MITOCHONDRIA MYSTERIES

Scientific data indicates that CBD & THC can affect mitochondria, the energy adaptors that power every multicellular organism. How do cannabinoids influence cellular function?

BY ADRIAN DEVITT-LEE ON DECEMBER 07, 2016

Homeostasis, renewal and the endocannabinoid system

Photo credit: National Human Genome Research Institute

In 2012, French scientists reported the presence of cannabinoid receptors on the membranes of mitochondria, the energy-generating organelle within cells. This discovery laid the groundwork for subsequent investigations into the role of the endocannabinoid system in regulating mitochondrial activity, which is critical to how cells function. Defects in mitochondria have been linked a wide range of neurodegenerative, autoimmune and metabolic disorders—Alzheimer's, schizophrenia, autism, cancer, epilepsy, diabetes, cardiovascular and neuromuscular disease, and more.

A growing body of scientific data indicates that cannabidiol (CBD) and tetrahydrocannabinol (THC), two key components of the cannabis plant, can affect mitochondria, both directly and indirectly. It turns out that many of the biological pathways that involve mitochondria—including energy homeostasis, neurotransmitter release, and oxidative stress—are modulated by endogenous and exogenous cannabinoids.

But research on cannabinoids often seems to be riddled with contradictions. Cannabinoids are notorious (in science and lived experience) for exerting opposite effects in different situations. How are CBD and THC able to balance physiological excess as well as deficiency? Why does a small dose of cannabis stimulate while a large dose tends to sedate? How is it possible that cannabinoid compounds can destroy cancer cells while leaving healthy cells unscathed? Examining the role of mitochondria sheds light on these questions and other perplexing aspects of the endocannabinoid system.

What Are Mitochondria?

Mitochondria are universal energy adaptors that exist in the cells of every multicellular organism, including humans. The number of mitochondria in an individual cell can vary greatly depending on the organism and tissue type. (All human cells, except for red blood cells, contain mitochondria.) One of the main functions of mitochondria is to take high-energy molecules—such as sugars and amino acids—and convert them into a form of energy, called adenosine triphosphate (ATP), which the cell can use. For the cell, ATP is like a battery.

The process of extracting small bits of energy from high-energy molecules can be quite dangerous. Imagine trying to power a car by simply lighting the fuel tank on fire. A cell can't handle the microscopic equivalent of an explosion, so the cell must use finesse to harness this energy. Individual electrons are extracted from high-energy molecules by a process known as cellular respiration and their energy is gradually released.

This gradual release of individual electrons allows the cell to synthesize ATP from its precursors, adenosine diphosphate (ADP) and inorganic phosphate (Pi). The cleavage of ATP back into ADP and Pi releases a small amount of energy, which powers the proteins that allow each cell to function and communicate. ATP is the main energy source for the majority of cellular functions. While commonly referred to as the cell's powerhouse, mitochondria are also involved in other metabolism-related functions, but the goal is always the same—homeostasis, the maintenance of a stable internal environment despite external fluctuations.

Symbiosis

Originally, mitochondria were separate from other cells. At some point, one-and-a-half to two billion years ago, a cell engulfed an evolutionary precursor to a mitochondrion. But instead of digesting the mitochondrion, the two living entities formed a symbiotic relationship. The host

cell would provide nutrients and a safe place for the mitochondrion to exist, and the mitochondrion would perform the dangerous process of cellular respiration, giving the host a more useable form of energy. The result was so evolutionarily fundamental that this symbiotic relationship preceded the occurrence of multicellular organisms. All plants, animals and fungi are endowed with mitochondria.

This theory of how two different self-organized living systems began to collaborate symbiotically is supported by the fact that mitochondria have retained their own genome that is separate from the host cell's DNA. Mitochondria and the host cell replicate independently; they also have separate cellular membranes. Two other organelles are thought to have developed in a similar way: the chloroplast, which enables photosynthesis in plants, and the nucleus, which holds the cellular DNA and acts as a kind of coordinator of the cell.

Mitochondrial diseases can be caused by inherited mutations in mitochondrial DNA or defects in the nuclear genes that encode proteins that regulate mitochondrial division and DNA replication. Mitochondrial disorders can also develop due to the adverse effects of drugs, infections, environmental toxins or unhealthy lifestyle habits. Mitochondrial diseases are most severe when the defective mitochondria are present in muscle, brain or nerve tissue, as these cells require more energy (and hence more mitochondrial activity).

Free Radicals & Phytocannabinoids

Mitochondria Busters & Boosters

Deficiencies of omega 3 oils and probiotics, low vitamin D levels, and other nutritional imbalances contribute to mitochondrial dysfunction. Ditto for many prescription pharmaceuticals, artificial sweeteners, and toxic food additives. Rooted in poor dietary choices and lifestyle habits, cancer can be thought of as a mitochondrial metabolic disorder. Sugar feeds cancer, while saturated fats starve it.

Antioxidants found in many plants have long been promoted as natural food supplements to minimize harm from free radicals. A vitamin-like antioxidant known as CoQ10 or ubiquinone facilitates mitochondrial function. Foods rich in CoQ10 include broccoli, cauliflower, sesame seeds, oily fish, chicken, and grass-fed beef. In addition to a healthy diet, exercise is a great way to boost mitochondrial

repair and regeneration. Several scientific studies also show that low-level laser therapy can accelerate the healing process by triggering mitochondrial activity and ATPsynthesis. — Project CBD

Although mitochondria allow energy to be accessed at a measured pace in relatively small quantities, the process of cellular respiration, whereby cells extract energy from nutrients, still can be damaging. High-energy electrons offload their energy in a multitude of complicated steps, until the lower-energy electron is finally released onto an oxygen molecule. Ideally, the oxygen molecule will interact with hydrogen and form water, which is very stable.

But sometimes the ionized oxygen, called superoxide, can escape, resulting in oxidative stress. Similarly, other unstable molecules like peroxide and hydrogen peroxide, can form and escape. These unstable, renegade molecules are called reactive oxygen species (ROS) or free radicals. Free radicals cause damage by interacting with DNA, cell membranes, proteins, or other organelles.

By effectively neutralizing free radicals and mitigating oxidative stress, antioxidants confer a broad range of therapeutic benefits—from slowing down the aging process to reducing the risk of DNAdamage linked to cancer. THC and CBD are both potent antioxidants, according to the U.S. government, which filed a patent on the antioxidant and neuroprotective properties of cannabinoids based on research from 1998. This patent underscores one of the great hypocrisies of federal drug policy, which disingenuously maintains that cannabis has no medical value.

Autophagy & Apoptosis

Oxidative stress is a natural byproduct of mitochondrial activity. The creation of oxidative stress is necessary for obtaining energy and sustaining cellular function. Inevitably this will take its toll on an organism. But oxidative damage can be repaired to a certain extent through an adaptive process known as autophagy, whereby faulty cell parts – misfolded or aggregated proteins, dysfunctional mitochondria, etc.—are removed and replaced by newer, better-working components. Cell survival is dependent on this ongoing regenerative mechanism.

Oxidative stress is not exclusively bad. At low levels, reactive oxygen species act as signaling molecules. Damaged neurons can shed their worn-down mitochondria, which neighboring cells interpret as an SOS. Immune cells in the brain, called astrocytes, respond by donating some of their own mitochondria to the impaired neurons. Lung cells can also secrete healthy mitochondria for damaged cells to use.

Low levels of oxidative stress may stimulate a necessary cellular housecleaning, but high levels of oxidative stress are an indication that something is going wrong in the cell. Too much oxidative stress is a signal for the cell to destroy itself in a regulated way, a process called apoptosis. It's as if there's a tipping point when oxidative damage exceeds the capacity of a cell to repair itself, so the cell pivots from survival mode and commits suicide for the betterment of the team. The fate of a cell—whether survival via autophagy or death via apoptosis—is contingent on the kind of stress it encounters and its duration.

Age-Related Neurodegeneration

While oxidative stress in moderation can be used by the cell, the dysregulation of oxidative stress results in illness. Disruption of the delicate interplay between autophagy and apoptosis allows free radicals and damaged cells to accumulate, which can lead to a wide range of pathologies. Mitochondrial dysfunction is involved in virtually all disease, especially age-related neurodegeneration. Since neurons use a tremendous amount of energy to transmit information throughout the body, they require highly active mitochondria, which means greater oxidative damage. This slowly leads to a loss of functioning and symptoms of age-related decline.

According to a 2016 report in *Philosophical Transactions of the Royal Society* (London): "Cannabinoids as regulators of mitochondrial activity, as anti-oxidants and as modulators of clearance processes protect neurons on the molecular level… Neuroinflammatory processes contributing to the progression of normal brain ageing and to the pathogenesis of

neurodegenerative diseases are suppressed by cannabinoids, suggesting that they may also influence the aging process on the system level."

Ageing, neurodegeneration, metabolic disorders, and cancers are all linked to mitochondrial activity—or lack thereof. (The discovery of minimal mitochondrial activity in cancer cells, called the Warburg effect, earned Otto Heinrich Warburg a Nobel prize in 1931.)[1] Preclinical studies indicate that THC can inhibit the formation of amyloid plaque in the brain, a hallmark of Alzheimer's dementia, by enhancing mitochondrial function. And CBD has been shown to stimulate mitochondrial biogenesis and reverse symptoms of memory loss in animals. But how do cannabinoids improve cognitive function? How do they interact with mitochondria and reduce brain inflammation?

Mechanisms of Action—Receptors & Membranes

There are three major ways that plant and endogenous cannabinoids can directly modulate mitochondrial function – by 1) activating CB1receptors on the mitochondria; 2) perturbing the mitochondrial membrane; and 3) binding to other (non-cannabinoid) receptors on the mitochondria's surface.

Mitochondrial CB1 receptors. Embedded in cell membranes, cannabinoid CB1 receptors are the most prevalent G-coupled protein receptors to populate the human brain and central nervous system. An estimated fifteen percent of all CB1 receptors in neurons exist on the mitochondria. In certain kinds of muscle tissue, half of the CB1 receptors are localized on the mitochondria. In order to directly activate a mitochondrial CB1 receptor, THC must penetrate the outer cellular membrane and be chaperoned through the cell's interior.
Mitochondrial CB1 receptors are not structurally distinct from the prolific CB1receptors that wrap around the cell's outer surface, but their effects can be quite different. (Light switches may look the same from room to room, but they are connected to different circuitry throughout the house, and so turning the switch on or off in different places causes different outcomes.) Preclinical science suggests that activation of mitochondrial CB1 receptors

usually decreases mitochondrial activity. This can protect the cell from oxidative stress and prevent apoptosis, but paradoxically it can also cause cell death in some conditions.

Membrane perturbation. The membrane of mitochondria are primarily made of lipids, such as fatty acids and cholesterol. As in the outer cellular membrane, the relative concentration of short and long chain fats, saturated and unsaturated fats, and cholesterol influences many aspects of the mitochondrial membrane. Lipophilic compounds like endocannabinoids and plant cannabinoids can also meld into the mitochondrial membrane, changing its fluidity and permeability. Mitochondria harness the energy of electrons by using proteins imbedded in the mitochondrial membrane; alteration of membrane fluidity can inhibit the mitochondria's ability to produce energy and allow free radicals to more easily escape into the cell. Stephanie Seneff, a senior research scientist at MIT, reports that Monsanto's Roundup herbicide interferes with ATP production by adversely affecting mitochondrial membrane permeability.

Non-cannabinoid receptors. Cannabidiol does not directly activate mitochondrial CB1 receptors. Instead, CBDbinds to different receptors, including the sodium-calcium exchanger (NCX), on the mitochondria's surface. Binding to NCX opens an ion channel, and ions, such as electrically charged calcium atoms, flow from high concentrations to low concentrations. Different levels of calcium ions have different effects.[2] In conditions of low cellular stress, characterized by low intracellular calcium surrounding the mitochondria, CBD will increase stress by allowing calcium to flow out of the mitochondria. But in high stress conditions, characterized by copious intracellular calcium, CBD will do the exact opposite, allowing the flow of calcium from outside to inside the mitochondria (where calcium is stored) by opening NCX. The bidirectional calcium flow regulated by NCX is one of the mechanisms whereby CBD facilitates cellular homeostasis and neuroprotection.

CBD, Calcium Channels & Homeostasis

Cannabinoids are well known among scientists for their trickster-like ability to exert opposite effects in different situations. In mitochondria, cannabinoid activity is even more complicated. At low-stress conditions, cannabinoids often increase mitochondrial activity

and cellular respiration, triggering autophagic cellular repair. Cannabinoids will also buffer high stress conditions and protect cells by decreasing mitochondrial activity. But that's not all. The dependence on stress is actually *trimodular*. In very high-stress conditions—such as those often present in cancer cells—endocannabinoids can create a positive feedback loop, increasing stress to the point where the cell undergoes apoptosis. Plant cannabinoids can also induce apoptosis under similar conditions. The death of cancer cells promotes homeostasis and the survival of the organism as a whole.[3]

The ebb and flow of calcium and stress, autophagy and cell death, the restoration of homeostasis on a cellular level are all regulated by CBD. A report by British researchers in the *Journal of Neuroscience* (2009) noted that "under pathological conditions involving mitochondrial dysfunction and calcium [Ca(2+)] dysregulation, CBD may prove beneficial in preventing apoptotic signaling via a restoration of calcium homeostasis."

Examining CBD's effect on mitochondria sheds light on how cannabidiol can protect against brain injury by regulating fluctuations in intracellular calcium. A November 2016 study in the *European Journal of Pharmacology* found that an "imbalance of sodium and calcium homeostasis trigger[s] pathophysiologic processes in cerebral ischemia, which accelerate neuronal brain death." The good news for stroke victims, according to Iranian scientists at Shahid Beheshti University in Tehran, is that CBD can reduce the severity of ischemic damage by enhancing NCX receptor expression on the mitochondrial membrane.

Ceaseless Regeneration

Cannabinoids promote neuroplasticity and mediate homeostasis through various bidirectional pathways. Consider, for example, the biphasic effect of THC. Whereas most drugs follow the trend that a higher dose causes a stronger effect, THC and other cannabinoids can trigger a biphasic dose response. A biphasic effect refers to two opposite responses to a single compound; this is not uncommon, especially among

cannabinoids. THC has been shown to increase mitochondrial activity at a mild psychoactive dose, while decreasing it at higher doses.[4]

Biphasic dose-responses often occur when a compound influences a cell through multiple channels. With respect to mitochondrial function, the biphasic effects of cannabinoids depend on cellular conditions as well as dosage. For example, at high doses THC will reduce mitochondrial activity by binding to CB1 receptors on the organelle's surface; but at low doses THC may cause an opposite effect by changing the fluidity of the mitochondrial membrane in way that promotes ATP synthesis and cellular respiration. Membrane fluidity and permeability are also modulated by other epigenetic factors, including different levels of cholesterol and dietary fats.

The actions of CBD and THC in the mitochondria highlight some of ways that the endocannabinoid system regulates cellular repair and renewal. Our body's default state is one of ceaseless regeneration. Continual turnover on a cellular level is the fulcrum of health, the dynamic underpinning of homeostasis. In times of illness, regenerative processes are overcome by dysfunction and degradation. Cannabinoids and other membrane-penetrating antioxidants can enhance mitochondrial function and restore physiological balance.

Adrian Devitt-Lee is a Project CBD research associate and contributing writer.

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[1] The reason for the Warburg effect, as understood today, is that cancer cells need materials to synthesize DNA, proteins, and the cell membrane. The need for biomaterials is more significant than the need for energy. If the mitochondria are not active, then a form of fermentation will occur instead. This produces a small amount of energy but leaves molecules that can be used as precursors to cellular materials.

[2] One measure of stress in a cell is the concentration of cytostolic calcium (calcium in the intracellular space). Calcium is an important secondary messenger inside of a cell; it modulates the activity and inhibition of various

proteins and influences many cellular signals, including apoptosis. On a cellular level, calcium is primarily stored inside the mitochondria and another organelle, the endoplasmic reticulum.

[3] Why is it that cannabinoids tend to be toxic in cancer cells, but protective in other cell types? (Note that this is a trend; there are many exceptions.) Chemotherapy regimens typically involve generating excessive oxidative stress to precipitate apoptosis.CBD has potent anti-tumoral properties, but it is a robust antioxidant. Curiously, several studies suggest that mitochondrial-specific antioxidants, such as CBD, work synergistically with conventional chemotherapy regimens in promoting cancer cell death, even though antioxidants typically interfere with chemotherapy. The combination of plant cannabinoids and pharmaceutical chemotherapy agents has been examined to some degree and shows promise.

[4] A mild psychoactive dose is 6.25 µM THC, as determined by A. Athanasiou, et al (2007). The high doses mentioned are 10-100µM THC. The relevant physiological doses in humans appear to be around 1-20 µM THC. Studies often use very high doses because small animals like mice have much quicker metabolisms, and so the drug is removed from their system faster. To counteract this, scientists sometimes give a very high dose, such that the target dose is achieved at a certain time point (e.g. 10 µM THC at 10 min). This is one of the many reasons that preclinical research does not always translate to human experience with drugs. The fact that relevant doses of THC are right on the cusp of its biphasic effect is likely one of the many reasons that research with THC and the mitochondria is so challenging.

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