SCIENTIFIC VIEWPOINT

The Endocannabinoid System and CBD: Implications in Inflammation and Maintaining Homeostasis

According to BDS Analytics and ArcView Market Research, projections show that the collective market for cannabidiol (CBD) sales in the U.S. will surpass $20 billion by 2024. As with many products that come to the supplement industry that create huge public interest, there is always the inevitable marketing hype that comes with it, and CBD is no exception as it is now a "buzz word." In order to understand the physiological effects of CBD away from the marketing hype for its use, we must understand how the body works and what the Endocannabinoid System is, its role in bringing about homeostasis, and its interaction with different body systems, particularly the immune system.

Receptor	Impact	Potential Pharmacologic Outcome
CB1	Direct antagonism and negative allosteric modulator antagonism	Attenuation of imparied learning, memory, hypothermic, and psychosis effects induced by delta-9-THC
CB2	Antagonist + inverse agonist	Anti-inflammatory effects
GPR55	Antagonist	Anticancer effects
5HT1-alpha	Agonist	Pain relieving (allosterically regulates mu and sigma opoid receptors) and antianxiety effects
TPVR-1	Agonist	Anti-inflammatory, pain relieving, and sebum producing effects
Adenosine A2A	Enhanced adenosine concentrations	Anti-inflammatory effects

CB1, cannabinoid receptor 1; CB2, cannabinoid receptor 2; CBD, cannnabidiol; GPR55, G-coupled protein receptor; 5HT1-alpha, serotonin 1a receptor; TPVR-1, transient receptor potential vanilloid receptor

CBD increases anandamide concentration, an endogenous CB1, CB2, and TPVR-1 agonist

An increasing number of people are using cannabis-derived ingredients such as the phytocannabinoids THC and CBD for their many therapeutic benefits with sleep, anxiety, pain, and more as well as a number of conditions ranging from autoimmune disorders to cancer. Many of the health benefits from these phytocannabinoids are due to their interaction with our Endocannabinoid System (ECS). The ECS acts as the "break" in an overactive and excitatory nervous system in times of stress and is a key regulator of the immune system, thereby bringing about homeostasis.

Originally identified from studies on the mechanism of action of the psychotropic ingredient of some varieties of cannabi, Δ9 -tetrahydrocannabinol (THC), the ECS has turned out, as more and more studies are done, to be full of complexity, redundancy, and promiscuity. The ECS was originally identified in the late 20th century and is composed of two main ligands (the endocannabinoids AEA and 2AG), their synthesizing metabolic enzymes (NAPE-PLD for AEA and DAGL for 2AG), their degrading metabolic enzymes (FAAH for AEA and MAGL for 2AG), and two main G-coupled protein receptors (CB1 and CB2).

Endocannabinoids AEA and 2AG, unlike classical neurotransmitters that are stored in vesicles, are synthesized "on demand" in response to a stimulus. Primary

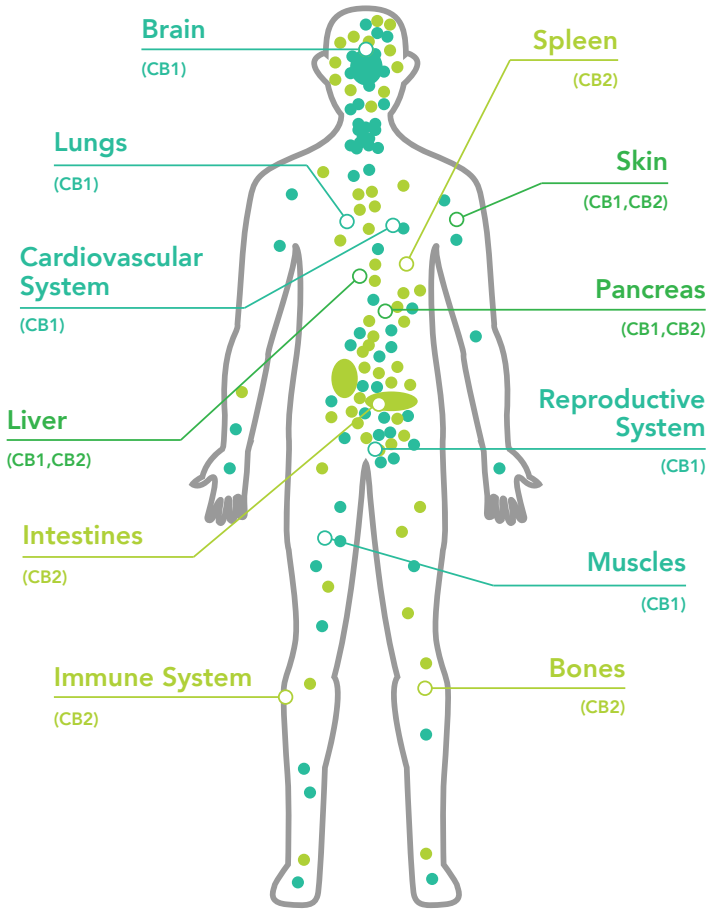
AREAS IMPACTED BY CB1 RECEPTORS

- Inflammation
- Stress response
- Blood pressure
- Mood
- Memory

AREAS IMPACTED BY CB2 RECEPTORS

- Inflammation
- Immune system
- Bone and skin health
- Cardiovascular system
- Liver and kidney function

Figure 1. Two main receptors of the ECS and its different locations in the body.



stimuli for their synthesis are an elevation of intracellular calcium and activation of a number of G-protein coupled receptors (GPCRs) that come by way of sympathetic responses or substances that over excite cells. It is basically a lipid-signaling system used for regulating many physiological functions in the body such as cell, tissue, organ, and organism homeostasis, brain development, neurotransmitter release, and synaptic plasticity as well as cytokine release from the microglia.

The ECS plays a crucial role in regulating a broad range of physiological processes that affect our everyday experience such as mood, energy level, gastrointestinal integrity, immune activity, blood pressure, bone density, glucose metabolism, pain perception, stress, hunger, and many more. It can be viewed as a widespread neuromodulatory network involved both in the developing central nervous system (CNS) as well as playing a major role in tuning many cognitive and physiological processes. The main physiological function of the endogenous cannabinoid system consists of inhibiting the release of other neurotransmitters (e.g., acetylcholine, dopamine, histamine, serotonin, glutamate, GABA, etc) in the nervous system, usually by stimulating CB1 receptors.

CB1 is mainly expressed in the brain and the enteric nervous system and is therefore the main mediator of the psychoactive effects of Cannabis, whereas CB2 has a greater expression in the immune system. Both receptors are spread throughout different organs and are affected by Endocannabinoids and Phytocannabinoids (see ***Figure 1***).

It is important to appreciate that most components of the ECS are multifunctional, and when dysregulated appear to be involved in many pathological conditions. Thus, rather than being a discrete, isolated system, the ECS influences and is influenced by many other signaling pathways. This is especially important to consider when assessing the effects of ECS-targeting substances such as CBD which interacts with this as well as other neuromodulatory systems. For example, as is true with most systems in the body, CBD not only interacts with CB1 and CB2 receptors but also with other receptors that play a vital role in human physiology (see ***Table 1***).

Taking into account that the ECS is affected not only by endocannabinoids (AEA and 2AG) but also by phytocannabinoids (THC and CBD), that these cannabinoids also interact with other receptors in addition to CB1 and CB2 (e.g., GPR55, GPR18, GPR119, TRPV1, GABA A, glycine receptors, and the PPAR-γ), and that depending on the CBD and the receptor interaction the outcome could be agonistic or antagonistic, this system is complex, redundant, and promiscuous.

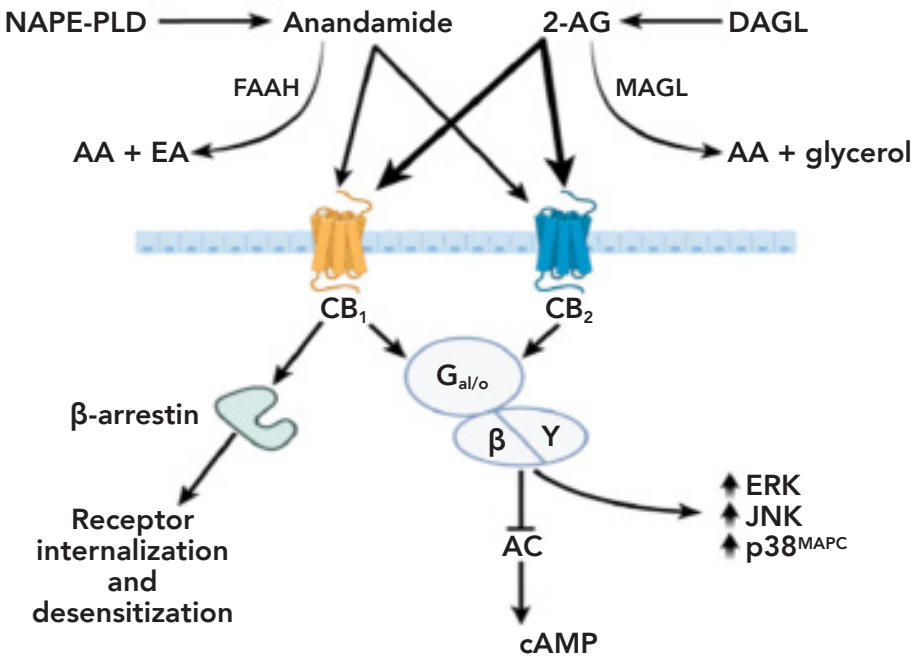


Figure 2: Signaling pathways induced by endocannabinoid receptor activation. Increasing ERK and p38MAPK pathways induces cell growth and increasing JNK induces apoptosis.

Human health is never linear, and the body will take its available resources and decide where to use them in an "as needed" basis. In the case of the ECS, it is "on demand" and lasts from seconds to minutes when it is triggered by an overstimulation of the nerves of the CNS, enteric nervous system, and immune cells in times of stress, infection, inflammation, or pathology in general in order to bring about homeostasis. When a system that is "on demand" is constantly triggered with exogenous cannabinoids, it can have some unintended consequences if not done with caution, especially when the body is in a chronic state of disease.

Normal Physiology vs Pathophysiology

Normally a cell is in a resting state, and when triggered by a substance such as a hormone or a neurotransmitter in times of need or stress, it activates the stimulatory G-coupled protein receptor (GPCR) which then signals a membrane-bound enzyme called adenylate cyclase (AC) to activate cyclic adenosine monophosphate (cAMP) which allows an influx of calcium. This stimulates the cell to carry out the desired effect of the hormone or neurotransmitter until this process is shut off and the cell returns to its normal resting state. This is normal physiology. However, when the stress signal continues from a state of disease or a hyper-sympathetic trigger it becomes chronic, creates havoc, and can lead to disease.

This is where the ECS is called into action by releasing the endocannabinoids AEA and 2AG from the cell wall to trigger the inhibitory GPCR (CB1 and CB2). Upon endocannabinoid or phytocannabinoid binding, both receptors activate G-alpha inhibitory subunits, which then initiate downstream signaling. The primary response is inhibition of adenylate cyclase (AC) and therefore reduction in the cytosolic levels of cAMP so that there will be no more influx of calcium to continue stimulating the cell. As a consequence, we now achieve homeostasis. There is evidence suggesting that these receptors can also activate MAP kinase (MAPK) pathways including ERK, JNK, and p38MAPK which are involved in cell growth and apoptosis (see ***Figure 2***).

Although the ECS pathway is very important for purposes of homeostasis, as mentioned earlier it is naturally "on demand" and lasts from seconds to minutes, so when it is chronically stimulated via phytocannabinoids, it can have unintended consequences. This is especially true when considering that since it is promiscuous and its metabolites interacts with other pathways, it can increase the chances of stimulating pro-inflammatory pathways.

Modulating EC levels can produce anti-nociceptive effects. However, bioactive lipids such as AEA and 2-AG are promiscuous and can be metabolized by multiple enzymes. Artificially elevating EC levels can unmask alternative metabolic routes, producing additional bioactive products. For example, when Fatty Acid Amide Hydrolase (FAAH) and Monoacylglycerol Lipase (MAGL) metabolize AEA and 2AG respectively, they produce arachidonic acid which can then trigger the arachidonate pathway towards inflammation (see ***Figure 3***).

Interestingly, pathological conditions such as chronic pain states are associated with changes in levels of enzymes such as cyclooxygenase (COX), lipoxygenase (LOX), αβ hydrolase, and members of the cytochrome P450 family, which can metabolize the EC to novel lipid-signaling molecules. COX-2 metabolites of AEA and 2-AG have been shown to have pro-nociceptive actions in the spinal cord. COX-2 metabolizes AEA to Prostamide F2α, whose spinal application increases the firing of nociceptive neurons and reduces paw withdrawal latencies, and levels of Prostamide F2α are elevated in spinal cord tissue in mice with knee inflam-

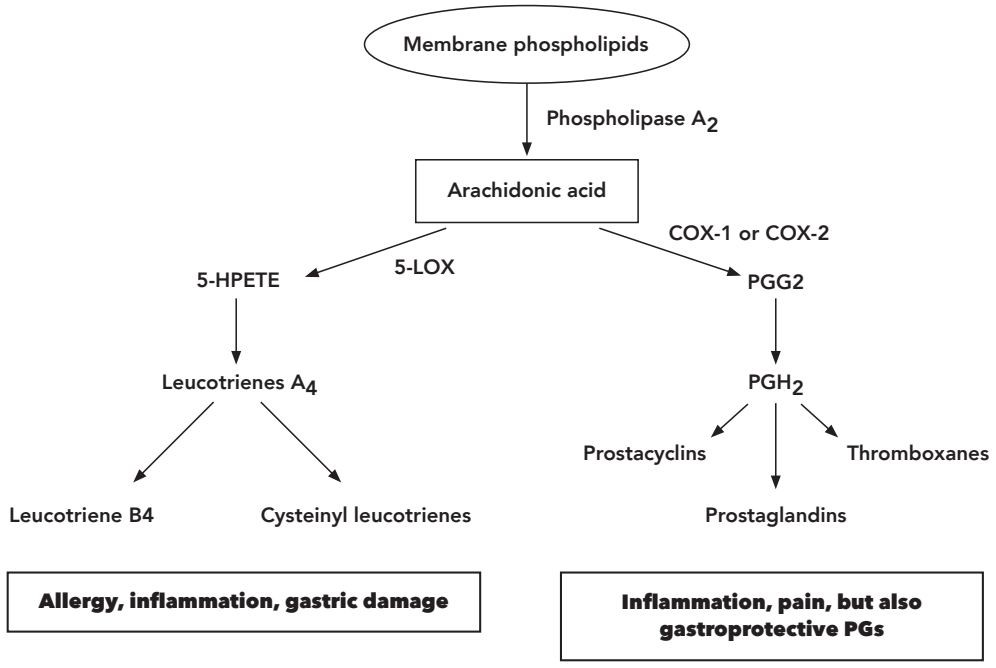


Figure 3: Products and enzymes of arachidonic acid metabolism involved in the inflammatory process (Martel-Pelletier et al., 2003)

mation. Similarly, the COX-2 metabolite of 2-AG, PGE2 glycerol ester, is endogenously generated in rat tissue, and induces mechanical allodynia and thermal hyperalgesia following intra-plantar administration.

Based on these reports, it is clear that determining the levels of these potential ligands in pain states is of great interest, as many of these metabolites may have effects on pain processing. These findings need to be kept in mind in chronic pain states, as they may increase substrate levels for generation of alternative pro-nociceptive EC metabolites, and thus counteract the anti-nociceptive effects of AEA and 2-AG. Previous work has shown that, in addition to being a CB1 receptor agonist, at higher concentrations AEA binds to and activates the pro-nociceptive TRPV1 channel. Since AEA can be also converted into pro-nociceptive signaling molecules in the presence of COX-2 activity, then it is feasible that under pathological pain states where COX-2 activity is up-regulated (e.g., osteoarthritis), pro-nociceptive effects of AEA at TRPV1 may outweigh the anti-nociceptive actions. It has previously been suggested that the development of dual FAAH and COX-2 inhibitors or substrate selective inhibitors of COX-2 would be advantageous in terms of uncoupling the pro- and anti-nociceptive actions of AEA, and producing compounds with superior analgesic profiles.

Benefits of CBD with Enzymes

If upregulation of COX-2 is of concern in disease states when applying phytocannabinoids such as CBD, one should consider combining with proteolytic enzymes. One example is bromelain which has been shown to decrease COX-2 activity along with NF-kB in a number of studies. Studies of prostaglandin metabolism during acute inflammation showed that orally administered bromelain reduces the level of both PGE2 and thromboxane B2 dose-dependently. Another study performed at Baylor University using a multi-proteolytic blend also observed significantly lower levels of COX-2, the primary signal for the production of prostaglandins, in the protease group. This result not only indicates a potential beneficial effect on health but also seems to support the hypothesis that proteolytic enzyme supplementation may reduce inflammation by inhibiting the arachidonic acid cascade.

The therapeutic potential of phytocannabinoids as it interacts with the ECS is of great clinical value as long as there is awareness of the effects of manipulating such a complex system. In respecting biochemical individuality, clinical applications should follow a "start low and go slow" dosing protocol. Low-dose CBD appears effective for stress and its manifestations and has a good safety and tolerability profile with few adverse effects. Unlike THC, CBD is not psychomimetic and does not cause intoxication, euphoria, addiction, psychomotor impairment, or cognitive impairment. Importantly, low-dose CBD (less than 150 mg/day) does not cause the hepatocellular injury observed for higher dose CBD (>600 mg/day). CBD has also been shown to have no potential for abuse or dependence in humans. High-dose CBD has drug–drug interactions with medicines metabolized by the cytochrome P450 pathways.

Because CBD is poorly water soluble, ingestion typically provides poor absorption, and most of the CBD that is absorbed undergoes first-pass metabolism, which results in a bioavailability of only 6%. Systemic exposure to CBD is increased four-fold by ingestion with a high-fat meal and five-fold with severe hepatic impairment. The main reason for the large increase in absorption with a high-fat meal is that micelles are naturally formed in the small intestine by the mixing of bile salts with fatty

acids from the high-fat meal, and these micelles carry CBD into intestinal epithelial cells and the portal circulation. The single-dose half-life of CBD is around 3 hours. However, CBD accumulates in tissues, including adipose tissues due to its lipophilicity, and after repeated doses, its half-life is 2–5 days. CBD is mainly metabolized in the liver by CYP3A- and CYP2C-dependent phase I metabolism to its active metabolite 7-OH-CBD, which is then metabolized and excreted in feces and urine after phase II metabolism by uridine 5'-diphospho-glucuronosyltransferase (UGT) enzymes from endoplasmic reticulum.

Conclusion

As our understanding of the endocannabinoids improves, so does the awareness of their complexity. During pathological states, the levels of these mediators in tissues change, and their effects vary from those of protective endogenous compounds to those of dysregulated signals. When moving to the clinic, however, the pleiotropic nature of endocannabinoid functions will require careful judgement in the choice of patients and stage of the disorder for treatment. A good rule of thumb when dosing patients with CBD is a "start low and go slow" approach, and to be aware of the upregulated COX pathway in many disease states. Clinicians should also consider diet as an important factor, as an over consumption of Omega 6 fatty acids versus Omega 3 will favor the arachidonate pathway and possibly trigger the inflammatory cascade. Much research is still needed on this fascinating system as there are many components involved and it seems to interact with a great amount of phytocannabinoids, where each bring about a different effect.

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Milton Bastidas, DC, CIHP, was born in Colombia and is a graduate of Texas Chiropractic College with additional certification in Functional Medicine. He is Founder of True Lifelong Wellness Center and has been in practice since 1998 treating skeletal conditions, sports injuries, and systemic disorders. He is also Vice President of the College of Integrative Medicine (CIM) as well as Director of Research and Development at Transformation Enzyme Corporation. Dr. Bastidas has a passion for soccer which put him through college at Texas Lutheran University and is very sought after in the soccer community for rehabilitation and nutritional counseling. He has co-directed clinical studies showing the benefits of enzymes on inflammation and GI dysfunction and is an expert in the use of laboratory analysis and enzyme nutrition as part of a functional and natural approach to wellbeing.

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