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Spoznavanje biokemijskih temeljev endokanabinoidnega sistema

POVZETEK

Odkritje endokanabinoidnega sistema (ECS) je bilo temeljnega pomena ne le pri razumevanju učinkov rastlinskih kanabinoidov, amapk je vodilo tudi do precej širšega biokemisjkega delovanja našega telesa in odprlo velike terapevtske potenciale. Fitokanabinoidi in tudi sintetični kanabinoidi delujejo preko našega endokanabinoidnega sistema in razumevanje osnovne biokemije tega ključnega signalnega sistema omogoča vpogled v koristne in terapevtske učinke teh molekul. Na voljo je veliko raziskovalnih in znanstvenih člankov na temo kanabinoidov, ECS ter njihove vloge in vpliva na zdravje in potek bolezni. Številni podatki so tudi zbrani iz epidemioloških raziskav in iz Life science laboratorijev in vsi ti podatki dajejo bolnikom in zdravstvenim delavcem dobre osnove za uporabo kanabinoidov v medicini. Toda razumevanje biokemije ECS ter vloge tega signalnega sistema v človeški fiziologiji je ključ do pravilne uporabe teh močnih molekul.

Ključne besede: biokemija, receptorji, endokanabinoidni sistem, kanabinoidi.

1. Introduction

From a biochemical perspective human beings are very complicated. If we look only at one basic unit of life- the cell – biochemistry is already rather complex at this level. Cells are not just simple building blocks, unconscious and static as bricks in a wall. Cells can detect what's going on around them, and they can respond in real-time to cues from their neighboring cells and environment. At any moment, cells are sending and receiving millions of messages in the form of chemical signaling molecules and at any given moment there are over 10.000 biochemical reactions taking place inside each cell. All of these are coordinated and tightly regulated. Energy is reasonably used only for vital processes. But in our human biochemistry a single cell is not an individual unit of life, but rather a part of a tissue, an organ or a physiological system. So for a cell to be able to function as a part of a whole system, it is vital for the cell to communicate with its environment. A cell is divided from its surroundings by a semipermeable membrane, a lipid bilayer with embedded proteins. The basic function of the cell membrane is to protect the cell from its surroundings. The cell membrane controls the movement of substances in and out of cells. In this way, it is selectively permeable to ions and organic molecules. In addition, cell membranes are involved in a variety of cellular processes such as cell adhesion, ion conductivity and cell signaling.

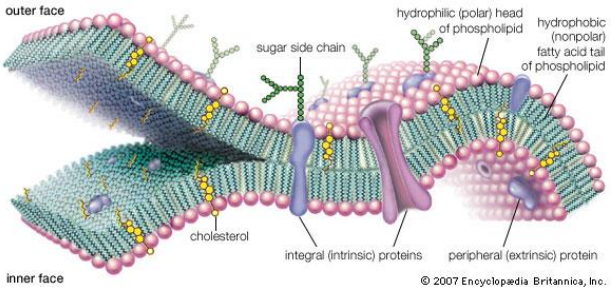

Figure 1: structure of the cellular membrane (source: https://www.britannica.com/science/cell-membrane)

Cellular communication is vital for all multicellular organisms and the more complex and evolved organisms are the higher is the importance of cellular communications. The basic setup needed for cellular communication or signaling is similar as in all communications. We need to know what message we want to send (a signaling molecule) and to who we want to send it to (who has the right receptors or antennas). Cells typically communicate using chemical signals. These are different types of molecules (cannabinoids are just one of many) produced by a sending cell and released into the extracellular space. There, they can float – like messages in a bottle – over to neighboring cells or into circulation.

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Not all cells can "hear" a particular chemical message. In order to detect a signal a cell must have the right receptor for that signal. When a signaling molecule binds to its receptor a shift takes place triggering a change inside of the cell. Signaling molecules are often called ligands, a general term for molecules that bind specifically to other molecules (such as receptors).

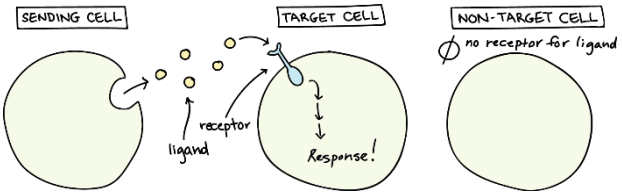

Figure 2: Schematic representation of cell signaling (source: https://www.khanacademy.org)

A signaling molecule and receptor recognize each other based on a unique 3D molecular structure. In essence a receptor will bind a molecule if its structure fits the receptors binding site in a very similar way as a key fits a keyhole. If it's a match the doors will open and if not, nothing will happen. If a signaling molecule and a receptor are a match, a cascade of downstream reactions will take place, ultimately, leading to a change in the cell, such as alteration in the expression of a gene or even the induction of a new process, such as cell division, apoptosis... Such communication not only enables the cells to respond to changes in the extracellular environment, adapt to these changes and thrive but also exchange signals between cells, tissues, organs, and whole body.

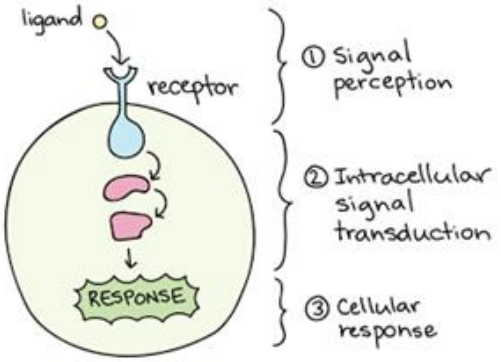

Figure 3: schematic representation of signal transduction (https://www.khanacademy.org)

Different types of cells have different set of receptors. And the cells are very economical in this sense, each cell expresses on its surface only the types of receptors that are very vital for their survival and only in the numbers that are needed. In cellular biochemistry there is not a molecule or a reaction too much, all of its function is highly optimized and adjusted according to the environment, stimuli and needs. Each specific cell type in our body has a specific set of receptors, the types and density of the receptors can change during the life of a cell, depending on the conditions a cell is exposed to.

In 1988, more than 4 decades after the first plant cannabinoid was discovered (CBD) and its structures elucidated, the first cannabinoid receptor was found. The existence of cannabinoid receptors puzzled scientists. More so after it was found that these receptors were very abundant on the membranes

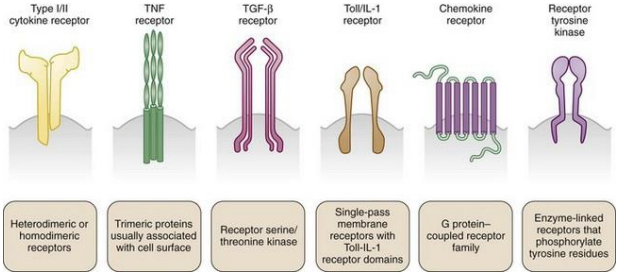

Figure 4: 3D structure of different types of receptors (source: Iain B. McInnes https://musculoskeletalkey.com/cytokines/)

of our cells. It did not make much sense that our bodies would be so sensitive and fine-tuned to these molecules, since there is only little chance that we might encounter and consume cannabis in our lifetimes. Another 20 years passed before the discovery of endocannabinoids, cannabinoids that are produced by human bodies, and not only by human, all vertebrates produce endocannabinoids. This was a major discovery that led to intense research into the role and functioning of this signaling system.

So in a way our endocannabinoid system is comparable to our hormonal system or our neurotransmitter system, although it is much more than that. The endocannabinoid system seems to be the enhanced version of an ancestral intercellular communication system that has been passed on evolution since plants appeared on the planet earth.

2. The biochemistry of the endocannabinoid system

All that we now know about cellular signaling, is true for the endocannabinoid system. We have cannabinoid receptors and ligands (cannabinoids). Taking into consideration that the research field of cannabinoids and endocannabinoid system is rather new and that the research was and is held back by legislation hurdles, we now know for sure is that we have at least 3 cannabinoid receptors: CB1, CB2 and CB3 (formerly known as the GPR55). There are several endocannabinoids know thus far, the best studied are the N-arachidonylethanolamide or anandamide and 2-arachidonoylglycerol or 2-AG (analogs to plant derived THC and CBD). For the production and the degradation of endocannabinoids we need enzymes that are pivotal for the optimal functioning of the endocannabinoids system. So the endocannabinoid system is composed of receptors, endocannabinoids and involved enzymes.

Cannabinoids are essentially messenger molecules, their role is to send signals, convey a message and the underlying biochemistry of the functioning of endocannabinoid system is similar as for majority of signaling systems (described in previous chapter).

The endocananbinoids anandamide and 2-AG are released upon demand from cell membrane-embedded phospholipid precursors. The primary biosynthetic enzyme of AEA is N-acyl-phosphatidylethanolamine phospholipase D (NAPE-PLD). 2-AG is biosynthesized by two isoforms of diacylglycerol lipase, DAGLα and DAGLβ. AEA and 2-AG work in a homeostatic fashion, thus they are broken down after they activate CB1 or CB2. AEA is catabolized primarily by fatty acid amide

hydrolase 1 (FAAH1), and 2-AG is catabolized by monoacylglycerol lipase (MAGL), and, to a lesser extent, α,β-hydrolase-6 (ABHD-6), cyclooxygenase 2 (COX2), and FAAH1. So cannabinoids are not molecules to circulate in our system and be present in high concentration, rather they are synthesized where and when they are needed and then degraded. In this respect they differ from hormones for example. Their low physiological concentration was one of the reasons why it took researchers so long to prove the existence of endocannabinoids.

There is another specificity to the mode of action of cannabinoids. They are retrograde messengers, sending the signal from the postsynaptic cell to the presynaptic. Cannabinoids are released from depolarized postsynaptic neurons presumably in a calcium-dependent manner and act retrogradely onto presynaptic cannabinoid receptors to suppress neurotransmitter release. So cannabinoids modulate neuronal excitability by inhibiting synaptic transmission. So these endogenously synthesized cannabinoids, but also phytocannabinoids and synthetic analogs appear to act as retrograde signalling agents, reducing synaptic inputs onto the stimulated neuron in a highly selective and restricted manner. This being one of the reasons why cannabinoid receptors were found later then for example opioid receptors. Just as a comparison the major active ingredient of opium was discovered in 1799 and in 1973 the receptor, whereas THC as the active ingredient of cannabis was discovered in 1964 and the receptor in 1988.

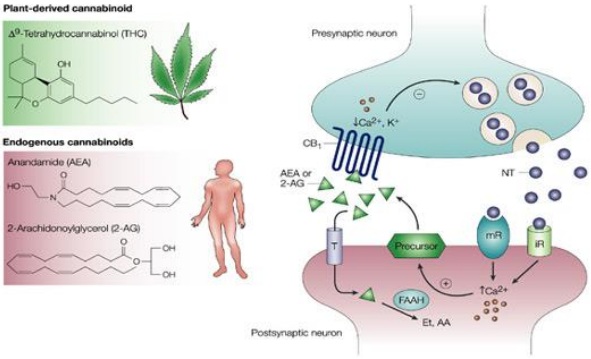

Figure 5: Schematic representation of the endocannabinoid system (ECS) (source: https://www.nature.com/articles/nrc3247)

2.2 Cannabinoids receptors

Now let's have a closer look at the cannabinoid receptors. What all three cannabinoid receptors have in common is that they are all G-protein coupled transmembrane receptors (GPCR). When a ligand (cannabinoid) binds to the GPCR it causes a conformational change in the receptor, which allows it to act as a guanine nucleotide exchange factor. The GPCR can then activate an associated G protein by exchanging the GDP bound to the G protein for a GTP. The G protein's α subunit, together with the bound GTP, can then dissociate from the β and γ subunits to further affect intracellular signaling proteins or target functional proteins directly depending on the α subunit type. GPCRs are an important drug target and approximately 34% of all Food and Drug Administration (FDA) approved drugs target this family of proteins.

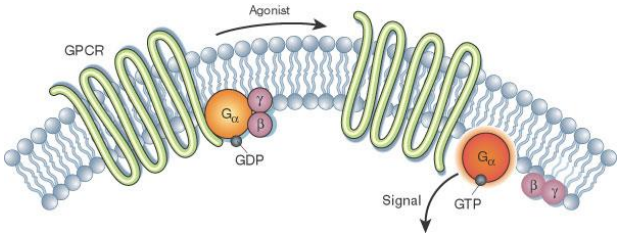

Figure 6: The structure of G coupled receptors or GPR's and their activation by agonist (source: http://gpcr.utep.edu/background)

So basically a part of the receptor is on the outside of the cell, facing the extracellular space (the environment) sensing changes in the concentration of cannabinoids, and a part of the receptor is on the inside of the cell (conveying the message on what is going on outside). All three receptors cross the membrane 7 times and are coupled with a G-protein that sends the signal towards the cell nucleus. Thereby enabling the cell to respond to changes in its environment. Through a complex biochemical cascade the message is send to the nucleus and changes in gene expression take place and enable cell response. What kind of a response a cell will give depends on many factors, including cell type, the chemistry of the cannabinoids, concentration of cannabinoid molecules, presence of other molecules and also the number or density of cannabinoid receptors on the cell surface. To fully understand the physiological roles the endocannabinoid system has in our body, we have to take a look at where anatomically we have cannabinoid receptors, so what organs or tissues can hear the message cannabinoids are sending.

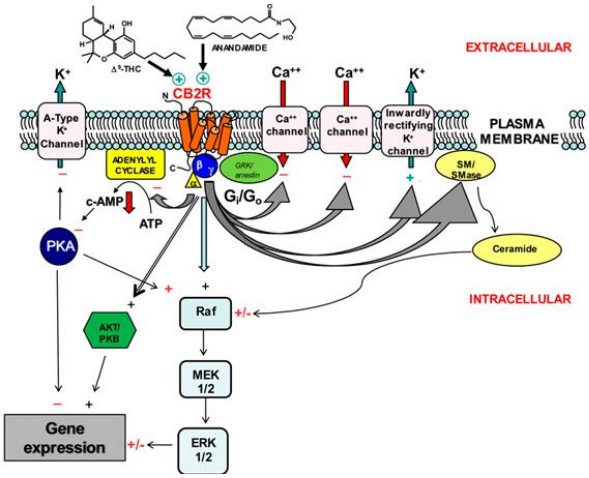

Figure 7: Activation of the cannabinoid receptors and following intracellular signaling (source: https://www.researchgate.net/figure/Activation-of-CB-2-receptors-by-natural-or-synthetic-ligands-favors-a-range-of-receptor_fig1_264631508)

The CB1 receptor is one of the most abundant G protein-coupled receptors (GPCRs) in the central nervous system and is found in particularly high levels in the neocortex, hippocampus, basal ganglia, cerebellum and brainstem. They are less expressed in the amygdala, hypothalamus, nucleus accumbens, thalamus, periapeduncular grey matter and the spinal cord, as well as in other brain areas, mainly in the telencephalon and diencephalum. CB1 receptors are also expressed in several peripheral organs. Thus, they are present in adipocytes, liver,

lungs, smooth muscle, gastrointestinal tract, pancreatic ß -cells, vascular endothelium, reproductive organs, immune system, sensorial peripheral nerves and sympathetic nerves. There are very few CB1 receptors in the brain stem, in the centers that regulate breathing and cardiovascular functions. This is one of the reasons cannabinoids have a very good safety profile, since their overdose does not adversely affect these brain centers. Attesting to this is also the fact that up to date there has not been a single proven case of death due to overdose with cannabis.

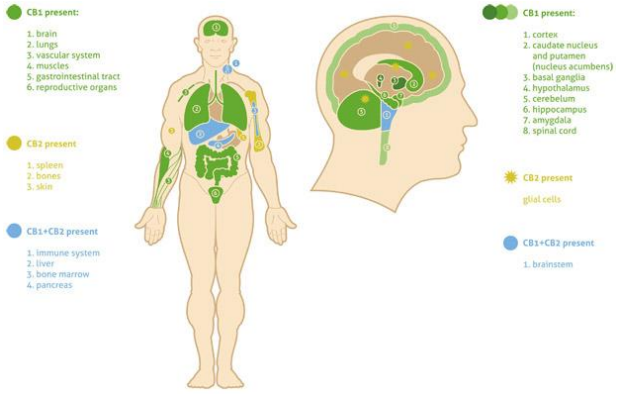

Figure 8: Distribution of the cannabinoid receptors CB1 and CB2 in the body (source: https://www.fundacion-canna.es/en/endocannabinoid-system)

The distribution of CB2 receptors is quite different, the highest density is found on the periphery in the immune system cells, such as macrophages, neutrophils, monocytes, B-lymphocytes, T-lymphocytes and microglial cells. Recently, CB2 receptor expression has also been shown in skin nerve fibers and keratinocytes, bone cells such as osteoblasts, osteocytes and osteoclasts, liver and somatostatin secreting cells in the pancreas. The presence of CB2 receptors has also been demonstrated at the CNS, in astrocytes, microglial cells and brainstem neurons. There is evidence of CB2 also on the surface of neurons. Recent evidences suggest that the CB2 receptor mediates emotional behaviours, such as schizophrenia, anxiety, depression, memory and nociception, supporting the presence of neuronal CB2 receptors or the involvement of glial cells in emotional behaviors.

CB3 receptor or GPR 55 is a general cell signaling receptor, its specific physiological role is unclear, because mice with a target deletion of the GPR55 gene show no specific phenotype. GPR55 is widely expressed in the testis, spleen and brain, especially in the cerebellum. It is expressed in the gastrointestinal tract, especially jejunum and ileum. Osteoblasts and osteoclasts express GPR55 and this has been shown to regulate bone cell function. GPR 55 has a list of ligand, only one class of them being cannabinoids.

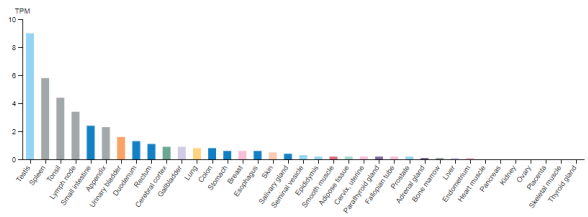

Figure 9: Distribution of the GPR55 or CB3 receptor in the human tissues (source: https://www.proteinatlas.org/ENSG00000135898-GPR55/tissue)

Already from the distribution of the three receptors in our body, we can see that the effects of cannabinoids that bind the CB1 or CB2 or CB3 will be very different. Taking into account that we now know that cannabinoids on top of having clear binding affinities to cannabinoid receptors, also have some degree of affinity to other types of receptor and also receptor independent effects, it is a complex mode of action. Much more complex then we usually see in pharmaceutical medication, where there is a known specific target and highly predictable effect.

So at the baseline the endocannabinoid system is defined as the ensemble of the two cannabinoid receptors; their two most studied endogenous ligands, the endocannabinoids N-arachidonoylethanolamine (anandamide) and 2-arachidonoylglycerol (2-AG); and the enzymes responsible for endocannabinoid metabolism (the primary 5: NAPE-PLD, the two DAGLs, FAAH, and MAGL). However, anandamide and 2-AG, and also the phytocannabinoids, have more molecular targets than just cannabinoid receptors. Furthermore, the endocannabinoids, like most other lipid mediators, have more than just one set of biosynthetic and degrading pathways and enzymes, which they often share with "endocannabinoid-like" mediators that may or may not interact with the same proteins as phytocannabinoids. In some cases, these degrading pathways and enzymes lead to molecules that are not inactive and instead interact with other receptors. Finally, some of the metabolic enzymes may also participate in the chemical modification of molecules that have very little to do with endocannabinoid and cannabinoid targets.

So the classic definition of the ECS has expanded with the discovery of secondary receptors, ligands, and ligand metabolic enzymes. For example, AEA, 2-AG, N-arachidonoyl glycine (NAGly) and the phytocannabinoids Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD) may also serve, to different extents, as ligands at GPR55, GPR18, GPR119, and several transient receptor potential ion channels (e.g., TRPV1, TRPV2, TRPA1, TRPM8). The effects of AEA and 2-AG can be enhanced by "entourage compounds" that inhibit their hydrolysis via substrate competition, and thereby prolong their action. Entourage compounds include N-palmitylethanolamide (PEA), N-oleoylethanolamide (SEA), and cis-9-octadecenoamide (OEA, oleamide).

2.3. Endocannabinoidome

This narrow definition of the ECS presented a few semantic problems:

1) of the > 80 cannabinoids naturally found in cannabis (with different relative composition depending on the cannabis variety), only THC and its less abundant Δ9-tetrahydrocannabivarin (THCV), are capable of binding with high affinity to CB1R and CB2R (with agonist and antagonist activity for THC and THCV, respectively); hence, these 2 receptors should not be defined as "cannabinoid" receptors, but rather as THC/THCV receptors

2) as a consequence, "endocannabinoids" should not be the endogenous ligands of CB1R and CB2R, but rather the ligands of all those "cannabinoid receptors" that uniquely and

selectively bind to cannabinoids in general (thus, anandamide and 2-AG might not be the only endocannabinoids); and

3) again, as a consequence, "endocannabinoid enzymes" would not only be NAPE-PLD, the two DAGLs, FAAH, and MAGL, but also other enzymes responsible for the biosynthesis and inactivation of the other mediators to be eventually included in the list of the endocannabinoids.

Although this broader view would seem like the natural "evolution" of the definition of the "endocannabinoid system", things are likely to be even more complicated. First, endocannabinoids, and also cannabinoids, have more molecular targets than just CB1, CB2, CB3 and thermo-TRPs, and these receptors appear to extend also to proteins that are targeted by other endogenous and exogenous substances. Furthermore, anandamide and 2-AG, like most other lipid mediators, have more than just 1 set of biosynthetic and degrading pathways and enzymes each which they often share with "endocannabinoid-like" mediators that may or may not be part of the extended definition of "endocannabinoids" provided above, that is, they may or may not interact with the same proteins to which non-THC cannabinoids bind. In some cases, these degrading pathways and enzymes lead to molecules, such as the prostamides and prostaglandin-glycerol esters which are not inactive but instead interact with other receptors, that is, these enzymes are "degrading" for endocannabinoids and "biosynthetic" for other mediators. Finally, some of these enzymes may also have additional completely different functions, for example participate in the chemical modification of molecules that have very little to do with endocannabinoid and cannabinoid targets.

As a result of the above reasoning, some authors now use an extended definition of the endocannabinoid system, such the "enlarged endocannabinoid system". For the sake of clarity a new term was introduced the "endocannabinoidome" and represents the ensemble of endocannabinoids, endocannabinoid-like mediators, and their several receptors and metabolic enzymes.

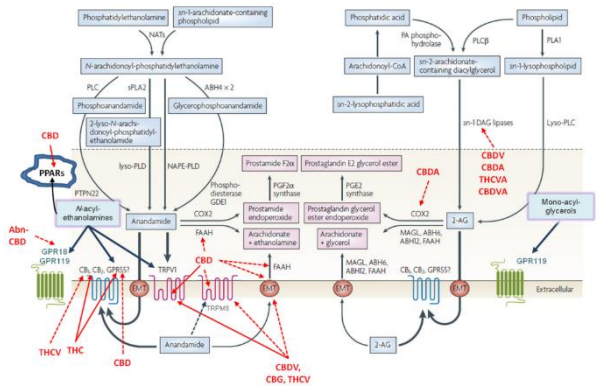

Figure 10: The endocannabinoidome (source: https://www.semanticscholar.org/paper/The-Endocannabinoid-System-and-its-Modulation-by-Marzo-Piscitelli/a5b336dc28cb961a9375d03b57e10c1ff37241de)

3. The physiological role of the ECS

So all in all the role of the endocannabinoids system is very complex. It affects the majority of the systems in our bodies and the cannabinoid receptors are expressed (in different density) on majority of cell types. So describing what exactly it does, is not an easy task, as it regulates the biochemistry of vast majority of 37 trillion cells in our body. Research has shown that the endocannabinoids system functions as an SOS mechanism that is activated whenever our bodies are out of balance for whatever reason. So example it is activated when we suffer from a physical injury, when we encounter pathologic microbes and also when we feel emotional pain or are under stress.

It seems that the ECS serves as a general protective mechanism, starting at the cellular level, proceeding to the tissues, organs, body and our general well-being. The ECS's salient homeostatic roles have been summarized as, "relax, eat, sleep, forget, and protect". It is known to modulate embryological development, neural plasticity, neuroprotection, immunity and inflammation, apoptosis and carcinogenesis, pain and emotional memory, and most importantly from the viewpoint of recent drug development: hunger, feeding, and metabolism. It is well worth noting that human breast milk contains significant amounts of endocannabinoids (less of AEA and high levels of 2-AG). The oral administration of endocannabinoids produces calming properties. Experiments with suckling models in mice showed that when newborn mice are fed the CB1 antagonist (SR141716A), they stop suckling and die. So the disruption of the ECS in the first 24 hours after birth seems to be lethal.

Albeit the cells seem like very dynamic structures, with many 1000 biochemical reactions happening simultaneously, all the parameters are very tightly regulated. From temperature, to osmotic pressure, pH level, redox potential, concentration of ions, nutrients, enzymes… So it's a dynamic structure, but within strict limits. For example when the intracellular pH value changes by 0,1 pH unit many cellular processes are activated to return the value to optimum. This a termed cellular homeostasis. This is the tendency of cells or organisms to auto-regulate and maintain their internal environment in a stable state. The stable condition is the condition of optimal functioning for the organism. It is brought about by a natural resistance to change in the optimal conditions and homeostasis or equilibrium is maintained by many regulatory mechanisms, some general (like the ECS) and some very specific.

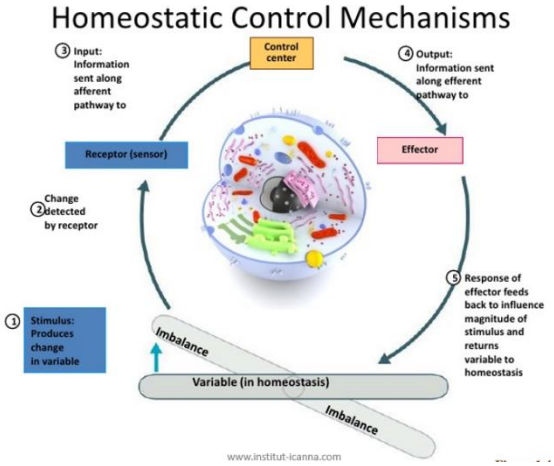

Figure 11: The cycle of homeostatic control mechanisms (source: https://antaology.wordpress.com/2012/10/08/what-is-homeostasis/)

So starting from a cellular level, the ECS is a protective mechanism that is turned on, like a SOS mechanism, when the cellular homeostasis is out of balance. It is like the first line of defense to go off, activating all other mechanisms needed to return to homeostasis as soon as possible.

Living in today's modern society is giving many challenges to our endocannabinoids system and leading to exhaustion of the supply of endocannabinoids. If we take a look at one ordinary day, getting up, getting ourselves and kids ready for work and school, all in a time stress, being in traffic, responsible and stressful jobs, challenging relationships, toxic environment, contaminated food, water and air…it is obvious that in one ordinary day our ECS is facing more challenges then it would in a month or longer even 100 years ago. If our endocannabinoid system is constantly challenged over a longer period of time, this vital SOS mechanism starts to dysfunction. It can dysfunction either not producing endocannabinoids when we need then, or producing endocannabinoids when we do not need them. This is usually one of the first steps in the development of chronic disease, the first dominoe dice in a complex dominoe structure to fall, leading to symptoms and disease development. And in such cases, where the ECS is malfunctioning, use implementation of exogenous cannabinoids can be very beneficial.

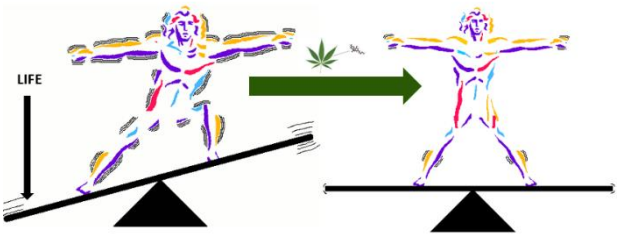

Figure 12: Representation of homeostatic mechanism and the role of cannabinoids (source: Institute ICANNA)

Some emerging literature documents the "ECS deficiency syndrome" as an etiology in migraine, fibromyalgia, irritable bowel syndrome, psychological disorders, and other conditions. The theory of clinical endocannabinoid deficiency (CED) was presented in 2001. The theory of CED was based on the concept that many brain disorders are associated with neurotransmitter deficiencies, affecting acetylcholine in Alzheimer's disease, dopamine in parkinsonian syndromes, serotonin and norepinephrine in depression, and that a comparable deficiency in endocannabinoid levels might be manifest similarly in certain disorders that display predictable clinical features as sequelae of this deficiency.

All humans possess an underlying endocannabinoid tone that reflects of levels of anandamide (AEA) and 2-arachidonoylglycerol (2-AG), the centrally acting endocannabinoids, their synthesis, catabolism, and the relative density of cannabinoid receptors in the brain. If endocannabinoid function were decreased, it follows that a lowered pain threshold would be operative, along with derangements of digestion, mood, and sleep among the almost universal physiological systems subserved by the endocannabinoid system The CED theory also posits that such deficiencies could arise due to genetic or congenital reasons or be acquired due to intercurrent injury or disease that consequently produces characteristic pathophysiological syndromes with particular symptomatology. The greatest evidence for CED is present for migraine, fibromyalgia, and irritable bowel syndrome (IBS).

Other diseases are also associated with suboptimal functioning of the ECS, Fride speculated that a dysfunctional ECS in infants contributes to "failure to thrive" syndrome. Hill and Gorzalka hypothesized that deficient ECS signaling could be involved in the pathogenesis of depressive illnesses. In human studies ECS deficiencies have been implicated in uncompensated schizophrenia, migraine, multiple sclerosis, Huntington's, uncompensated Parkinson's, irritable bowel syndrome, uncompensated anorexia, and chronic motion sickness.

Conclusions

There is a large body of evidence that the endocannabinoids regulate mood, emotion, motivation, memory, pleasure perception, appetite, metabolism and more. The connection between cannabinoids and health/disease has long been empirically and scientifically established. Once we understand the functioning and the role of the unique system in our bodies, we need to employ action to nourish and support the function of this system and start with cannabinoid intervention in situations where it is evident that the ECS is no longer performing its protective function. It is high time for medicine worldwide to catch up with research findings and patient's demands and harness the effects of cannabinoids to support or restore the cellular biochemical homeostasis, which serves as the cornerstone of health.

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