, that would more likely yield quantifiable levels of CBD. The Food and Drug Administration has previously tested the products used in this study and found no cannabinoids at all in some products and others that did not match the label claim of potency. The quality of the product used in this study comes into question based on the three previously mentioned studies and the hypothesis of the first pass effect.

Another study performed was a more comprehensive PK study. Thirteen healthy, fasted, adult horses were administered a cannabinoid oil (0.1 mg/kg) transmucosally.

The oil contained the following concentration of cannabinoids: CBD (12.6 mg/mL), CBC (0.77 mg/ml), Δ9-THC (0.5 mg/mL), CBG (0.2 mg/mL), and CBN (0.2 mg/mL). Terpene profile was not mentioned. Serum samples were collected intermittently for 72 h post administration. After OTM administration, C_{max} was 27.2 (13–53.9) ng/mL at T_{max}: 2.9 (1.9–4.3) hours. MRT and disappearance half-life were 18 h (11–94–28) and 15 h (9–25), respectively after undergoing significant first pass effects. AUC was 247.1 (166.1–367.8) ng/mL/h. The oil (0.1 mg/kg) was well tolerated for administration to horses. These doses did not provide elucidation of a pharmacodynamic effect. In a recently published study, In a recently published study, Ryan et al. (2021) investigated the pharmacokinetics of a CBD powder in sesame seed oil, administered with an oral dosing syringe at either 0.5, 1 or 2 mg/kg to twelve thoroughbred horses. CBD was well tolerated at each dosing level and terminal half-lives were 10.7, 10.6, and 9.88 h for each respective dose. Blood was collected at time 0 (immediately before administration), 15, 30, and 45 min, and 1, 2, 3, 4, 5, 6, 8, 12, 18, 24, 30, 36, 48, and 72 h following administration and evaluated for CBD and metabolite concentrations. This study also evaluated urine for the presence of CBD and its metabolites at time 0 (before administration), 24, 48, and 72 h after administration. In addition to measuring bioavailability of CBD and its metabolites, Ryan et al. also examined blood samples for inflammatory biomarkers (5- hydroxyeicosatetraenoic acid (5-HETE), leukotriene B4 (LTB4), 15-hydroxyeicosatetraenoic acid (15(s)-HETE), thromboxane B2 (TXB2), max prostaglandin E2 (PGE2) and prostaglandin F2 alpha (PGF2alpha)). Unfortunately, the researchers did not note inhibition of COX or LOX enzymes at the doses provided. There were no behavioral effects noted at any dose administered in the study. More research is needed to examine the use of higher doses, or a broad- or full-spectrum product, on inflammatory pathways.

12.6 Indications

Based on the authors' and other experienced veterinarians' clinical experience utilizing cannabinoid therapy in horses, we have been able to determine dosing ranges for ailments. This information should not be used to prevent, mitigate, or cure any disease, but rather as an integrative and, at times, alternative route of therapy for animals as has been described previously in this text. Further controlled safety and efficacy studies in horses would be necessary to obtain an FDA drug label for a particular formulation.

12.7 Anxiolytic

As noted previously, cannabidiol is known to be a potent anxiolytic in humans. An anxiolytic effect has been repeatedly observed in horses receiving CBD therapy. As prey animals by nature, horses are often anxious when placed in new or unique situations, especially if not in proximity to other horses. As such, the events

necessary for horse health, exhibition, or showing place physiologic and emotional stress on equines. Many horses are adept or well-schooled at handling new situations, while others are constantly anxious and can become dangerous to horse handlers or themselves. Common uses of CBD as a calming aid for horses include trailering, clipping, working during rehab from an injury, introducing horses to a new barn, clinics, and for veterinary or farrier appointments. Most horses (average weight of 545 kg) respond to 80–125 mg CBD (with additional trace cannabinoids ideally in a whole plant or full spectrum formulation) sublingually in oil or paste form within 20–30 min of dosing. This effect seems to last 8–12 h and can be repeated as needed; no loading dose is necessary. For equids weighing less (such as ponies and miniature horses), a lower dose of approximately 0.2 mg/kg CBD orally elicits good responses. Drafts and large Warmbloods are typically responsive to the 125 mg dose, as they are often more sensitive to medications than standard mg/kg dosing would suggest.

The United States Equestrian Federation, FEI, and many other horses show regulatory bodies are testing for cannabinoids and are penalizing owners of horses with positive cannabinoid results. Similarly, the Kentucky Racehorse Commission is assigning B penalties to any horses with positive CBD tests. Owners should be advised that cannabinoids are not safe for showing; many owners are using hemp and cannabis products to calm horses in show settings and should be cautious in utilization during their showing season. In our testing of CBD elimination times, a conservative withdrawal time of 7 days would be advised before showing. Larger doses or smaller doses may amend withdrawal times, but this is an area that requires more study.

12.8 Analgesia

Osteoarthritis in multiple joints is a common finding in horses by the time they are middle-aged (15+ years). Common therapies involve daily COX2-selective nonsteroidal anti-inflammatory medications (NSAIDs) (e.g. Equioxx®), intra-articular injections of corticosteroids or biologics, and oral nutraceuticals (e.g. glucosamine, chondroitin sulfate, ASU, etc.). The ubiquity of oral supplements for joint health in horses has grown exponentially in the last 10 years, likely due to the lack of effective options with anti-inflammatory benefits outside of prescription medications. Long term NSAID use can lead to gastric ulceration and renal impairments. Repeated corticosteroid intra-articular injections are deleterious to cartilage health and tend to lose duration of efficacy after many uses.

As covered previously, cannabinoids (especially CBD) have been shown to have anti-inflammatory properties by decreasing inflammatory cytokines. Oral CBD decreased levels of key cytokines (IFNγ and TNFα) in synovial fluid in a murine model with collagen induced rheumatoid arthritis (Malfait et al. 2000). Cannabinoid receptors CB1 and CB2 along with three putative cannabinoid receptors (PPARα, TRPA1, 5-HT1aR) were found in dorsal root ganglia from the cervical spine in

6 horses (Chiocchetti et al. 2020). As the dorsal root ganglia are clusters of sensory neurons, the presence of cannabinoid receptors in this region suggest that cannabinoids are a possible target for pain management in horses. Studies have demonstrated successful CBD use in reducing inflammation in dogs and humans (Gamble et al. 2018; Blake et al. 2006), though the recently published prospective equine study (Ryan et al. 2021) did not show a reduction in inflammatory biomarkers. In the authors' experience, notable improvement in baseline lameness scores and response to flexion has been achieved using 0.25–1 mg/kg CBD every 24 h. The wide dosing range is due to patient variability in response to CBD therapy dependent on product profile and ECS tone. It is important to note that all mammals have the potential to respond to CBD therapy differently. Further applications with cannabigerol (CBG) and cannabichromene (CBC) are promising. Average-size horses (545 kg) with mild osteoarthritis that do not tolerate daily NSAID therapy have been reported by owners to be equally if not more comfortable on 125 mg of CBD powder in grain daily (unpublished survey data). Loading doses of 250 mg CBD daily for 3–4 days or until effect is seen can be used for moderate osteoarthritis to achieve comfort.

Colitis in horses is another painful and significant disease process which seems to be highly responsive to CBD therapy. Equine clinicians should consider cannabinoid therapy for cases of chronic colitis or inflammatory bowel disease, especially in those patients who cannot tolerate systemic corticosteroid therapy. Based on a review of cannabinoids' role in colitis in humans and mice, there is strong evidence to show that phytocannabinoids can decrease inflammation within the gastrointestinal tract and lead to improvement in outcomes. Notably, cannabigerol (CBG) was most effective at reducing MPO activity (marker of inflammation) in a group of ten affected mice (Couch et al. 2018). Other companion animal studies have shown the presence of endocannabinoid receptors in the gastrointestinal tract which is assumed to be true for equid species as well.

Earlier in the chapter we noted a case for use of CBD for allodynia. The study describes a mare with apparent cutaneous hyperaesthesia and mechanical allodynia, predominantly around the withers/shoulder region. After treating with dexamethasone, gabapentin, magnesium/vitamin E, prednisolone, and aquapuncture with no improvement, the horse received 250 mg of a CBD isolate twice a day orally. After 36 h on the CBD isolate, the mare exhibited significant improvement in clinical signs, including allowing more firm touching over the neck, withers, and shoulder region. She was able to be tacked for lunging without negative behavior. Approximately 60 days after dosing was initiated, the CBD dosage was decreased by half which resulted in recurrence of the clinical signs within 24 h. The initial dose of 250 mg twice daily was resumed and tapered more gradually over the next 60 days without any subsequent recurrences of clinical signs. The mare is currently maintained on 150 mg CBD by mouth once daily with the owner reporting a 90% improvement of clinical signs overall (Ellis and Contino 2019).

Laminitis is a significant disease in horses with severe inflammation of the laminae (sensitive layers of tissue) within the hooves. While the underlying cause of laminitis can be varied (equine metabolic syndrome, pituitary pars intermedia dysfunction, mechanical trauma, support limb, septicemia, carbohydrate overload),

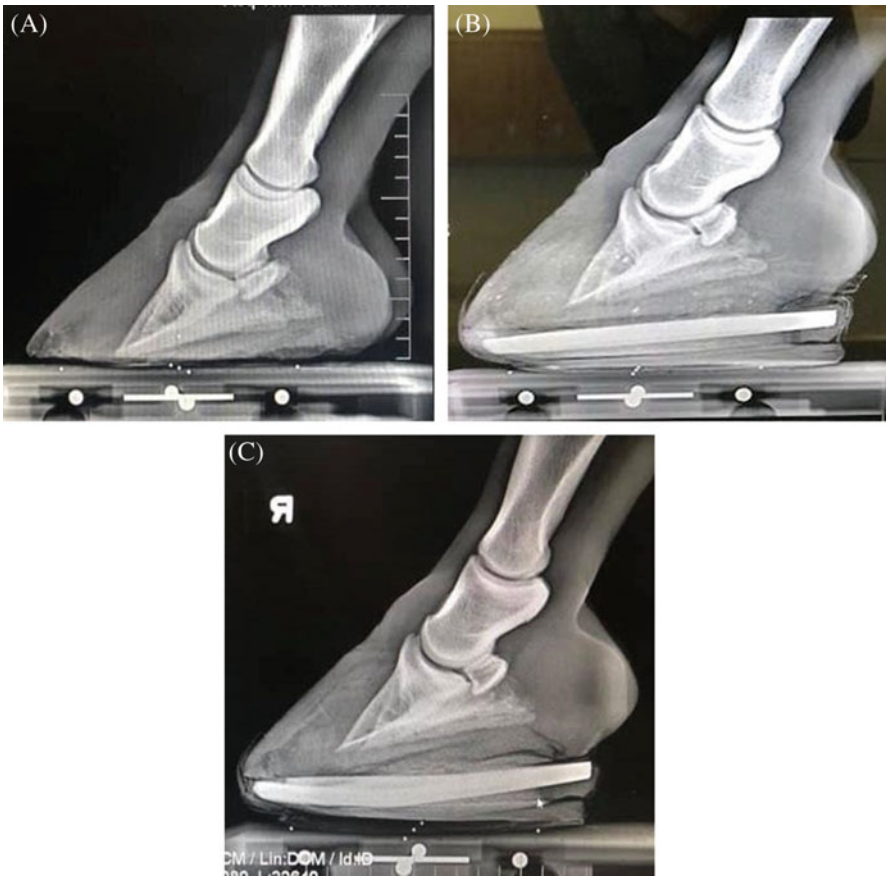

Fig. 12.2 (**a**–**c**) Recovery from acute severe laminitis in a 9-year-old warmblood mare following infectious disease. Severe rotation of the third phalanx (P3) is observed, with absent sole between ground surface and bone (**a**). Substantial sole depth improvement in 6 weeks in (**b**) and improved P3 positioning and sole depth in (**c**) 4 months later (work from authors, permission granted)

ultimately it is difficult to control the horse's pain levels while the primary underlying cause is being addressed (Patterson-Kane et al. 2018). Standard therapeutics include NSAIDs, gabapentin, pentoxifylline, cryotherapy of the distal limbs, corrective farriery, supportive bedding, and various other remedies. Laminitis often leads to uncontrollable pain and ultimately humane euthanasia for a significant number of horses. In this author's experience, higher than average doses of CBD (2–5 mg/kg q12–24 h) can be very useful for analgesia during a laminitic event as an adjunct to traditional therapies and is well tolerated by patients even at high doses.

Case Highlight: A 9-year-old Warmblood mare was diagnosed with acute onset laminitis post severe infectious disease. The coffin bones in both forelimbs had rotated severely and were at risk of rotating through the sole of the hoof (Fig. 12.2). Laminitis is a particularly debilitating condition: this mare did not tolerate

phenylbutazone well and was kept on 500 mg CBD paste daily for 6 weeks, then 250 mg CBD paste daily for 4 additional weeks. Prior to the onset of CBD administration, the mare had severe pain and was non-ambulatory. Within 3 days of receiving daily CBD, the mare's ability to ambulate significantly improved, as did her mentation and appetite. Veterinarians managing the case attribute her improvement to several factors (appropriate farriery, biologic therapy, and CBD). In this specific patient, CBD was integral in pain management, during which time local therapies were employed to improve the prognosis. This patient has returned to her previous level of work as a show hunter.

12.9 Future Avenues and Conclusion

Opportunities to advance our knowledge of *Cannabis* therapy in equids are vast. As in all mammals with an ECS, numerous avenues exist to improve the health of horses. A study published in early 2020 by Sanchez-Aparicio et al. explored the ECS receptor CB2 as a promising target in horses that suffer from chronic and degenerative painful conditions. The future of using phytocannabinoids, given their many potential therapeutic effects in horses, carries endless applications. Other classes of pharmaceuticals offer reliable results but often at the risk of adverse effects on gastrointestinal, hepatic, and renal systems. Our current understanding of product testing, dosing, and cannabinoid profiles will allow cannabis therapy in horses to come to the forefront and offer a safer alternative or integrative therapy option. Significant investments in research funding are needed to continue exploration of cannabis efficacy and dosing; with the improved legal landscape for hemp cultivation and processing, progress in clinical research will begin to unveil further applications and data in horses.

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Chapter 13
Product Selection and Dosing Considerations

Robert Silver, Sarah Silcox, and Danielle Loughton

13.1 Introduction

The phytoconstituents of *Cannabis sativa* L. can be classified as pharmaceutical ingredients, nutrients, nutraceuticals, supplements, or herbal products. In the US the regulation of each constituent, in terms of veterinary use, is the responsibility of the Food and Drug Administration's Center for Veterinary Medicine (FDA-CVM). In Canada these constituents and products are regulated by Health Canada. For designation as a pharmaceutical there is a lengthy and expensive approval process defined by the FDA-CVM and Health Canada Veterinary Drugs Directive (VDD) for animal drugs.

"Nutraceutical" is a term that was created in 1989 by DeFelice by joining the two words "nutrition" and "pharmaceutical" (Kalra 2003). It has no legal definition but refers to those compounds that are neither nutrients nor approved pharmaceuticals. In 1996 the North American Veterinary Nutraceutical Council (NAVNC) defined a veterinary nutraceutical as a "[non-drug] substance which is produced in a purified or extracted form and administered orally to a patient to provide agents required for normal body structure and function and administered with the intent of improving the health and well-being of animals" (Booth 2009). This definition is not legal, nor is it accepted by any regulatory agency, though it is used frequently in marketing and other materials. The FDA-CVM works with the National Animal Supplement

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Council (NASC) to regulate nutraceuticals or herbal products designed for animal health. At the time of publication, hemp is an unapproved feed ingredient for animals and, as such, cannot be included as a nutritional ingredient in any animal feed formulation.

13.2 The NASC and the Regulation of Animal Supplements

In 2000 Iowa State Feed Control officials began to remove nutritional products containing glucosamine from the shelves of veterinary offices. Glucosamine is not approved by the FDA-CVM for its intended use in joint health. At that time a trade group was formed, the National Animal Supplement Council (NASC) to work with regulators at both state and federal levels to develop workable guidelines for companies to follow that would satisfy the regulators' concerns about these products that are commonly referred to as nutraceuticals.

As a result of this work by the NASC, animal supplements, defined by the FDA-CVM as "dosage-form animal health products", may be marketed to veterinary consumers provided companies act responsibly. Guidelines for responsible behavior include but are not limited to: the labeling of products appropriately; making claims that are not in violation of Sec. 201(g)(1)(b) of the Federal Food, Drug, and Cosmetic Act (FFDCA) (Food Drug and Cosmetic Act n.d.); following current Good Manufacturing Practice (cGMP) standards; implementing effective risk monitoring/management systems; ensuring product safety; and protecting the health of both animals and people.

This trade group established a website for veterinary nutraceutical adverse event reporting (AER). This database, the NASC Adverse Event Reporting System (NAERS™), now contains risk management data, product labels, and statistical analyses on hundreds of animal nutraceutical and nutritional products as well as individual ingredients. NAERS™ contains many millions of recorded administrations of a given supplement and can accurately report the percent and type of adverse events recorded over these large numbers of animal administrations. NAERS™ is currently the most advanced database in the world for supplements for dogs, cats, and horses.

The NASC began with 18 company members in 2001 and has grown steadily to over 350 members as of June 2020. Member companies must comply with cGMP standards, follow label template guidelines, not make medical claims, and can use structure and function statements in their marketing materials and on their labels.

NASC members must undergo regular audits by a third party that includes random testing of their products to verify they meet label claims. Member companies that pass the audit are allowed to display the NASC seal on their products and marketing material. This seal has become a symbol of product quality to the consumer as many pet owners will only select those products.

13.3 History of Veterinary Cannabis Products

Cannabis preparations have historically been used as home remedies, medicine, functional food, and a source of nutrition. Reports of cannabis products being used in animals date back to the 1800s when cannabis became a popular herbal remedy. These commercial products, sometimes known as "patent medicines", containing cannabinoids usually in an alcohol-based tincture, were typically high in THC and low in CBD, and were sold to horse owners for colic and other equine ailments. Cannabinoid products also included topical ointments, salves, and poultices that were used externally for joint and lameness problems. The use of cannabis in horses became less common as cannabis was made illegal and as pharmaceutical medicines became more available.

The use of cannabis products in companion animals gained popularity in the mid-1990s as cannabis was legalized (on a state by state basis) for medicinal purposes in humans. Pet owners, learning of the many benefits of cannabis in people, understandably began to explore its use in their pets as substitutes for pharmaceuticals or in situations where pharmaceuticals were not well tolerated or were ineffective.

Today pet owners are likely to use cannabis products for their pets for a variety of reasons. In two studies, Kogan et al. used surveys to assess the reasons pet owners use hemp products (Kogan et al. 2016, 2018, 2019):

- Epilepsy that is resistant to pharmaceutical remediation
- Pain that is poorly responsive to opiates, steroids, and non-steroidal anti-inflammatory drugs
- Cancer treatment side effects such as nausea and vomiting
- Cancer therapy
- Alternatives to drugs that are toxic to the patient
- Alternative to drug use
- To reduce inflammation

13.4 From Biomass to Finished Product: Considerations for Product Selection

The authors recommend the following six criteria to govern product selection:

1. The cultivar of hemp plant, in terms of its content of cannabinoids, terpenes, and flavonoids
2. The drying and handling of the plant biomass in terms of the acidic or neutral cannabinoids and delicate terpenes
3. The method of extraction of plant constituents
4. The method of concentration and separation of plant constituents
5. The type of extract product
6. The format of the product that is administered to the veterinary patient

13.5 Plant Cultivar-Dependent Product Consideration

Although the predominant cannabinoid found in hemp cultivars is cannabidiol (CBD), the entire mixture of minor cannabinoids, terpenes, and flavonoids will determine the clinical conditions best addressed by this cultivar-specific fingerprint of phyto-constituents. Ultimately, the considerations involved in the selection of a hemp or cannabis product for a practice or for a specific clinical presentation need to be supported by objective data demonstrating its clinical value. Currently, the literature contains only experimental data regarding the clinical usefulness of specific phyto-constituents. Products that promote the value of these cultivar-specific constituents will need to support these claims with objective research.

13.6 Drying, Decarboxylation and Storage Methods' Influence on Product Consideration

Historically, the plant biomass is harvested and dried, then subjected to moderate temperatures to convert the acidic/raw forms of the cannabinoids into their neutral forms. Heating of the biomass is termed "decarboxylation". This technique evolved over the years as a way to "activate" the acidic form of THC, which is not intoxicating, into its intoxicating, neutral form. This heating of the plant material became the standard for processing cannabis biomass due to cannabis' use primarily for "recreational" purposes. Until the advent of legal medical cannabis and hemp, minimal investigation into the value of the acidic, raw forms of the cannabinoids was pursued, since all cannabis and hemp biomass was dried and decarboxylated.

Plant material that is dried but not subjected to the heating process of decarboxylation will still contain substantial acidic/raw cannabinoids and most of the volatile terpenes are also conserved. This biomass needs to be stored in a cool place and sealed from exposure to air in order to conserve these delicate molecules and avoid oxidation. The conservation of these molecules is not as complete in the dried biomass as in the raw material.

An optimal time and temperature for decarboxylation of THCA is 6 minutes at 145°C (Wang et al. 2016). The use of lower temperatures for longer periods of time can be effective for converting the cannabinoid acids THCA and CBDA to neutral THC and CBD molecules. (e.g., 110°C for 30 min). Lower temperatures can help spare terpenes, particularly the more volatile monoterpenes. Slow decarboxylation of THCA to THC can occur at room temperature as cannabis dries and ages. One study determined that after a year in storage 50% of the THCA in cannabis was converted to THC (Brenneisen 1984). Storage will also lead to a decrease in total THC content via oxidation to cannabinol (CBN). In another study it was found that, after 47 weeks, THC content decreased by 7% with dark, dry storage at 5 °C and by 13% at a temperature of 20 °C. With additional light exposure the THC loss increased to 36% (Fairbairn et al. 1976).

13.7 Extraction Methodology and Product Selection Considerations

A number of methods exist to extract the active molecules in cannabis from the raw, dried, and/or decarboxylated forms of the plant. Each method has its advantages and disadvantages in terms of the preservation or loss of individual components of each harvest's active molecules (cannabinoids, terpenes, and flavonoids). Each method can also contribute contaminants, such as residual solvents, or can reduce the overall active phytochemical content. Extraction methods employ chemicals or temperature and pressure in their methodology. This compares to concentration and separation methodologies which commonly use temperature and the differences in vapor pressure and boiling points to separate the individual components of an extract.

13.7.1 Raw Plant Juicing

Juicing of cannabis leaves extracts acidic cannabinoids, terpenes, and flavonoids. The cold extraction of raw cannabis preserves the heat-sensitive acidic cannabinoids that otherwise undergo degradation through the decarboxylation pathway. The monoterpenes, which are the smallest and most volatile of the terpenes, are well conserved by this extraction of raw, fresh plant material. Juicing of cannabis plant flowers and leaves can be performed with a juicing apparatus adapted to leafy plant material such as wheat grass. The liquid material created by juicing can be frozen for 100% retention of acidic and volatile plant components or can be freeze-dried which will also conserve the bulk of the raw plant molecules, depending on the freeze-dry process used.

13.7.2 Ethyl Alcohol Extraction

Alcohol extraction using (ethyl alcohol (ethanol) is a common solvent used to extract phytocannabinoids, terpenes, and flavonoids from cannabis biomass, although other forms of alcohol can be used. This can be an efficient process, potentially removing nearly 90% of the active material present in the biomass under optimal conditions. The ethanolic extract is then subjected to a number of different possible concentration technologies by evaporating the ethanol and further refining the product with distillation and chromatographic techniques.

13.7.3 CO_2 Extraction

In this process, pressurized liquid carbon dioxide is used to extract the desired phytochemicals from the cannabis plant. Extraction with carbon dioxide is

adjustable by altering pressures and temperatures to selectively isolate a wide range of target compounds. The pressures and temperatures can greatly affect the solubility of terpenes and cannabinoids. Subcritical conditions are inefficient at cannabinoid extraction but are well-suited for terpene extraction. Higher pressures and temperatures in the supercritical range can also extract unwanted impurities without the ability to selectively extract the cannabinoids.

Supercritical extractions (>88 °F), due to their higher extraction temperatures, are more efficient in removing larger molecules such as waxes and fats. Temperatures exceeding 120 °F can result in decarboxylation of cannabinoid acids with prolonged extraction times.

Subcritical extractions (<88 °F) run at lower temperatures, lower pressures, take a longer time to complete, and allow the terpenes to be selectively extracted. These conditions are typically not sufficient to extract cannabinoids without the use of a second solvent (Reverchon et al. 1995; Azmir et al. 2013).

13.7.4 Liquefied Hydrocarbon Extraction

The use of non-polar liquefied hydrocarbon solvents**,** such as butane or propane**,** is a common method of extraction of phytochemicals from the cannabis biomass. These solvents are highly volatile and, when mixed with air, have the potential for ignition with a high risk of explosion. Industrial chemical technology uses a closed loop extractor system that prevents the vapors of these solvents from mixing with air. In these systems the hydrocarbon solvent is refrigerated and blended with the biomass to extract the phytochemical constituents. The use of a vacuum and/or indirect heating of the solvent in this closed loop system allows for evaporation; the solvent can then be condensed and recycled.

Hydrocarbon extraction is not commonly used for extraction from the cannabis plant for oral consumption, although it is a less expensive technology and some edible products may still use extracts from this technology. Residual solvents in the resultant extract are usually not measurable. Extraction with ethanol or CO_2 are more commonly used for ingestible plant extracts. Hydrocarbon extractions are still extensively used for commercial and high-THC products (e.g., “shatter”, “wax” or other highly concentrated products designed for inhalation) due to efficiency and relative low cost.

13.7.5 Fixed Oil Extraction

Fixed oils, such as coconut oil, grape seed oil, olive oil, butter, etc., can be used to extract the lipophilic phytochemicals from the cannabis plant. This technique requires milder temperatures and more time compared to hydrocarbon extraction. The oil infusion of cannabinoids and terpenes is commonly used for salves or topical

products, or it may be blended or baked into a treat to create edible dosage form animal health products.

13.7.6 Thermal/Pressure Extraction

Technology that uses heat and pressure to "express" or squeeze the fatty components from the fresh, undried biomass without using solvent is not a very efficient process but produces an extract called "live" or "fresh" resin (or rosin). The primary application for this type of concentrate is combustion and inhalation although it can be ingested if dissolved in alcohol, oil, or butter.

13.8 Concentration and Separation of Plant Constituents

13.8.1 Distillation

Distillation is a physical process, utilizing vapor point and volatility differences to separate the multiple components of the extracted cannabis.

13.8.2 Simple Distillation

This process is used to separate two liquids based on their relative boiling points. The boiling points of the two liquids must differ by a minimum of 25 °C. This technique can also be used to separate a liquid from a non-volatile compound with a higher viscosity. This process typically involves creating a vacuum in order to reduce atmospheric pressure and lower the vapor point of the mixture's components. This allows for a lower temperature to be used in distillation which conserves the more heat labile components. Upon heating, the vapors are collected through a process of condensation. This can result in an impure distillate.

13.8.3 Fractional Distillation

This form of distillation is more effective in separating the individual components of a liquid mixture. It uses a fractionating column that is placed in the distillation apparatus, between the heated container and the condenser. The column is filled with materials that encourage condensation due to their increased surface area. This condensate is then re-evaporated by the rising hot gases from the heating container and the process repeats itself, resulting in an increased concentration of molecules

with higher vapor pressure. In this way, fractional distillation achieves better separation with less material loss versus multiple simple distillations to achieve the same result.

Fractional distillation allows for the separation of the different constituents of the liquid mixture based on their molecular weights, which translates into their vapor pressures and boiling points. Lighter molecules, such as the monoterpenes, will separate out at lower temperatures and the heavier molecules, such as cannabinoids, will separate out at higher temperatures.

"Short path distillation" is a form of fractional distillation using a specific type of laboratory apparatus configuration.

13.8.4 Steam Distillation

This process is used to distill compounds that are sensitive to heat. Some organic compounds that are temperature sensitive can be denatured at the temperatures required for simple distillation. Steam distillation uses the chemical properties of liquids that are immiscible; the process can be conducted at lower temperatures than those used for simple distillation. Using steam at ambient atmospheric pressures ensures that the temperature of distillation does not exceed 100 °C.

This distillation technique is performed by running steam through the biomass or the liquid mixture to be distilled and then condensing the vapor. This results in two layers being produced: a layer of water and a layer of the compound being distilled. This condensate is then separated based on the immiscibility of these liquids by physically separating them via decanting or the use of a separation funnel.

Steam distillation releases the volatile and the fixed oils contained in the biomass. The smaller, more volatile molecules are extracted first. This process is used extensively in the production of essential oils. The disadvantage of this process is its poor efficiency in terms of a less than complete removal of the active molecules contained in the plant material when compared to other extraction techniques.

13.8.5 Vapor Distillation

This technology is a variation on steam distillation, using hot air versus steam to liquefy and extract the lipophilic components of the plant. Proponents of this technology claim that it does a better job of sparing the more volatile terpenes and cannabinoids, resulting in a better representation of the plant's cannabinoid, terpene, and flavonoid fingerprint. The extract derived from this technology still needs to be winterized (see below) to remove waxes, and then heat distilled for further concentration if desired.

13.8.6 Winterizing

This technique is used to isolate and remove plant waxes that may cause cloudiness in cannabis oils. Extractors take advantage of the limited solubility of these plant waxes in alcohol solvents to separate them from the liquid mixture using chilling and filtration methodologies. The mother liquid is then evaporated to leave the cannabis oil translucent, wax-free, and with slightly higher concentrations of cannabinoids than the pre-winterized extract.

13.8.7 Chromatographic Purification and Separation

Industrial production methods to produce purified, single component extracts typically use a chromatography method to separate and isolate constituents to produce a nearly pure form for commercialization. This process is also used to remove THC in order to produce broad-spectrum extracts with very little THC present. This technology depends on the difference in molecular weight among cannabinoids in an extract, giving each specific molecule a unique transit time through a vertical column; this allows individual fractions of the extract to be removed for commercial purposes. This technology is similar to fractional distillation in that it separates the individual components of an extract, but chromatographic processes do not involve heat.

13.9 Types of Extract Products

Extraction and concentration usually result in a thick, resinous oil with concentrations of CBD ranging from 30 to 90%. With concentrations higher than 90% CBD will crystallize at room temperature, which is the last step before it becomes a purified 99.7%-pure powder. The oil form of the extract may have its THC removed chromatographically, be blended with a carrier oil to produce a specific concentration of phytochemicals, or further concentrated and crystallized to become a purified isolate. It is these forms of concentrated extract that are then manufactured into final commercial products, whether those be liquid oil infusions, commonly known as "tinctures", or blended into dosage form animal health products like biscuits, soft chews, paste, or pellets.

13.9.1 Hash Oil/Hemp Oil

These are concentrated oils that result from a variety of extraction methodologies. If this concentrated oil is derived from marijuana it is popularly called "hash" oil. If derived from hemp it is called "hemp" oil or, more imprecisely, "CBD oil". The basic difference between hash oil and hemp oil is the content of THC.

13.9.2 Distillate

Distillates employ a given extraction technology to the cannabis biomass and then, through one or several of the distillation processes, create a 30–99% pure oil. Typically, this oil is ingested in a small amount, or combusted and inhaled through a high temperature technique using either a vaporizer or a process called "dabbing". The actual dosage delivered with a grain-of-rice-sized distillate is based on the concentration of the distillate and the volume of that small bead of oil. Other names for distillates include Rick Simpson Oil (RSO), Phoenix Tears or oil concentrate. There are other locally popular names for products made in this manner.

13.9.3 Isolate

Similar to distillates, isolates are concentrated forms of a single cannabinoid. Generally, isolates are 99.7% pure at their highest concentration. Isolates are manufactured by subjecting distillate to further extraction using hydrocarbons and acetic acid, which is then further filtered and processed in a rotary evaporator. The extraction is then subjected to an industrial chromatographic process. This process is repeated with a different solvent and further chromatography and drying, which results in a highly purified cannabinoid extract. THC isolate is generally seen in a liquid format, whereas CBD isolate is a crystalline powder in its purest form (United States Patent Application. . . n.d.).

13.9.4 Full or Complete Spectrum

"Full" or "complete" spectrum refers to a cannabis extract that contains all of the cannabinoids, terpenes, and flavonoids extracted from an individual cannabis plant. The mistaken belief that THC is required for CBD and other cannabinoids and terpenes to work exists among many dispensary workers. THC is not necessary to "activate" CBD. There is no scientific basis for this statement based on our current understanding of cannabinoid receptor physiology or chemistry. "Activation" is decarboxylation, the heating of the plant biomass or extract to convert the acidic forms of cannabinoids to their neutral form. This process converts the minimally intoxicating THCA into THC.

Due to the wide-spread use of decarboxylation in the past, acidic cannabinoids have only marginally been scientifically investigated. Recent studies suggest that the acidic cannabinoids may possess properties of increased anti-inflammatory activity and they may have improved bioavailability over their neutral forms. This research is in its early stages and studies are underway to more fully understand their role in health and disease (Deabold et al. 2019).

13.9.5 Broad Spectrum

Full spectrum hemp extracts that have had their THC removed to extremely low levels are defined as "broad spectrum". This is advantageous for patients who are excessively sensitive to THC. These products are often labeled as "Zero THC". In fact, unless they are derived from isolate, there will be a trace amount of THC present. There is no regulatory guidance expressing the actual upper limit of THC allowable in this definition. This term was created by the industry to tag products that have reduced the THC content post-extraction to differentiate them from products that have not had their THC content modified.

13.10 Veterinary Product Formats

Product formats for veterinary applications must be adapted to specific considerations for each veterinary species with respect to palatability, routes of administration, and species-specific product ingredients.

13.10.1 Raw Plant Material (Flowers)

The plant material itself can be administered to veterinary patients. If the plant material is derived from hemp, it will, by legal definition, have less than 0.3% THC. Some patients may still be sensitive to that small amount of THC. The flowers (buds) of the plant have the highest concentration of cannabinoids, which are found in the sticky, resinous trichomes. The plant material can be ground and encapsulated, mixed with the animal's food, or given directly to the animal by mouth.

There are problems associated with using plant material, such as inconsistency in potency from one flower to the next, or inaccurate analyses leading to administering unknown amounts of cannabinoids. Potential contamination with microbial overgrowth, pesticides, herbicides, or heavy metals is also a possibility.

13.10.2 Capsules

Capsules contain a fixed volume of active materials and are limited in efficacy to specific weight ranges of patients. This limitation necessitates manufacturing and packaging different capsule sizes to accommodate the many different sizes of veterinary species. Capsules are convenient to keep and administer and are a dosage form that is familiar to most consumers. They can be hidden in a small amount of food to facilitate administration but this may subject the cannabinoids to first pass

liver metabolism. A comparative pK study from Colorado State University measured the bioavailability of three different cannabis preparations. It was determined that oral administration of hemp-derived oil in capsule form was less bioavailable than an oil administered orally; this may be attributable to increased absorption of the oil through the oral mucosa (Bartner et al. 2018).

13.10.3 Tinctures

These are oil infusions of lipophilic extracts of the cannabis plant and can contain either high THC cannabis ("marijuana") or low THC cannabis ("hemp"). Carrier oils can be any fixed oil. The word "tincture" is derived from botanical medicine to denote a liquid extract of botanical material (Wynn and Fougere 2007). Oil-based tinctures may contain cannabis alone, or may be compounded with additional terpenes, other botanicals, nutraceuticals, or pharmaceuticals for a more targeted effect. Tincture labels should include the potency of the formulation in total milligrams of CBD and THC as well as other measurable levels of cannabinoids. The label should also list any other active ingredients in the bottle and any preservatives that have been added to improve shelf life. Tinctures have the advantage of being scalable in terms of dosing different sizes of animals from a single bottle since all that is needed for a larger or smaller patient would be more or less volume, respectively. Tinctures can also be added to or mixed with a small amount of tasty food to facilitate administration.

Extended stability studies of cannabis products are needed to determine the shelf life of various formulations. Existing studies support a range of viability of 12–24 months given optimal storage conditions. This "outdating" should be expressed on the label as an expiration date, manufacturing date, or a "best by" date so as to inform the consumer of how recently the product has been manufactured. This dating of the formulation guides the consumer in selecting a product based on its aging over time and the subsequent gradual deterioration of the delicate active materials contained therein.

13.10.4 Soft Chews and Biscuits

Treat-like products that contain nutraceuticals are officially termed "dosage-form animal health products." This format is very popular among pet-owning consumers. Dosage-form animal health products must be labeled as nutraceuticals and not as nutritional compounds, despite the fact that the nutraceuticals are contained in edible treats. The FDA-CVM is very specific with the language that can be used to describe this category of product and the agency will enforce action against companies that use a nutritional label such as "treat" or "biscuit" which would imply that the

cannabis contained in the product is an FDA-CVM approved ingredient when it is not.

Currently, the Hemp Feed Coalition (n.d.), a non-profit group of regulatory, industry, and academic members, is submitting a Feed Additive Petition (FAP) to the FDA-CVM and ingredient definitions to the AAFCO (American Association of Feed Control Officials) to obtain approval of hemp seed oil and hemp seed cake as acceptable feed additives for dogs, cats, and horses. It will take several more years before a similar FAP will be submitted for each of the cannabinoid-bearing parts of the cannabis plant.

The label of dosage-form animal health products is based on NASC and FDA-CVM guidelines. Their guidance dictates that the label for these animal products must contain a listing of "active ingredients" and "inactive ingredients" in order of decreasing weights of the materials present. "Inactive ingredients" include the nutritional ingredients of the treat. Hard biscuits and soft chews currently are the most popular dosage-form animal health products available.

An advantage of dosage-form animal health products is the relative ease of their administration. Potential disadvantages are:

1. Unequal distribution of active ingredients in each treat
2. Patient hypersensitivity to the ingredients in the treat
3. The fixed amount of active ingredients in each treat creates the need for multiple treats or fractional treats to treat larger or smaller weight patients or a patient whose condition necessitates a higher or lower dosage
4. Reduced shelf life (when compared to tinctures or capsules)
5. Manufacturing processes that involve baking or heating and extruding the material could cause heat adulteration of the product or an uneven distribution of the active ingredients or both
6. Cost per chew is generally higher than cost per dose of oils or tinctures.

13.10.5 Pellets and Powders

Pellets and powders are scalable for dosing a wide variety of target species with varying weights. Powders can be manufactured from: plant material; pharmaceutical modification of the lipophilic extract of the plant material into a powder format; or an inert or active powder that is infused with the lipophilic extract of cannabis.

Powders can be sold in wide-mouthed jars with a measuring scoop used to estimate effective dosages. Standard kitchen measuring spoons can also be used. Powders can be packaged into pouches, sticks, sachets, and, of course, capsules. Individual serving sizes contained in sachets or sticks make these products more convenient for dosing but remove the scalability benefit of powder over dosage-form animal health products and add additional expense to the product.

Pellets are pressed from dried cannabis plant material with or without standard pelleting matrix excipients. They can be given to horses, goats, sheep, and other farm

animals, as well as poultry, swine, zoo animals, caged birds, and pocket pets. Pellets can be pressed either from unextracted biomass or from the post-extraction biomass.

Many thousands of tons of post-extraction biomass of cannabis are produced annually in the US. This is material which has been harvested, dried, and has had the bulk of its cannabinoids and terpenes removed for use in other products, leaving a potential source of CBD (with negligible content of THC) in a natural plant format. This material can be powdered for easy measurement and administration or pelletized for horses and other species that are adapted to eating pellets. Currently hemp is not an approved feed ingredient for animals, and is not allowed to be found in a product intended for nutritional use. Any product resulting from this use must be labeled compliantly as an animal nutraceutical.

13.11 Considerations Regarding Administration

13.11.1 Oral Administration

Ingested routes are very common and quite effective. Pharmacokinetic studies documenting the bioavailability of oral cannabis extracts, specifically the major cannabinoids THC and CBD, in both humans and dogs, demonstrate that 13–35%, depending on the cannabinoid profile, of the lipophilic drug reaches its intended site of action (Samara et al. 1988; Harvey et al. 1991). Recent studies suggest that administration of a full spectrum tincture either with a small amount of fatty food, in conjunction with or immediately followed by feeding, or in a soft chew, may increase plasma levels of cannabinoids (Deabold et al. 2019; Boothe et al. 2020). The acidic forms of CBD and THC, CBDA and THCA, appear to have better absorption compared to the neutral forms CBD and THC (Wakshlag et al. 2020b).

In spite of variations in product and patient bioavailability, good clinical responses have been anecdotally reported in many species. The majority of products currently available for animal species have been formulated specifically for oral consumption, although transdermal formats are currently under development.

13.11.2 Transmucosal/Sublingual Administration

Transmucosal routes of administration can be very effective in most contexts as it is fairly easy to apply small volumes of cannabinoids in a liquid format to the oral mucous membranes. There is some debate about the volume of liquid administered to the oral mucous membranes. It is questioned whether there is a size of volume large enough that would be swallowed versus small enough to be able to reside in the oral cavity long enough to be absorbed there. Bartner measured the comparative plasma levels of an intended transmucosal route against oral and transdermal routes in 30 beagle dogs. The transdermal method of administration demonstrated 12–32%

bioavailability at a dose between 75 and 150 mg/day, based on the measured C_{max} values. The oral transmucosal method of administration demonstrated 55–68% bioavailability. It was concluded from this study that the most bioavailable administration method among these three dosage formats was the transmucosal approach (Bartner et al. 2018). It has been hypothesized, in criticism of this study, that some volume may be too large to completely absorb through the oral mucous membranes (transmucosally). Instead, this volume of liquid was sufficiently large enough to transit through the upper GI tract. Deglutition by the mouth, gravity, and esophageal peristalsis carry the volume distally through the upper GI tract, and therefore, it is not exclusively absorbed through the oral membranes. This larger volume's pharmacokinetics and subsequent blood levels may be delayed due to its GI transit and distal absorption. One way to avoid this potential problem is to use an oil with higher concentrations of the target cannabinoid(s), meaning that lower volumes can be used.

13.11.3 Transmucosal Administration via Suppositories

A small study compared the absorption and effect of both oral THC capsules (Marinol) and THC hemisuccinate suppositories in human patients with muscle spasticity. After measuring peak plasma levels of both THC and its major metabolite, it was determined that plasma levels after oral administration were 45–53% lower relative to the rectal route of administration due to lower absorption and higher first-pass metabolism with oral administration. The relative effectiveness of the oral vs. rectal formulation on spasticity, rigidity, and pain (as determined by patient rating protocols and objective neurological tests) was 25–50%. This difference could be caused by the elimination of first-pass metabolism via oral dosing (Brenneisen et al. 1996). Several other studies in humans, primates, rabbits, and dogs confirm that the rectal route of administration can be effective (Mattes et al. 1993; ElSohly et al. 1991a, b). Currently there are no studies supporting the absorption of CBD via this route in any species. It is likely, based on the similarity in THC and CBD molecules, that rectal suppositories of CBD would show efficacy comparable to rectal suppositories containing THC. While this route of administration may not be practical for most veterinary applications, it could be considered in recumbent animals or those who cannot be easily medicated orally due to oral cavity pain, esophageal disease, nausea, or vomiting.

13.11.4 Topical Administration

Topical applications of cannabinoids have low systemic bioavailability but can penetrate locally to benefit regional anatomy. These products can be difficult to use in veterinary patients due to thick coats or feathers that interfere with local

absorption. Products that are alcohol-based (liniment) or contain emulsifiers in a water-soluble compound (cream or lotion) are much more likely to penetrate the coat and be absorbed. As with many topically applied veterinary products, some of the product is likely to also be ingested so it is important to ensure that all ingredients found in any topical product are safe for consumption by the target species.

13.11.5 Transdermal Administration

Transdermal products involve the use of a bipolar material**,** such as a phospholipid**,** to carry the highly lipophilic cannabinoids across the epidermal barrier into the local circulation and then into the systemic circulation to be transported throughout the body. Transdermals can be creams applied topically to a bald or minimally hairy part of the anatomy or can be manufactured in special "patches" that use a membrane to separate the transdermal solution from the skin which releases the drug slowly into the patient's circulation. In order to apply transdermal patches to veterinary species, most patients will need a section of hair clipped to provide a hairless area for application. Transdermal liquids and creams can be applied topically to the inside of the pinna of the ear, where the patient is unable to get to the application. Transdermal medication absorption is dependent upon local vascularity to carry the medication from the skin into the systemic circulation.

To date, only two published studies have examined the pharmacokinetics of transdermal cannabinoids in dogs. One study by Bartner et al. (2018) compared the pharmacokinetics of CBD when administered orally (as either microencapsulated oil beads or CBD-infused oil), versus CBD-infused transdermal cream applied topically. In this study, the relative bioavailability of the transdermal cream was less than ten percent compared to the CBD-infused oil administered orally. The AUCs from this study were 135.6 min × mcg/mL and 11.7 min × mcg/mL respectively at the 5 mg/kg input dosage of CBD. Transdermal absorption may have been incomplete due to diffusion barriers such as skin thickness or absorptivity of the cream. It is possible that better results could be obtained using different carrier vehicles to address the challenges of transporting a lipophilic substance through the hydrophilic stratum corneum. In the second study by Hannon et al. (2020) six healthy research beagles were treated with a transdermal CBD/CBDA emulsified with Pencream (HUMco) base in a 1:7.5 ratio. The final concentration was 32 mg/ml CBD, 33 mg/ml CBDA, 1.3 mg/ml THC, and 1.0 mg/ml THCA. The transdermal product was delivered in 0.1 cc increments onto the pinna of the ear (approximately 0.6 cc per treatment). Serum concentrations of CBD, CBDA, THC, and THCA were examined prior to and at the end of weeks 1 and 2. Results found that a 4 mg/kg dose of total cannabinoids twice daily resulted in appx 10 ng/ml of CBD, 21–32 ng/ml of CBDA, trace amounts of THCA, and unquantifiable amounts of THC in serum at both weeks 1 and week 2 of treatment. Results showed that CBDA and THCA were absorbed better systemically than CBD or THC. Transdermal products need to be supported by absorption studies in the target species to insure product effectiveness in providing therapeutic blood levels of cannabidiol.

13.11.6 Inhalation

Although inhalation methods provide a rapid onset of action and bypass first pass metabolism in the liver, these methods are currently impractical for veterinary species.

As water-compatible versions of cannabinoids are developed, the possibility exists that a nebulization method could be employed to allow for absorption through the large surface area of the lungs, without involving combustion of cannabis and subsequent potential heat damage to the lung mucosa. Alternatively, future developments may include devices that utilize metered dose inhalers (MDIs), in conjunction with a spacer device (such as an Aerochamber™) that would allow for more convenient inhaled dosing for animals requiring rapid onset or pulmonary targeted therapy.

13.12 Consideration in Selection of Dispensary Products for Veterinary Applications

The availability of cannabis products containing THC for human use is becoming more common in the United States and Canada. In the US, as of January 2021, 37 states, including the District of Columbia, have passed legislation allowing cannabis for medical uses in humans, and 16 states, including the District of Columbia, allow adult-use sales of cannabis. Fourteen states allow only CBD to be dispensed with a medical license, and in six states, all cannabis is illegal.

Hemp has been legalized at the federal level in the United States, but the FDA has yet to issue regulatory guidance for products containing hemp-derived CBD; many states' veterinary medical boards and the American Veterinary Medical Association have urged that veterinarians use caution when recommending, prescribing, or dispensing CBD products for their patients. This guidance should be applied to any OTC animal supplement that is not FDA approved. Forty-seven states have legalized hemp cultivation and commercialization.

Canada has federally legalized cannabis, but Health Canada, like the US FDA, has not yet issued regulatory guidance for veterinary use of cannabis products. Thus, Canadian veterinarians are without regulatory guidance for cannabis use in veterinary practice.

Many US dispensaries and black-market producers in Canada are offering products labeled for use in pets. There is no regulatory oversight for these products for pets in either the US or Canada with regard to quality control or the type or number of cannabinoids they contain. Additionally, most dispensaries and provincial stores are uninformed about the potential hazards of administering THC to veterinary species. Pet owners are looking to extend the benefits they may be experiencing themselves and therefore want to give dispensary products to their pets as well. Pet owners can easily become overwhelmed by conflicting information from dispensary employees ("budtenders") or may be misguided in thinking that cannabis is capable

of curing every condition imaginable. The selection and administration of the wrong product to their pet could create serious problems. In the absence of reliable advice from dispensaries, it is incumbent upon veterinarians to be knowledgeable about dispensary products.

Products most suitable for veterinary applications are products with low THC levels such as CBD isolates, hemp-derived products that naturally have <0.3% THC, and ratio products with low THC levels (such as CBD:THC 20:1). In some instances products that contain higher THC concentrations (such as CBD:THC 1:1) can be beneficial but any such product should be administered under close veterinary oversight to reduce the risk of adverse effects. Edibles for human use may contain toxins such as xylitol, chocolate, raisins, or macadamia nuts; for this reason, human edibles from dispensaries are nearly always inappropriate for veterinary use.

13.12.1 Commercial Dispensary Products

The most common dispensary products include dried flower, oils, and concentrates. Dried flower that is not decarboxylated may be available as trimmed "buds" or may be powdered and available in capsule format. This product has the highest number of acidic cannabinoids, including THCA. The acidic form of THC is generally not intoxicating, and thus is commonly found in products labeled for animal use in dispensaries.

Dispensaries may offer capsules filled with hemp or hash oil. The oil contained in capsules, tincture bottles or syringes marketed for pet use may be full spectrum extracts of marijuana or hemp, broad spectrum hemp extracts, isolates (most commonly CBD isolates), or as ratio products with varying CBD:THC ratios. Ratio products are now being manufactured with other cannabinoids, such as CBN for sleep or pain, and may have terpenes added to impart a specific therapeutic direction to the formulation.

Concentrates are, as the name implies, undiluted cannabis oils that can be found with a range of cannabinoid/terpene profiles. As a result of their high concentration and thick viscosity, these products are commonly sold in 1 ml syringes. In the dispensary, they usually contain high amounts of THC for human medical or recreational use. In most cases, concentrates are smoked, or 'dabbed', although they may also be ingested orally in the amount of a grain of rice. These products are much more difficult to dose appropriately for veterinary species due to the minute quantities needed for smaller patients. The amount of THC, if present, is usually much higher than is safe to use for a smaller pet, as they are designed for human use. As a result, none of these products are appropriate for veterinary species when used "as is" right out of the syringe. If the syringe is warmed up to 70 C to facilitate flow, it can be diluted into a carrier oil, which then would allow for smaller doses to be administered. To create a custom ratio product, a broad-spectrum hemp formula for which there is an accurate analysis of CBD content can be combined with a pure THC distillate syringe for which there is an accurate analysis of THC in the

appropriate amounts to create the precise ratio between CBD and THC needed for a specific veterinary patient.

Additional formats that may be available in the dispensary include transdermal patches and creams, topical products, vaporizer cartridges, pre-rolled dried flower, suppositories, and edibles. Most of these are not appropriate for veterinary species in that they can contain substantial amounts of THC compared to the dosage requirements for veterinary species. Likewise, transdermal creams or patches are adapted for human use and may not be as well absorbed through the skin of a veterinary patient as through human skin.

13.13 Veterinary-Labeled Products

As the interest, demand, and research into veterinary cannabinoid medicine continues to develop, we will no doubt see novel veterinary-labeled cannabis products coming to market. These products have the potential to be FDA-CVM/Health Canada-VDD approved pharmaceuticals. Currently, in the US, these products can be marketed as whole herbal extracts, or veterinary nutraceuticals, although in both the US and Canada, regulation of CBD as a nutraceutical has yet to be finalized. Herbal and/or nutraceutical products must be labeled and sold without medical claims. This compares to products that are US FDA or Health Canada approved drugs, wherein the manufacturer can make medical claims specific to that drug's approval.

In the US, there are currently few veterinary-labeled cannabis products on the market and they represent full-spectrum or broad-spectrum extracts of federally legal hemp plants with high percentages of CBD. These products are sold exclusively to veterinarians directly or through distributors; they may also be sold directly to consumers. Products that are sold exclusively to veterinarians must have a higher standard of quality control than those sold for consumer use. The NASC has been working with the FDA-CVM to establish these standards and member companies of the NASC may display the NASC seal if they have met the high-quality control standards represented by that seal.

While clients of veterinary practices have access to hemp products online and in many local retail outlets, the quality of these products is suspect, considering the current low level of regulatory oversight. Veterinarians should endeavor to dispense (US) only products that are veterinary exclusive or those backed by rigorous scientific evidence, and in Canada advise only on products legally available through provincially licensed cannabis retailers. It is incumbent on veterinarians to educate their clients with regards to the potential problems that could arise from unregulated products with poor quality control being sold as veterinary products.

13.14 Pet Consumer Products

As a result of the currently booming CBD market, and with the rising status of pets in the household, products marketed directly to pet owners are playing a significant role in the cannabis industry. Pet consumer products in the US need to be hemp-derived or CBD-isolate products as it is illegal to sell products with >0.3% THC outside of a dispensary. In Canada however, it is illegal to sell any cannabinoid containing product, including hemp derived products, outside of a licensed cannabis provider. Pet consumer products come in formats that are designed to make administration to pets as easy as possible: oils (some with pet-preferred flavorings), dosage-form treats, capsules, powders, pastes, and pellets.

The quality control surrounding pet-specific consumer phytocannabinoid products does not usually match the standards for regulated veterinary-labeled products by the FDA-CVM or products sold through licensed Canadian cannabis providers.

This is due in part to the large number of companies entering this market with intentions of large profits without regard to the purity or quality of the product. Additionally, labeling language on these products often makes illegal medical claims. Label declarations of potency may also be erroneous.

In a recently published study (Wakshlag et al. 2020a), 29 US commercially available products for animals labelled as "full spectrum" or "broad spectrum" hemp were analyzed for THC, CBD, other minor cannabinoids, and terpenes, as well as for heavy metals. All of these products were found to be within the federal limits of <0.3% THC. 22/29 products were able to supply Certificates of Analysis (CoAs) from third-party, independent laboratories based on batch or lot numbers. Two products did not provide CBD or total cannabinoid potency on their labeling. 10/27 products analyzed fell within 10% of their label claims for cannabinoid content. Heavy metal contamination with lead and/or arsenic was found in 4/29 products. Commercial products must be accompanied by a third-party, independent laboratory CoA detailing potential contaminants and accurate amounts of active molecules. Without the accompanying lot or batch number, there is no chain of custody whereby the analysis can be linked to the specific product. Based on the findings of this study, and on anecdotal reports of product analysis inconsistencies, there is high potential for a commercial product to be inappropriately analyzed, mislabeled, or contaminated in the absence of clear regulatory guidelines. Quality control guidelines for veterinary cannabis products are covered in the last section of this chapter.

13.15 Therapeutic Dosing Considerations

Evidence-based dosages for THC and CBD in the dog, cat, horse, and other species through dosage-tiered Phase I studies have yet to be published. Several published randomized clinical trials have used CBD dosages extrapolated from published studies in human and laboratory animals, verified by single-dose pharmacokinetics

to ensure detectable blood levels over a therapeutic period of time. The studies in dogs used CBD from full spectrum hemp or isolate products. The published safety studies used doses of 2–10 mg/kg (Deabold et al. 2019; McGrath et al. 2018; Gamble et al. 2018). The published efficacy studies have used doses of 1 and 2.5 mg/kg CBD BID in patients with osteoarthritis or refractory epilepsy respectively (Gamble et al. 2018; McGrath et al. 2019).

Objective studies in human patients and multiple case reports from physicians trained in clinical applications of medical cannabis have supported the dosages of THC and CBD currently being recommended for human patients (MacCallum and Russo 2018). Due to the current regulatory considerations affecting veterinarians' clinical use of cannabinoids, there has been little 'hands-on', empirical dose establishment by veterinary clinicians. Dosages have been anecdotally determined largely by hemp companies, pet owners, and those few veterinary professionals willing to assume potential legal risk of their veterinary licenses in a regressive regulatory environment. The reports from veterinarians who recommend phytocannabinoids is that veterinary species have a biphasic response to cannabis dosages in the same way that humans do. "Biphasic" means that a low dosage will generate one set of effects and address one set of clinical issues and a high dosage will produce a second set of effects and address a different set of clinical issues. In practice, the biphasic dose response supports the empirical establishment of the appropriate therapeutic window for an individual patient and condition.

In his presentation at the Second Annual Conference at the Institute of Cannabis Research at Colorado State University in 2017, Tishler discussed the value of low dosages (microdoses) of cannabinoids in the human patient. Microdosing serves to reduce or eliminate the intoxicating effects of THC by the development of tolerance to THC adverse events (Tishler 2018). Using this microdose strategy in veterinary patients for whom the use of THC is necessary and therapeutic (e.g., in severe pain and neoplastic disease) will facilitate the development of tolerance to THC's adverse effects. Once tolerance is achieved, THC dosages can be escalated to achieve the desired clinical benefit(s).

Microdoses for CBD are arbitrarily considered to be 0.5 mg/kg BID or less, while macrodoses are considered to be 2 mg/kg BID or greater (Hartsel et al. 2019). Doses between 0.5 and 2.0 mg are in a grey area with respect to this categorization. Dosing for THC initially is much lower than dosing for CBD due to the potential for adverse effects, even at microdoses of THC in veterinary species. THC needs to be administered initially at an ultra-low dosage, such as 0.05–0.1 mg/kg BID, in order to establish tolerance to its adverse effects. Once tolerance has been established after 7–14 days of ultra-low dosages, then the dosage of THC can be titrated up. As a general rule, due to the intoxicating properties of THC, the use of macrodoses should be very gradually titrated. It is rare that a dose of THC as high as 2 mg/kg would be used in a veterinary patient. Physicians recommending cannabis to human patients are advised, with respect to dosing THC, to "start low, go slow, and stay low" (MacCallum and Russo 2018). This is advice that would be well-heeded by veterinarians as well. For veterinary species in which objective dosing studies have not been performed, dosage ranges have been established empirically through thousands

of veterinary uses. The empirical dose starts low (0.25–0.5 mg/kg), with slow and steady dose titration based on the patient's response. Currently, in the absence of objective dosing data, this scheme yields the best clinical results. It is recommended to give any particular dose for 10–14 days to allow the development of steady-state blood levels.

13.15.1 Macrodosing and Safety

McGrath evaluated the safety of administering high doses of CBD to 30 dogs over a 6-week period. One result of this study was that the macrodose levels (10–20 mg/kg/day divided into two doses) were associated with a greater frequency of adverse reactions. Diarrhea (100%), sedation, and serum alkaline phosphatase (ALP) elevations (36%) were recorded in these purpose-bred research beagles receiving 10 and 20 mg/kg/day. ALP elevations were more common in the 20 mg/kg/day dosage tier (McGrath et al. 2018).

Deabold et al. evaluated the safety of administering 2 mg/kg BID of 1:1 CBD/CBDA from a full-spectrum hemp in a soft chew format to eight dogs for 12 weeks. In a second wing of this study, eight cats were administered 2 mg/kg of 1:1 CBD/CBDA in an encapsulated oil tincture blended with fish oil. The dogs tolerated this macrodose well, which was approximately 60–80% lower than the dosages used in the McGrath study. There was no evidence of elevation of ALP in these dogs outside of normal reference ranges, the development of loose stools was substantially less (3.3%), and no sedation was reported. In this first study of full spectrum hemp extracts in cats, Deabold found substantially lower C_{max} and AUC in the pharmacokinetic arm of the study. The feline participants presented technical difficulties in acceptance of the oral encapsulated dose and in allowing multiple blood draws. Out of eight cats initially enrolled, only four were able to complete the pK part of the study. The pK results from these cats demonstrated substantially lower C_{max}, AUC, and higher T_{max} values, suggesting that cats may need higher dosages for clinical results. In the safety portion of this study, one of the four feline participants had measured ALT levels in the upper limits of the reference range throughout the study period (Deabold et al. 2019). In speaking with the study authors, the cat's ALT levels normalized by the end of the study with no clinical manifestations of concern.

A Canadian study compared the safety of administering CBD alone, THC alone, CBD+THC, and a placebo in dogs. Dose escalation of each of the four treatments was performed every 3 days between administrations. Dose escalation for each group was terminated when adverse events became severe/medically significant in two out of the four subjects in the group. The CBD-dominant, full spectrum oil was shown to be as safe as the placebo and safer than the dose escalation of both the THC-dominant oil and the THC/CBD ratio oil. Researchers were able to escalate the THC/CBD oil up to the fifth dose, whereas all other groups were able to escalate to the tenth dose. Moderate adverse effects (AEs) (4.4% of all AEs) and severe/medically significant AEs (0.8% of all AEs) such as lethargy, hypothermia, or ataxia

occurred mainly in the two groups receiving THC in the oil. The ratio product tested contained a ratio of CBD:THC of 1.5:1 (Vaughn et al. 2020). This study did not employ a technique for developing tolerance to the THC, hence the high rate of AEs recorded.

13.15.2 Ratio Dosing

Ratio dosing uses cannabis formulations that contain both THC and CBD in specific proportions to each other. The goal of this approach is two-fold:

1. Allow for the development of tolerance to THC through the gradual introduction of increased amounts, balanced against the anti-intoxicating effects of CBD. Tolerance enables the use of higher doses of THC with fewer adverse reactions.
2. Once tolerance is established, the dosage of the THC in the ratio can be slowly increased for improved clinical response to those conditions poorly responsive to CBD alone, or to the low level of THC (<0.3%) found in hemp.

It is important to remember that the dose of CBD administered as part of a ratio product will increase as the THC dose is also escalated. Therefore, depending on the dosages of each cannabinoid required, moving to a ratio formulation with a higher amount of THC in the ratio (a lower CBD:THC ratio) may be more efficacious. It may also make sense to administer the THC and CBD as separate products. Ratio formulations of CBD: THC (1:1) are used empirically and effectively for moderate to severe pain and in oncology patients for whom the low or zero THC ratio product does not provide sufficient anti-nociceptive or anti-neoplastic activity. In rare situations in veterinary species, it may be necessary to provide a high THC formulation for improved pain management or to better address the needs of the patient. For this high THC ratio, a CBD:THC ratio of 1:4 is most commonly used clinically. The CBD in the ratio should reduce the AEs that higher doses of THC commonly cause in veterinary patients.

In a recently published study (Curtis et al. 2019), 11 beagles were administered a single oral dose of one of three oil-infused formulations. Group 1 (n = 4) received THC (0.24 mg/kg) and CBD (0.12 m/kg), a 1:2 CBD:THC ratio. Group 2 (n = 4) received THC (0.12 mg/kg) and CBD (0.24 mg/kg), a 2:1 CBD:THC ratio. Group 3 (n = 3) received a placebo. The dogs were observed for clinical effects for 3 days, with blood samples obtained at 15 time points over the 72 h study period to measure THC and CBD concentrations, as well as biomarkers for pain and inflammation. None of the dogs in this study exhibited any AEs or intoxication at these doses. The results of blood testing concluded that both THC and CBD were bioavailable following oral administration and had the potential to control pain and inflammation in dogs. This study suggests that using THC doses between 0.12 and 0.24 mg/kg are unlikely to pose significant AEs when used in combination with CBD. However, the golden rule of "Start Low, Go Slow" is always advised, due to the unpredictable nature of individual responses. This study also shows the importance of the initial dosage of THC relative to the development of AEs. This is evident when compared

to the Canadian study (Vaughn et al. 2020) cited above in which the dose was escalated every 3 days, precluding the development of tolerance. The lower doses of THC in the latter study (Curtis et al. 2019) were insufficient to cause AEs in this study group. These results are consistent with anecdotal reports from veterinarians and pet owners experienced in using ratio products.

13.16 Adverse Events (AEs) and Dosage

The NASC maintains an Adverse Event Reporting website, that is available for the FDA-CVM to review and contains confidential information regarding specific commercially available animal supplements. In the Ingredient Risk Report for all hemp products, the number of reported adverse events over the 10 years that the use of hemp in dogs, cats and horses has been reported in this website's data was 131 AEs. This was out of a total of 68,860,368 administrations. There were no serious AEs reported for this 10-year time period for hemp use in animals (National Animal Supplement Council n.d.).

McGrath's CBD safety study (McGrath et al. 2018) determined that dosages of 5 or 10 mg/kg of CBD BID, using a full-spectrum hemp-derived product, produced a greater incidence of AEs than those reported at lower dosages in other studies (Gamble et al. 2018). McGrath found elevations of serum alkaline phosphatase (ALP), diarrhea and sedation to be side effects in her study using higher dosages. Other liver function tests, such as bile acids and ALT, were within normal limits. Other studies investigating lower dosages have demonstrated increases in ALP with no other AEs reported. More research is needed but it appears that AEs are primarily dose dependent.

13.17 Drug Interactions with Cannabis

In human medical cannabis patients, physicians have only rarely observed significant drug interactions between cannabis and pharmaceuticals. A recently published human review article states: "there is no drug that cannot be used with cannabis, if necessary" (MacCallum and Russo 2018). In human patients, the main AEs have been associated with the concomitant use of CNS depressants. Drug interaction studies for each individual drug and cannabinoid are lacking but the studies that currently exist have found no increased toxicity or loss of effect with concomitant medication use.

Depending on the strength of the affinity of a substance for the metabolizing cytochrome, serum levels of cannabinoids or pharmaceuticals may increase with enzyme inhibitors or decrease with enzyme inducers. It is known that THC and CBD in humans are oxidized by the p450 cytochromes (CYP2C9, 2C19, and 3A4). It is assumed that this is similar to the metabolic pathways in the dog and other veterinary

species. Studies still need to be conducted in each species to detail possible interspecies variation in the p450 cytochromes that are involved with cannabinoid metabolism.

McGrath, in her refractory epilepsy study, measured both phenobarbital levels and potassium bromide (KBr) levels at the end of the 6-week study period. She found no statistical difference between the treatment and placebo groups for either anticonvulsant. This may indicate that, in spite of the theoretical potential for interaction between CBD and CYP450-metabolized drugs, the interference may not be clinically relevant in the dog. McGrath stated in her discussion that the small study group size may not have given an accurate indication of these interactions (McGrath et al. 2019).

Regardless, it is good medical practice to retest important serum drug levels 2 weeks following initiation of CBD or THC therapy, especially if using macrodose protocols. If drug tests for the interacting pharmaceutical are not available, then clinically evaluating the patient for the effectiveness of that pharmaceutical is recommended.

13.18 Quality Assurance

Veterinarians should be able to identify products that are the result of current good manufacturing practices (cGMP); have third-party, independent laboratory analyses; and follow FDA/Health Canada-compliant labeling practices. FDA guidance for non-approved products includes making no medical claims on the label that suggests the OTC product can treat, prevent, or mitigate a disease or condition.

13.19 Third-Party Laboratory Testing

Quality control (QC) testing is a critical aspect of any manufacturing process. Without QC testing, manufacturers would not be able to sell products with standardized concentrations. In addition, contamination can be introduced at a number of points in the manufacturing process and the only way to assure that the product remains unadulterated is through validated testing methods. Important considerations in the production of a clean cannabis product include organic cultivation methods, Good Agricultural Practices (GAP), and cGMP protocols.

The analytical laboratory performing the analysis must be validated for accuracy and precision in its measurements in order for the standardization of product potency and safety to be effective. Currently there are no established federal standards for low-THC cannabis testing. The ISO/IEC 17025 standard for analytical testing laboratories has become a requirement for labs providing testing services in multiple US states and is a foundational part of the interim USDA Hemp Farming and Testing guidelines (Interim USDA Hemp Farming and Testing Guidelines n.d.). Companies

that use in-house laboratories must have those results confirmed through the use of validated, third-party analytical laboratories. It is also up to the analytical laboratory to apply statistically valid sampling methods to obtain a representative sample of a batch in order to generate meaningful data (Hartsel et al. 2019).

In Canada, the production, sale, and export of cannabis is strictly regulated and requires that license holders follow Good Production Practices. GPP includes general provisions that encompass a set of standard operating procedures, the controlled use of only approved pest control products, requirements for appropriate storage and distribution, air filtration, equipment, sanitation, and quality assurance programs. It also requires specific sets of analytical testing using validated methods. Testing of each batch or lot of cannabis must include analysis for residual solvents (where applicable), contaminants (microbes, heavy metals), dissolution or disintegration (for certain formats), and the quantity or percentage of THC, THCA, CBD, and CBDA (Justice Laws Website; Government of Canada n.d.).

Any test performed on cannabis products should culminate in a Certificate of Analysis (CoA) that should be provided rapidly on request to the veterinarian or consumer. The CoA should include:

- A breakdown of all cannabinoids present in the product
- The CBD:THC ratio in the product
- Terpene analysis
- Heavy Metal testing
- Herbicide / fungicide / insecticide testing
- Microbial testing
- Mycotoxin testing
- Residual solvent testing

13.19.1 Cannabinoid and Terpene Potency Testing

Accurate analysis of any given product's active ingredients is absolutely essential in order to achieve effective and consistent dosing of a patient. An accurate analysis for each manufactured batch is needed to meet strict food and drug guidelines. The contents of the product (including active and inactive ingredients) need to be listed on the product label and in marketing materials to allow the clinician to accurately establish an effective dosing strategy for the patient. Accurate labeling allows the pet-owning consumer to better select the appropriate potency of product and the duration of therapy the content of active ingredients will supply to their pet.

Due to the lack of federal regulations, manufacturers can make label claims regarding the cannabinoid and terpene content of their products in order to mislead consumers and inflate the perceived value of their products. This can pose a serious health risk if a patient is not getting the dosage necessary to alleviate their condition. Certificates of analysis not only convey the concentration and purity of cannabis derived products but also allow for more accurate dosing of that product.

13.19.2 Pesticide Contamination

The application of pesticides has long been a standard practice in the cultivation of cannabis plants despite market demand for organic products. The pesticides that are commonly sold in hydroponic stores can present a specific health risk to both animals and humans. For example, Eagle 20 (a commonly sold fungicide) contains myclobutanil that produces cyanide as a reactive by-product to heat exposure. Paclobutrazol, another fungicide and plant growth regulator, has been shown to cause liver and downstream kidney damage in animal case studies. California has designated it as a Category I pesticide, meaning no detectable amount is allowed in a cannabis product. It is imperative to insist on a multi-residue pesticide analysis from a third-party laboratory for any cannabis product.

13.19.3 Microbiological Contamination

Food testing has long set the standard for ensuring microorganisms are not present in sufficient quantity in products meant for consumption. While it is fairly rare to find organisms like *E. coli* or *Salmonella* in cannabis, it is quite common to find *Aspergillus*, a mold that is prevalent in cannabis grown outdoors. Mycotoxins are not removed by product sterilization once they have been produced by the contaminant mold. Additional microbial contamination can also occur if the harvested product is improperly stored or handled.

13.19.4 Heavy Metal Contamination

Cannabis is known to be a bioaccumulator, meaning it will absorb contaminants from the soil. While this can be useful in some applications, such as remediating contaminated soils, it poses a huge risk if the plant will be used for medical or recreational applications. Arsenic, cadmium, lead, selenium, and mercury are the principal heavy metals that can be found in cannabis. Heavy metal testing should be conducted on any plant-based product to ensure a clean product. A recent study (Wakshlag et al. 2020a) found that, out of 29 commercial pet CBD products analyzed for heavy metals, 4 products had excessive amounts of lead and arsenic contamination.

The geographical location where the plants are grown can help determine the potential for bioaccumulation of heavy metals in plants grown outdoors. One instance of this would be in selenium-rich soils, where cannabis can bioaccumulate enough selenium to produce toxicity.

13.20 Product Selection Considerations

13.20.1 Guidelines for Product Selection: Questions to Ask the Vendor

There is no single product that is appropriate for all patients and all applications. As discussed earlier**,** each consideration listed below needs to be evaluated when a product is selected for a specific patient for a specific application. In this way the practitioner can match the product to the patient for optimal outcomes, taking into consideration the following: pet owner compliance; species; patient size; severity of condition being treated; condition being treated; price point that is acceptable to client; duration of therapy.

13.20.2 Product Considerations

- Legal status in area sold
- Quality control procedures
- Independent third-party analysis
- Certificate of Analysis (CoA) (In the USA the results should come from an ISO/EIC 17025 certified accredited laboratory)
  - Cannabinoid profile with potency
  - Terpene profile
  - Solvent residues
  - Microbial contamination
  - Pesticides/Fungicides/Herbicides
  - Microbiological contaminants
  - Heavy metals/Elemental Analysis
  - Pathogenic bacterial contaminants
  - Mycotoxin testing
- Plant cultivation
  - Organic (preferable)
  - Indoor /Outdoor
  - Grow medium
  - Hemp versus higher THC cannabis
- Extraction methodology
- Concentration methodology
- Product Format
- Compliant labels and labeling language

  - Contact number for company
  - Full disclosure on label of ALL ingredients (both active and inactive)
  - Labeled as nutraceutical, not nutritional (US)
  - NASC seal on label (preferable)
- Product backed by evidence or peer-reviewed published scientific research

13.21 Harm Reduction Education

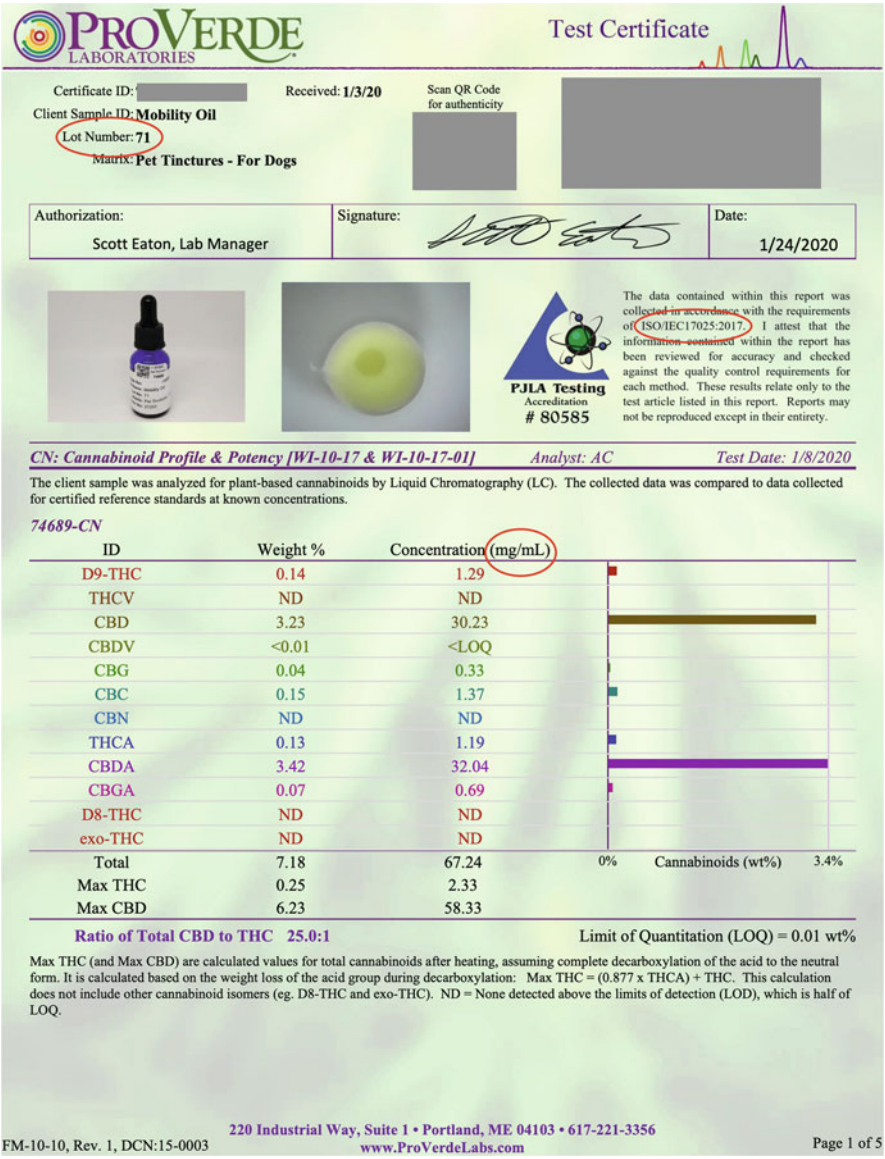
The increasingly widespread availability of cannabinoid containing products and the willingness of pet owners to administer these products to their animals highlights the need for the veterinary community to provide their clients with safety guidelines and education on harm reduction principles.

Veterinary practitioners should consider summarizing topics related to the animals specific condition, product selection and potentially nuanced dosing described in this text for discussions with pet owners on the use of cannabis/hemp to increase patient safety and reduce potential for harm.

13.22 Conclusion

We are just now opening the door to the many possibilities that cannabinoid therapies hold for veterinary patients. As we gain more experience with the use of cannabinoids and the marketplace becomes better regulated to provide more consistent, quality products, our discovery of applications and scientific substantiation for the biomedical action in veterinary species of cannabinoids, terpenes, and flavonoids will continue to gain speed and greater acceptance.

In the years to come, it is certain we will see FDA/Health Canada-approved cannabinoid drugs for veterinary use, both from naturally occurring cannabinoids as well as patented synthetic cannabinoids. It is highly likely we will also see substantial growth of the OTC market for pet-adapted cannabinoid products as they become regulated by the FDA-CVM and Health Canada. Economic predictors show huge growth in the cannabis industry. The future holds exciting new discoveries as we explore this emerging group of plant-derived therapeutics (Figs. 13.1, 13.2, 13.3, 13.4, and 13.5).



PROVERDE LABORATORIES

Test Certificate

Certificate ID: [redacted] Received: 1/3/20

Scan QR Code for authenticity

Client Sample ID: Mobility Oil

Lot Number: 71

Matrix: Pet Tinctures - For Dogs

Authorization:	Signature:	Date:
Scott Eaton, Lab Manager		1/24/2020

PJLA Testing Accreditation # 80585

The data contained within this report was collected in accordance with the requirements of ISO/IEC17025:2017. I attest that the information contained within the report has been reviewed for accuracy and checked against the quality control requirements for each method. These results relate only to the test article listed in this report. Reports may not be reproduced except in their entirety.

CN: Cannabinoid Profile & Potency [WI-10-17 & WI-10-17-01] *Analyst: AC* *Test Date: 1/8/2020*

The client sample was analyzed for plant-based cannabinoids by Liquid Chromatography (LC). The collected data was compared to data collected for certified reference standards at known concentrations.

74689-CN

ID	Weight %	Concentration (mg/mL)
D9-THC	0.14	1.29
THCV	ND	ND
CBD	3.23	30.23
CBDV	<0.01	<LOQ
CBG	0.04	0.33
CBC	0.15	1.37
CBN	ND	ND
THCA	0.13	1.19
CBDA	3.42	32.04
CBGA	0.07	0.69
D8-THC	ND	ND
exo-THC	ND	ND
Total	7.18	67.24
Max THC	0.25	2.33
Max CBD	6.23	58.33

0% Cannabinoids (wt%) 3.4%

Ratio of Total CBD to THC 25.0:1

Limit of Quantitation (LOQ) = 0.01 wt%

Max THC (and Max CBD) are calculated values for total cannabinoids after heating, assuming complete decarboxylation of the acid to the neutral form. It is calculated based on the weight loss of the acid group during decarboxylation: Max THC = (0.877 x THCA) + THC. This calculation does not include other cannabinoid isomers (eg. D8-THC and exo-THC). ND = None detected above the limits of detection (LOD), which is half of LOQ.

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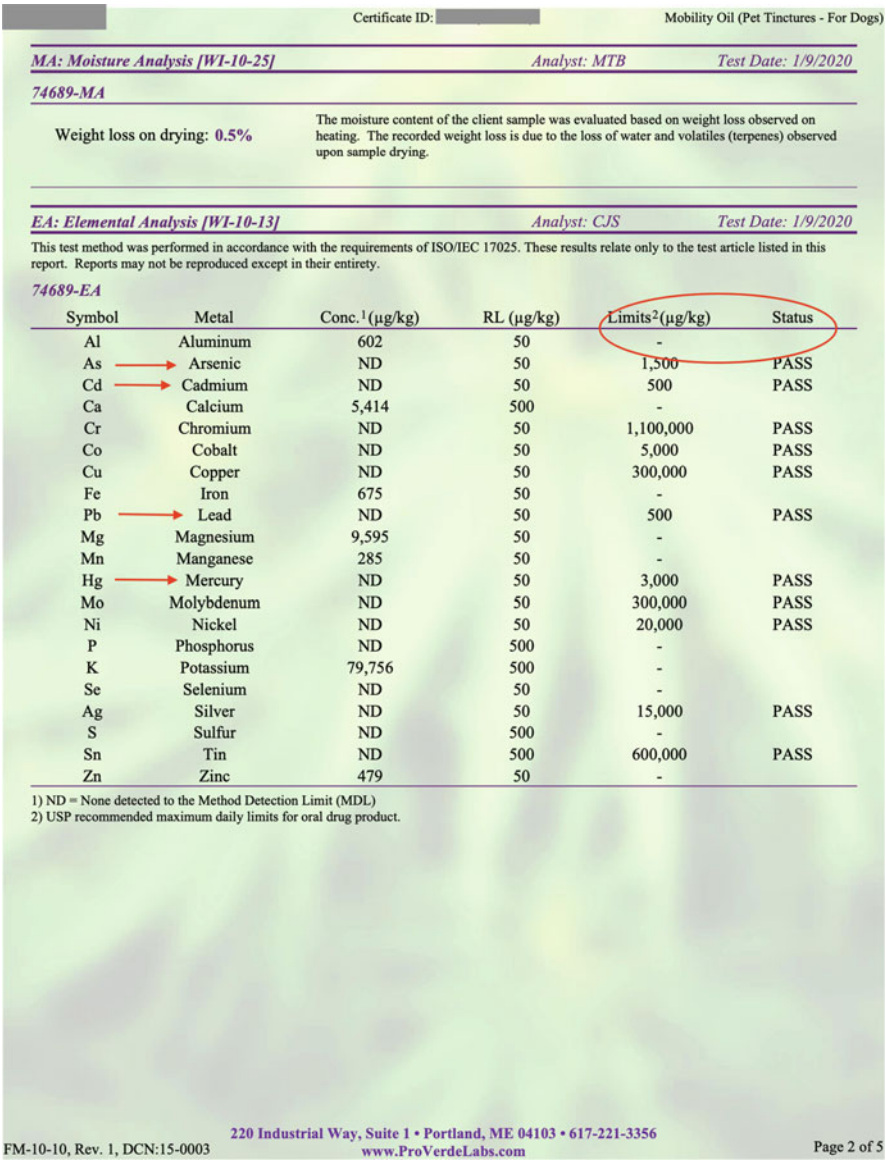
Fig. 13.1 When evaluating a certificate of analysis (CoA) it is critical to note that the company's name is on the certificate and that it appears on every page of the certificate. Ideally, the CoA contains another form of authentication, such as a QR code. Ensure the laboratory performing the CoA is accredited. The lot or batch number should match the product and concentration will ideally be in a mg/ml format. It is completely reasonable to ask the company to convert the concentration to this format for ease of use



Certificate ID: [redacted] Mobility Oil (Pet Tinctures - For Dogs)

MA: Moisture Analysis [WI-10-25] *Analyst: MTB* *Test Date: 1/9/2020*

74689-MA

Weight loss on drying: **0.5%**

The moisture content of the client sample was evaluated based on weight loss observed on heating. The recorded weight loss is due to the loss of water and volatiles (terpenes) observed upon sample drying.

EA: Elemental Analysis [WI-10-13] *Analyst: CJS* *Test Date: 1/9/2020*

This test method was performed in accordance with the requirements of ISO/IEC 17025. These results relate only to the test article listed in this report. Reports may not be reproduced except in their entirety.

74689-EA

Symbol	Metal	Conc.[1] (µg/kg)	RL (µg/kg)	Limits[2] (µg/kg)	Status
Al	Aluminum	602	50	-	
As	Arsenic	ND	50	1,500	PASS
Cd	Cadmium	ND	50	500	PASS
Ca	Calcium	5,414	500	-	
Cr	Chromium	ND	50	1,100,000	PASS
Co	Cobalt	ND	50	5,000	PASS
Cu	Copper	ND	50	300,000	PASS
Fe	Iron	675	50	-	
Pb	Lead	ND	50	500	PASS
Mg	Magnesium	9,595	50	-	
Mn	Manganese	285	50	-	
Hg	Mercury	ND	50	3,000	PASS
Mo	Molybdenum	ND	50	300,000	PASS
Ni	Nickel	ND	50	20,000	PASS
P	Phosphorus	ND	500	-	
K	Potassium	79,756	500	-	
Se	Selenium	ND	50	-	
Ag	Silver	ND	50	15,000	PASS
S	Sulfur	ND	500	-	
Sn	Tin	ND	500	600,000	PASS
Zn	Zinc	479	50	-	

1) ND = None detected to the Method Detection Limit (MDL)
2) USP recommended maximum daily limits for oral drug product.

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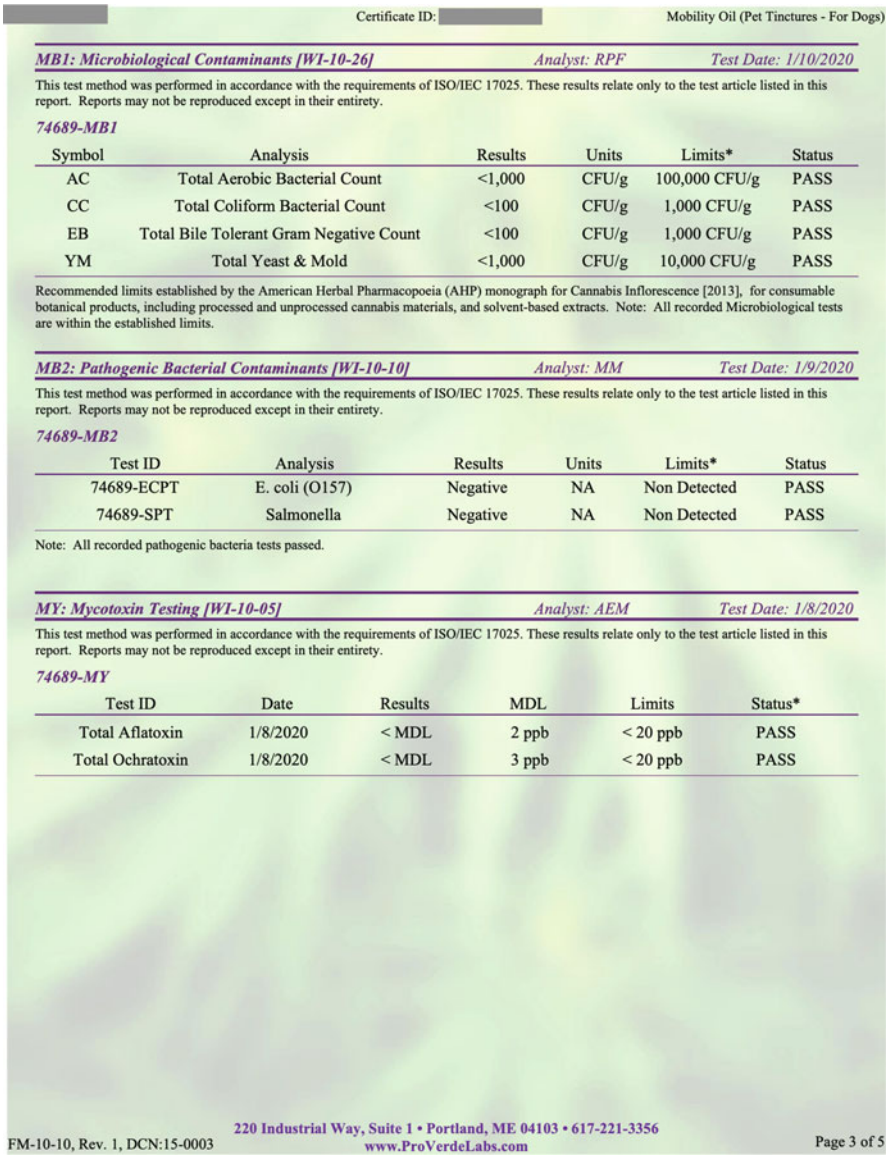
Fig. 13.2 The main elemental contaminants of concern are arsenic, cadmium, lead, and mercury. If a limit section is missing a value or status grade it means that there is no set reference range, not that the product is tainted. Some companies may provide this page, but with no values reported, instead listing "NT" (Not Tested) in the concentration column. Products lacking this information are not recommended



Certificate ID: [redacted] Mobility Oil (Pet Tinctures - For Dogs)

MB1: Microbiological Contaminants [WI-10-26] *Analyst: RPF* *Test Date: 1/10/2020*

This test method was performed in accordance with the requirements of ISO/IEC 17025. These results relate only to the test article listed in this report. Reports may not be reproduced except in their entirety.

74689-MB1

Symbol	Analysis	Results	Units	Limits*	Status
AC	Total Aerobic Bacterial Count	<1,000	CFU/g	100,000 CFU/g	PASS
CC	Total Coliform Bacterial Count	<100	CFU/g	1,000 CFU/g	PASS
EB	Total Bile Tolerant Gram Negative Count	<100	CFU/g	1,000 CFU/g	PASS
YM	Total Yeast & Mold	<1,000	CFU/g	10,000 CFU/g	PASS

Recommended limits established by the American Herbal Pharmacopoeia (AHP) monograph for Cannabis Inflorescence [2013], for consumable botanical products, including processed and unprocessed cannabis materials, and solvent-based extracts. Note: All recorded Microbiological tests are within the established limits.

MB2: Pathogenic Bacterial Contaminants [WI-10-10] *Analyst: MM* *Test Date: 1/9/2020*

This test method was performed in accordance with the requirements of ISO/IEC 17025. These results relate only to the test article listed in this report. Reports may not be reproduced except in their entirety.

74689-MB2

Test ID	Analysis	Results	Units	Limits*	Status
74689-ECPT	E. coli (O157)	Negative	NA	Non Detected	PASS
74689-SPT	Salmonella	Negative	NA	Non Detected	PASS

Note: All recorded pathogenic bacteria tests passed.

MY: Mycotoxin Testing [WI-10-05] *Analyst: AEM* *Test Date: 1/8/2020*

This test method was performed in accordance with the requirements of ISO/IEC 17025. These results relate only to the test article listed in this report. Reports may not be reproduced except in their entirety.

74689-MY

Test ID	Date	Results	MDL	Limits	Status*
Total Aflatoxin	1/8/2020	< MDL	2 ppb	< 20 ppb	PASS
Total Ochratoxin	1/8/2020	< MDL	3 ppb	< 20 ppb	PASS

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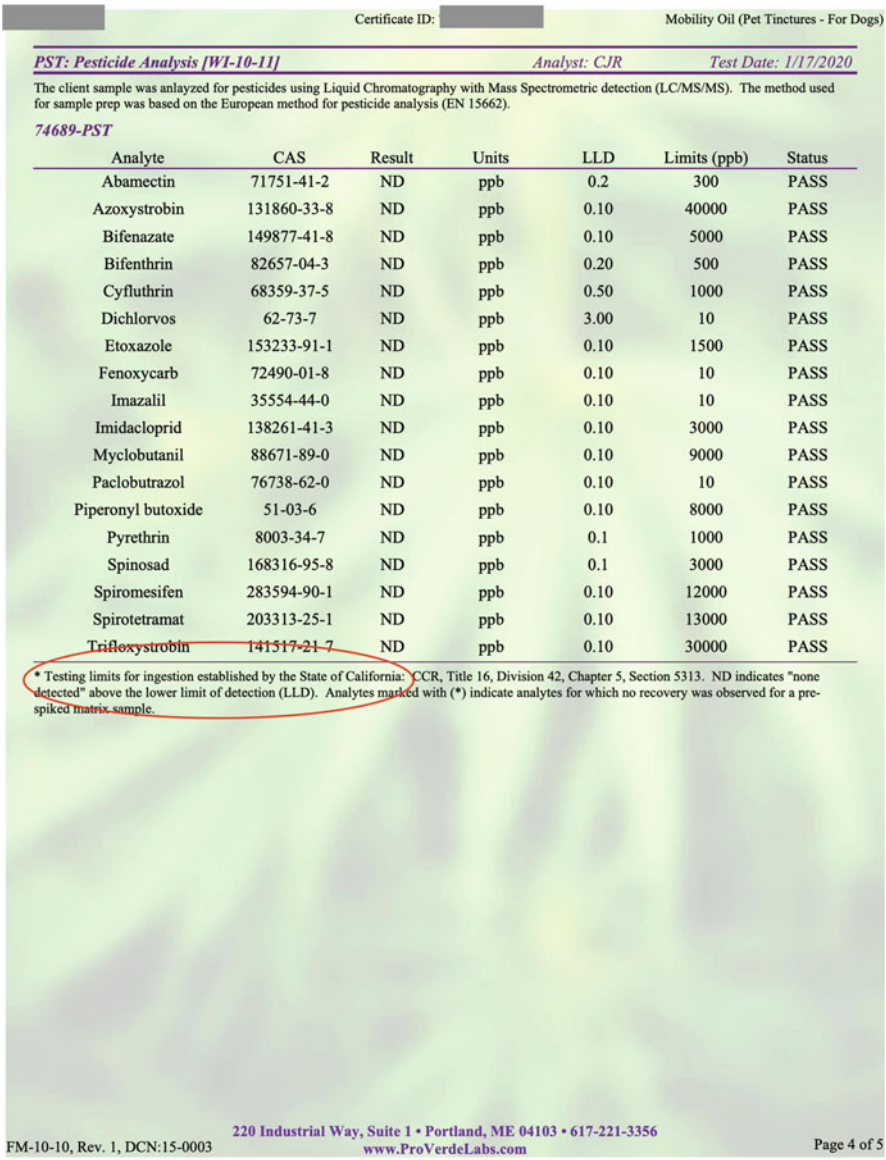
Fig. 13.3 This is an example of a good microbiological, pathogenic bacterium, and mycotoxin analysis. We see little to no growth of pathogens that could cause illness



Certificate ID: [redacted] Mobility Oil (Pet Tinctures - For Dogs)

PST: Pesticide Analysis [WI-10-11] *Analyst: CJR* *Test Date: 1/17/2020*

The client sample was anlayzed for pesticides using Liquid Chromatography with Mass Spectrometric detection (LC/MS/MS). The method used for sample prep was based on the European method for pesticide analysis (EN 15662).

74689-PST

Analyte	CAS	Result	Units	LLD	Limits (ppb)	Status
Abamectin	71751-41-2	ND	ppb	0.2	300	PASS
Azoxystrobin	131860-33-8	ND	ppb	0.10	40000	PASS
Bifenazate	149877-41-8	ND	ppb	0.10	5000	PASS
Bifenthrin	82657-04-3	ND	ppb	0.20	500	PASS
Cyfluthrin	68359-37-5	ND	ppb	0.50	1000	PASS
Dichlorvos	62-73-7	ND	ppb	3.00	10	PASS
Etoxazole	153233-91-1	ND	ppb	0.10	1500	PASS
Fenoxycarb	72490-01-8	ND	ppb	0.10	10	PASS
Imazalil	35554-44-0	ND	ppb	0.10	10	PASS
Imidacloprid	138261-41-3	ND	ppb	0.10	3000	PASS
Myclobutanil	88671-89-0	ND	ppb	0.10	9000	PASS
Paclobutrazol	76738-62-0	ND	ppb	0.10	10	PASS
Piperonyl butoxide	51-03-6	ND	ppb	0.10	8000	PASS
Pyrethrin	8003-34-7	ND	ppb	0.1	1000	PASS
Spinosad	168316-95-8	ND	ppb	0.1	3000	PASS
Spiromesifen	283594-90-1	ND	ppb	0.10	12000	PASS
Spirotetramat	203313-25-1	ND	ppb	0.10	13000	PASS
Trifloxystrobin	141517-21-7	ND	ppb	0.10	30000	PASS

• Testing limits for ingestion established by the State of California: CCR, Title 16, Division 42, Chapter 5, Section 5313. ND indicates "none detected" above the lower limit of detection (LLD). Analytes marked with (*) indicate analytes for which no recovery was observed for a pre-spiked matrix sample.

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Fig. 13.4 The authors prefer testing limits based on the most stringent testing limit guidelines. In the US there is often a large discrepancy between state and federal testing limits

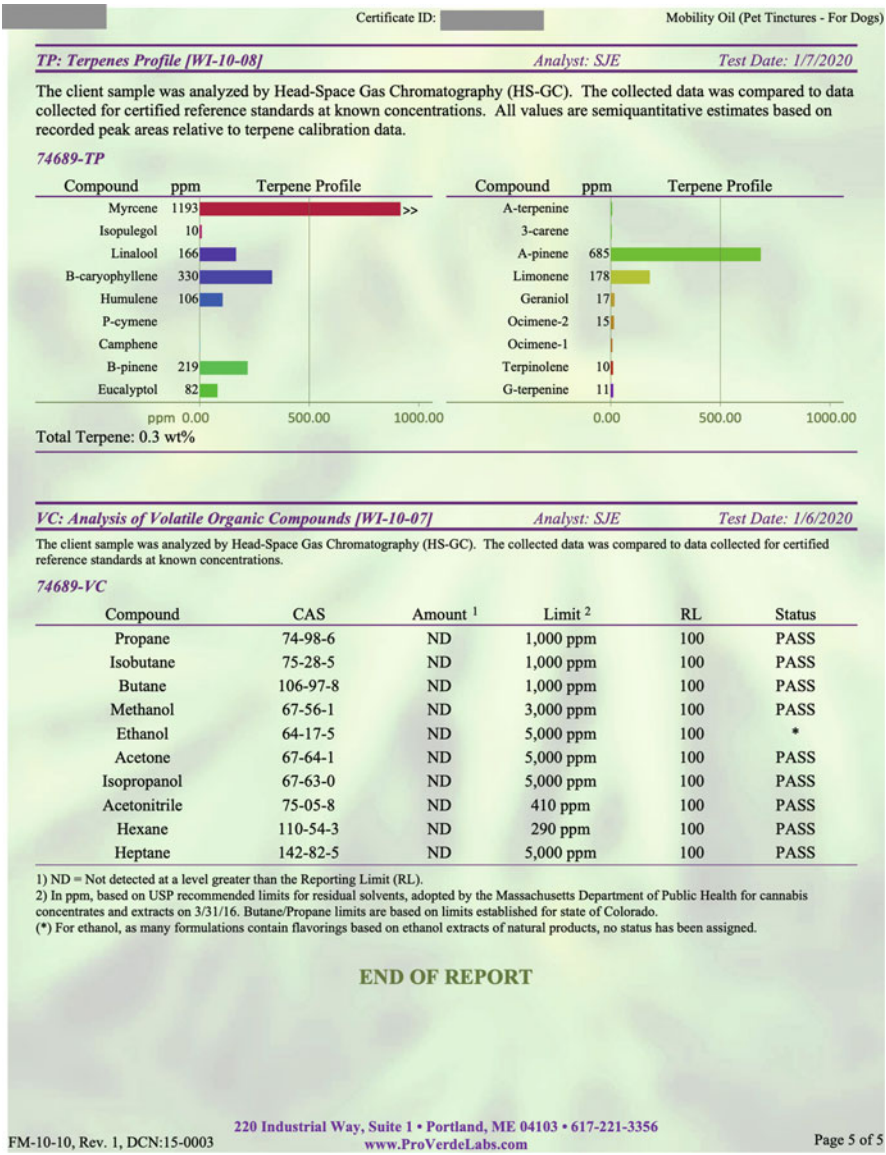

Fig. 13.5 A terpene analysis is important when evaluating which product might be best for a certain condition. Generally, total terpene concentrations should be above 0.05–0.1% for therapeutic advantages. Isolates and some CO_2 extracted products may lack terpenes

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