Care and Feeding of the Endocannabinoid System

What we eat and drink, the drugs we use, treatments we receive, exercise, the stresses we respond to —many factors augment or diminish cannabinoid neurotransmission within our bodies.

By John M. McPartland, DO, MS

The endocannabinoid (eCB) system consists of an "alphabet soup" of acronyms for receptors, ligands (compounds that bind to receptors), and ligand-metabolizing enzymes.

Cannabinoid receptor type 1 (CB_1) is primarily located in the brain, spinal cord, and peripheral nerves. CB_1 is also expressed in reproductive tissues, and in several cell types involved in metabolism, such as adipocytes (fat cells) and hepatocytes (liver cells). Cannabinoid receptor 2 (CB_2) is principally associated with cells governing immune function.

Two ligands known as anandamide (AEA) and *sn*-2-arachidonoylglycerol (2-AG) activate both CB_1 and CB_2.

Anandamide is released by an enzyme called NAPE-PLD, and it is broken down by an enzyme called FAAH.

2-AG is primarily released by an enzyme named DAGLα, and it is primarily broken down by MAGL. Other enzymes may also metabolize AEA and 2-AG.

THC functions like anandamide and 2-AG by sliding into CB_1 and CB_2 and activating the receptors.

Tetrahydrocannabinol (THC) is a plant compound that mimics our endocannabinoids, much the same as opioids from poppies mimic our endorphins. That is to say, THC functions like anandamide and 2-AG by sliding into CB_1 and CB_2 and activating the receptors.

Rodents trained to discriminate anandamide from other substances will accept THC as a substitute, and rats trained to discriminate THC will accept anandamide. "Who mimics who?" is a question of chronology. The eCB system evolved 600 million years ago, whereas Cannabis and THC are johnnie-come-latelies that evolved perhaps 25 million years ago.

Hopes and Caveats

Can the eCB system be augmented to provide therapeutic benefit? That is the topic of today's talk.

Ethan Russo has proposed that migraine, fibromyalgia, irritable bowel syndrome, and related conditions represent "clinical eCB deficiency syndromes." Ester Fride speculated that the "failure to thrive" syndrome in infants may be caused by a dysfunctional eCB system. Paola Sarchielli posited eCB "system failure" as a basis of chronic migraine. Matthew Hill hypothesized that deficient eCB signaling contributes to depressive illnesses. Compensating for eCB deficincies might help patients with these conditions. Enhancing the eCB system may reduce symptoms caused by multiple sclerosis, chronic pain, epilepsy, and many other diseases.

John McPartland learned about medicinal plants from Euell Gibbons at boy scout camp. He rediscovered THC in New Jersey about eight years after Raphael Mechoulam discovered THC.

In this article McPartland summarizes the lecture he presented at the Patients Out Of Time *conference in Tucson. The lecture itself summarizes a review article by McPartland and Vincenzo Di Marzo, which cites references for all the studies described here.*

There are two important caveats: some diseases, such as visceral obesity and cirrhosis, are worsened by chronic overactivation of the eCB system. Vincenzo Di Marzo and others have shown that overactivation is driven by excessive levels of eCB ligands. In these diseases, *down*regulating the levels of eCB ligands would be beneficial.

CB_1 receptors desensitize and downregulate when faced with constant activation.

Secondly, generating a chronic, continual, high-level rise in anandamide and 2-AG would be counterproductive, even in people with eCB deficiency syndromes. This is because CB_1 receptors desensitize and downregulate when faced with constant activation. A desensitized receptor loses its responsiveness; intake of cannabinoids such as THC results in less receptor-mediated signal transduction.

A downregulated receptor is not functional—it does not bind ligand, has internalized away from the cell membrane, or no longer exists. These are not good things; they have been observed in rodent studies. *Acute* blockade of the MAGL enzyme elevates 2-AG levels and provides pain relief, but *chronic* blockade of MAGL erases this analgesia, because the sustained elevation of 2-AG causes desensitization of CB_1. This leads to downregulation of the eCB system.

Complementary and Alternative Medicine

This review focuses on therapeutic approaches classified as "complementary and alternative medicine" (CAM). The National Center for Complementary and Alternative Medicine (NCCAM) defines CAM as "a group of diverse medical and healthcare systems, practices, and products, that are not currently part of conventional medicine."

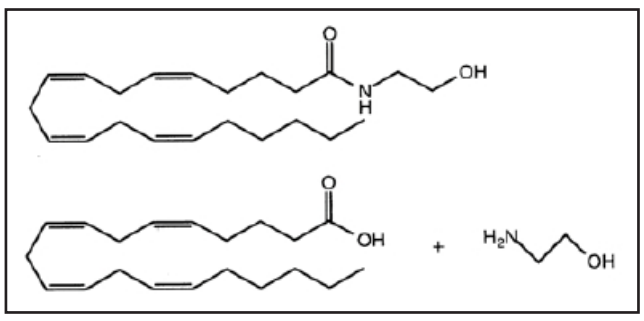
Running, biking, and hiking increase anandamide levels in the blood. The eCB system is primarily responsible for "runners high" (to a much larger degree than endorphins).

NCCAM categorizes CAM practices into three broad groups: "natural products" (dietary supplements and herbal remedies), "mind and body medicine" (meditation, yoga, and acupuncture), and "body-based practices" (massage, spinal manipulation). For the purposes of this review, we add "lifestyle modifications" (diet, weight control, exercise, and the use of psychoactive substances—ethanol, nicotine, caffeine, and cannabis).

We can enhance the eCB system by simply living a healthy lifestyle, beginning with aerobic exercise. Human studies show that running, biking, and hiking increase anandamide levels in the blood. The eCB system is primarily responsible for "runners high" (to a much larger degree than endorphins).

Rodent studies show that voluntary wheel running increases the expression of CB_1 in the brain. An interesting rat study by Hill and colleagues suggests that wheel running not only increases CB_1 expression, it also increases the sensitivity of CB_1 to activation by cannabinoids.

Diet is trickier. Overeating leads to obesity. Adipocytes produce excessive amounts of eCBs. The eCBs spill over into the blood, cross the blood-brain barrier, activate CB_1 in the brain, and cause the munchies.



Anandamide (top figure) is a combination of arachidonic acid plus ethanolamine (bottom figures).

The scenario becomes a feed-forward dysregulation of the eCB system. Our stereotype of the "happy fatman" gets erased after excessive amounts of eCBs cause CB_1 to desensitize and downregulate. It's not a happy or healthy picture—cardiovascular disease, diabetes, and systemic inflammation.

Caloric restriction (dieting, fasting) worsens the situation, at least initially: it leads to reduced eCB levels in the blood, and downregulated expression of CB_1 in the brain. Although some studies report conflicting results —and we need more research— a downregulated eCB system seems to fit the clinical picture of the "angry, depressed dieter."

But there is hope! A recent study of overweight women showed that dieting causes a decrease in CB_1 expression… but combining caloric restriction with aerobic exercise caused a net increase in CB_1 expression!

Trickier yet is the impact of polyunsaturated fatty acids (PUFAs) on the eCB system. The typical American diet contains excessive omega-6 PUFAs and lacks omega-3 PUFAs. Arachidonic acid is the archtypical omega-6. Some of its metabolites are bad actors —prostaglandins may cause pain and swelling, and leukotrienes may cause bronchoconstriction and asthma. But arachidonic acid is also required as a building block for endocannabinoids. *(See illustration at top right.)*

Many studies show that dietary supplementation with arachidonic acid increases serum levels of anandamide and 2-AG. There is a fine line here: we clearly need some arachidonic acid to biosynthesize eCBs. But excessive levels of AA may lead to excessive levels of anandamide and 2-AG, which eventually desensitize and downregulate CB_1 and CB_2.

The best-known omega-3 PUFAs are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Dietary supplementation with EPA and/or DHA increases the concentration of these compounds in tissues, cells, and plasma, and decreases the concentration of arachidonic acid.

Dietary supplementation with omega-3s decreases anandamide and 2-AG in tissues, cells, and plasma. Nevertheless, adequate levels of dietary omega-3s are required for proper functioning of the eCB system.

Mice supplemented with omega-3s, compared to mice on a control diet, express greater mRNA levels of CB_1 and CB_2. Another study with mice showed that omega-3 deficiency abolishes eCB-mediated neuronal functions. The omega-3 deficient diet led to CB_1 desensitization, because of a relative excess of arachidonic acid.

Omega-3 deficient mice did not respond to exogenous cannabinoids (in this case, the synthetic cannabinoid WIN55212-2; Δ^9-THC would have produced the same non-response). Omega-3 sufficient mice, however, showed normal cannabimimetic effects to WIN55212-2. Human breast milk contains small amounts of anandamide, but the biological significance of this is not known. Mouse milk also contains anandamide. Ester Fride showed that when newborn mice have their CB_1 receptors blocked (with the synthetic antagonist rimonabant), the baby mice don't suckle at birth, and die.

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"Probiotics" are microorganisms such as *Lactobacillus acidophilus* that confer health benefits upon humans. They occur in fermented foods, such as yogurt and kimchi.

Human intestinal cells exposed to *L. acidophilus* increase their expression of CB_2 mRNA. Mice fed *L. acidophilus* show less pain behavior following colonic distension than control mice, and this was reversed by a CB_2 antagonist. So probiotics likely protect us from irritable bowel syndrome and other gut problems by upregulating CB_2 receptors, whose activation decreases inflammation and visceral pain.

Several plants make compounds that selectively bind to CB_2 and modulate the immune system. But they have no affinity to CB_1 and do not elicit psychoactivity: Alkamides in Echinacea species bind to CB_2. Alkamides also inhibit anandamide breakdown. (E-)-**β**-caryophyllene in black pepper (and Cannabis) binds to CB_2, and its anti-inflammatory effects are reduced in CB_2 knockout mice.

A range of pesticides screw up the eCB system.

Eating organic foods may promote eCB homeostasis. A range of pesticides, from nasty (chlorpyrifos and diazinon) to relatively benign (piperonyl butoxide, which is often added to pyrethrum) screw up the eCB system. Phthalates are plasticizers added to water bottles, tin cans, food packaging, and even the enteric coating of pharmaceutical pills. Among their nefarious qualities, phthalates block CB_1 as allosteric antagonists.

Acupuncture is a good thing. Electroacupuncture (EA) upregulates the expression of CB_2 in skin tissues. EA also increases anandamide levels in the skin, and the pain-reducing effects of EA are attenuated by CB_2 antagonists. EA also treats

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pain by reducing GABA levels in the spinal cord (periaqueductal gray area), which is reversed by CB_1 antagonists.

Osteopathic manipulative treatment (OMT) employs massage, myofascial release, and spinal manipulation. In one study, a single OMT session increased serum anandamide levels by 168% over pretreatment levels, with no changes in control subjects. In another OMT study, anandamide did not change, but serum levels of PEA increased. (PEA is a molecule related to anandamide that enhances anandamide's activity.)

OMT may also improve CB_1 function in peripheral nerves. A schematic drawing of a c-fiber nociceptor is provided at right. The nerve originates in the dorsal horn (DH) of the spinal cord, with its cell body in the dorsal root ganglion (DRG). The illustration provides an enlarged view of the nerve's distal terminal, which is located in skin, muscles, joints, and other tissues. The distal terminal contains receptors that activate pain signals or sensitize the nerve to pain signals.

The distal terminal also contains CB_1, which dampens the effects of the pain-generating receptors. CB_1 receptors are synthesized in the DRG, and then carried by axoplasmic flow (a fluid flow of protoplasm) down the nerve fiber to the distal terminal. Andrea Hohmann showed that mechanical barriers that restrict axoplasmic flow will prevent CB_1 receptors from reaching the distal terminal, and functioning properly.

The mechanical barrier is analogous to carpal tunnel syndrome, thoracic outlet restriction, piriformis syndrome, or any other mechanical barrier that bodyworkers treat and eliminate. Eliminating the barrier restores axoplasmic flow, facilitating CB_1 transport to its peripheral site of action.

Chronic stress downregulates CB_1 expression and reduces the levels of eCB ligands in rat brains. Stress management may reverse the effects of stress on eCB signalling, although no studies have been done to date. Anecdotal information suggests that relaxation techniques, meditation, yoga, and deep breathing exercises may impart mild cannabimimetic effects.

Pharmaceutical medications

We briefly touch on analgesics (acetaminophen, NSAIDs, glucocorticoids, opiates), psychopharmaceutical (antidepressants, antipsychotics) and other medications.

Why Tylenol doesn't get people high is anyone's guess.

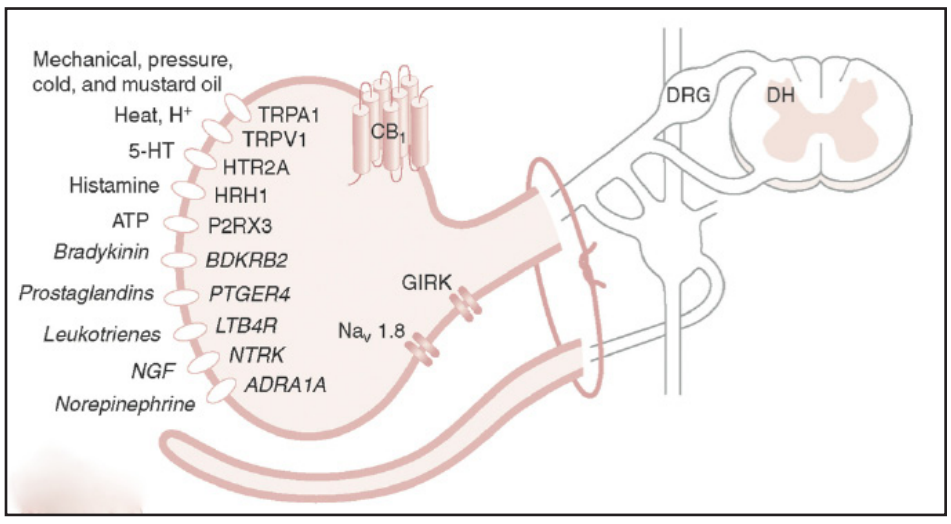
Acetaminophen (paracetamol, Tylenol) is converted into N-arachidonoylphenolamine by the liver, a compound that activates CB_1 and inhibits eCB reuptake. The analgesic activity of acetaminophen is blocked by CB_1 antagonists. Why it doesn't get people high is anyone's guess.

Non-steroidal anti-inflammatory agents (NSAIDs) such as ibuprofen (Advil, Motrin) inhibit cyclooxygenase (COX) enzymes COX1 and COX2, and thereby block the conversion of arachidonic acid into inflammatory prostaglandins.

NSAIDs also inhibit the breakdown of 2-AG and anandamide. This may explain why some people feel a mild buzz after taking ibuprofen.

Furthermore, anandamide and especially 2-AG are metabolized by COX2 into prostaglandin ethanolamides (PG-EAs) and prostaglandin glycerol esters (PG-Gs), respectively. These compounds produce effects opposite to those of their parent molecules —allodynia and thermal hyperalgesia. Blocking COX2 will prevent the conversion of eCBs into pain-generating PG-EAs and PG-Gs.

The acute administration of glucocorti-



Pain nerve (c-fiber nociceptor) originates in the spinal cord's dorsal horn (DH), with its cell body in the dorsal root ganglion (DRG). Distal terminal (enlarged at left) contains receptors that activate pain signals or sensitize the nerve to pain signals. CB_1 receptors are synthesized in the DRG, and then go with the flow of protoplasm down the nerve fiber to the distal terminal. Schematic drawing reproduced with permission from McPartland (2009) in Fibromyalgia, 3nd Edition, Churchill Livingstone, Edinburgh, UK.

coids (prednisone, dexamethasone) shifts arachidonic metabolism toward endocannabinoid synthesis. This may explain why acute glucocorticoids induce cannabimimetic psychoactive effects ("steroid mania"). Prolonged glucocorticoid administration in rats (21 days) reduces anandamide levels but not 2-AG, and decreases CB_1 density.

The coadministration of cannabinoids with opiates (codine, morphine) provide synergistic analgesia. However, chronic exposure to morphine leads to desensitization of opioid receptors.

SSRIs and tricyclic antidepressants probably enhance the sensitivity of CB_1 to THC.

Seratonin selective uptake inhibitors (SSRIs) such as Prozac are the most commonly prescribed antidepressant drugs. But tricyclic antidepressants (TCAs) such as Elavil are still prescribed for fibromyalgia and other chronic pain syndromes. Matthew Hill has published many studies that show, collectively, that SSRIs and tricyclics increase CB_1 density in rodent brains, without significantly altering anandamide or 2-AG levels. SSRIs and tricyclic antidepressants also enhance the sensitivity of CB_1 to synthetic cannabinoids, and probably to THC.

The antipsychotic drug haloperidol increases CB_1 density and the sensitivity of CB_1 to synthetic cannabinoids. Other antipsychotic drugs such as chlorpromazine or olanzapine do the same, whereas clozapine does the opposite. Several researchers have suggested that upregulation of CB_1 may explain appetite enhancement, weight gain, and CB_1 supersensitivity during antipsychotic drug treatment.

The co-administration of diazepam (Valium) and cannabinoids produces synergistic anti-anxiety effects, and the blockade of CB_1 with rimonabant prevents the anxiolytic effects of diazepam. Valproate, an anti-epileptic drug, increases CB_1 binding.

Psychoactive substances: ethanol, nicotine, caffeine, cannabis

Studies of chronic ethanol exposure in rodents usually demonstrate an increase in eCB ligand concentrations, and a downregulation of CB_1 and signal transduction. In a human study, alcoholics who died of

Photo by Todd McCormick

The interactions between nicotine and the eCB system are complex and paradoxical.

natural causes or motor vehicle accidents showed decreased CB_1 densities in autopsied brains. Brain slices showed decreased responses to cannabinoids. However, the same study showed that alcoholics who died of suicide showed increased CB_1 densities and CB_1 receptor-stimulated signal transduction.

Tobacco is often mixed with cannabis, and it enhances cannabimimetic effects such as sedation and analgesia. The interactions between nicotine and the eCB system are complex and paradoxical. One rodent study showed that chronic nicotine exposure increased anandamide levels in the limbic forebrain, and increased anandamide and 2-AG contents in the brainstem, but decreased anandamide and/or 2-AG contents in the the hippocampus, the striatum and the cerebral cortex.

Another rodent study showed that chronic nicotine increased CB_1 density in select regions of the brain in adolescent male rats, but not adult male rats. Chronic nicotine enhanced the locomotor-decreasing effects of THC in adolescent males, but not adult males.

Back in 1857, John Bell said that swallowing hashish with coffee increased the effects of hashish, and at the same time diminished its duration, by promoting a more rapid absorption.

Caffeine antagonizes neuroreceptors in the brain known as adenosine receptors. Adenosine receptors are tonically activated by adenosine, their endogenous ligand. Rodent studies indicate that adenosine receptors tonically inhibit CB_1 activity. Thus the antagonism of adenosine receptors by drugs like caffeine and theophylline will enhance the eCB system (increasing activation of CB_1 by 2-AG). Caffeine potentiates the stimulation of CB_1 by THC, at least when caffeine is consumed on a chronic basis. The acute blockade of the adenosine receptor A_1 with a single dose of an antagonist does not modulate the effects of THC.

The Effects of Cannabis

And now the big question: how does cannabis impact the eCB system?

Like other interventions, its impact likely varies according to dosage, and acute versus chronic administration. Several studies have showed that the acute administration of THC actually increases CB_1 densities in rodent brains. Acute THC stimulates in vitro anandamide biosynthesis in mouse neuroblastoma cells. Intuitively, THC could substitute for shortfalls of anandamide or 2-AG.

However, chronic, high dosing of THC causes a predictable desensitization and downregulation of cannabinoid receptors, accompanied by drug tolerance. Drug-tolerant individuals have to consume more THC to achieve the same effect. This has been demonstrated in many rodent studies and two human studies.

CB_1 receptors in different regions of the brain downregulate at unequal rates and magnitudes. This may explain the results published recently by D'Souza confirming that frequent users of cannabis develop tolerance to some effects of THC, such as anxiogenesis and cognitive impairment, but not to its euphoric effects.

THC causes less CB_1 desensitization and tolerance than synthetic full agonists, but it can antagonize 2-AG.

THC acts as a partial agonist of CB_1, compared to synthetic cannabinoids, which act as full agonists. Partial agonism can be a virtue: rodent studies show that THC causes less CB_1 desensitization and tolerance than synthetic full agonists.

But THC's partial agonism has a dark side: high concentrations of THC will functionally antagonize the effects of a full agonist when the two drugs are added together. 2-AG acts as a full agonist at CB_1 and CB_2, and rodent studies demonstrate that THC can antagonize 2-AG under some conditions.

The situation is very complex; one study indicated that THC oscillates between agonism and antagonism depending on neuron firing rate in response to stimulus. At low firing rates in rat hippocampal neurons, THC mimicked 2-AG and behaved like an agonist; at high firing rates, THC antagonized endogenous 2-AG signaling.

Acute versus chronic dosage also determines THC's impact on 2-AG signaling. Andrea Hohmann has studied "stress antinociception," where rodents become less responsive to painful stimuli following exposure to an environmental stressor. Stress antinociception is mediated, in part, by the coordinated release of 2-AG and anandamide.

The acute administration of THC potentiated eCB-mediated stress antinociception. The converse was also true: animals exposed acutely to foot shock, which elicits eCB-mediated stress antinociception, became sensitized to the effects of THC.

Chronic dosing with THC (daily use for 14 days in rodents) predictably dampened stress antinociception. The converse was not true: chronic exposure to foot shock (daily for 15 days) failed to dampen antinociception induced by either WIN-55,212-2 or by further footshocks.

In other words, the rats gained more pain relief by chronically upregulating 2-AG and anandamide and supplementing with acute THC, than by chronic dosing with THC and acutely boosting the eCBs.

In humans, the dividing line between "acute" and "chronic" THC is a gray zone and likely differs amongst individuals. Clinical trials with medical marijuana and cannabis extracts show that people in pain naturally titrate their dosage to levels that optimize pain relief—a level that probably preserves natural synergy with endogenous 2-AG and anandamide.

Lastly, we must remember that cannabis is more than THC. Adding cannabidiol (CBD) to acute THC administration enhances CB_1 expression in rodent brains. CBD stimulates the release of 2-AG, and CBD inhibits the breakdown of anandamide by FAAH.

"It was possible that God knew Paul would have forbidden smoking, and had purposely arranged the discovery of tobacco for a period at which Paul should be no longer living."

—Samuel Butler