Advances in Biochemistry & Applications in Medicine

Chapter 8

Obesity and Endocannabinoids

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1. Introduction

1.1. Obesity and its complications

In the modern-day world, obesity and its associated metabolic disorders like Type 2 Diabetes (T2DM) and other metabolic syndromes have skyrocketed and pose a serious global public health concern as seen in Fig 1. In United States, two thirds of the population are obese [1,2]. The problem of obesity not only exists in prosperous countries but is also present in developing countries like Mexico, China and Thailand [3] and hence serious interventions are required to solve this problem that exists across the world. According to National Institute of Health (NIH), obesity is complex and multifactorial condition. It is also considered as a condition of excess energy stores [4] (NIH). According to the definition of World Health Organization (WHO); in adults, "overweight" is defined as Body Mass Index (BMI) between 25-29.9 while "obesity" is defined by BMI greater than 30kg/m^2 [5]. BMI is defined as persons weight divided by his or her height in meters squared. It is known to correlate with percentage body fat in human subjects [6,7], however sometimes not considered a sufficient parameter [8]. Often waist circumference is also considered as a marker for obesity. There are several ways obesity can affect health. These complications include T2DM [9], Non -Insulin dependent Diabetes Mellitus (NIDDM) [10], hypertension [11], heart disease [12], dyslipidemia [13], osteoarthritis [14], high blood pressure [15], etc. A study [13] showed the distribution of obese individual affected in different diseases. The prevalence of dyslipidemia, hypertension and diabetes are profoundly correlated to obesity. Obesity is caused by the increase in the number and size of fat cells or by the dysfunction of adipose tissue which in turn lead to metabolic disease. Some of the important factors that lead to this pathophysiologic state are modern day sedentary lifestyles, environmental factors, easily available packaged foods, use of cheap soybean oil for food preparation. The metabolic dysfunction leads to altered uptake of nutrients and stor-

age of the same. Weight management often reduces the risk of T2DM by increasing insulin sensitivity [16]. It is also known to correct abnormalities in NIDDM [17,18]. However, there are no safe pharmacological therapies for the treatment of obesity so far. The development of effective therapies will be of priorities for the health systems. Among different targets for anti-obesity drugs, endocannabinoids remain at attention. This is because, endocannabinoids (ECs) are known to play a crucial role in the host, as in addition to act as neuromodulator, ECs elicit role in energy homeostasis [19,20], cardiovascular function [21] etc. There are evidences that dysregulated endocannabinoid system play a major regulatory role in energy balance by affecting both central and peripheral nervous systems [20].

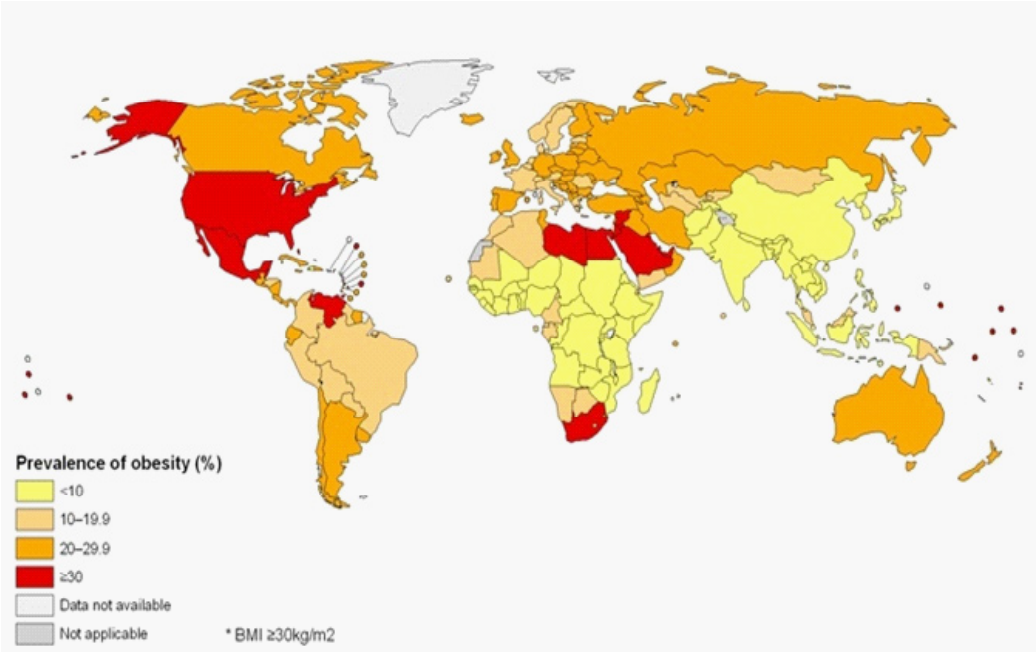

Figure 1: Prevalence of obesity across the world (Source: WHO)

2. The Endocannabinoid system: Overview

2.1 History of cannabinoids

Long back, *Cannabis Sativa*, a herbaceous flowering plant was used as medicine for nausea from arthritic pain, epilepsy etc. *Cannabis Sativa* has originated in Neolithic China. It contains 400 chemicals, 60 of them being cannabinoids [22]. The mechanism of action of cannabis could be known recently because the active compound was isolated, purified and characterized chemically. Cannabinol (CBN) was the first of the cannabinoids to be isolated from red oil extracts of cannabis. Elucidation of its structure was performed in 1930s and it was synthesized in the year 1940. A second cannabinoid, Cannabidiol (CBD) was isolated by Thomas Wood and later its structure was solved by Robert Cahn, Lord Allan Todd [23] and simultaneously by Roger Adams [24]. Although CBD was not the most pharmacologically active compound of cannabis, it led to the discovery of other active compounds present in Cannabis. Later, active compound from marijuana was extracted and named as Δ^9 Tetrahydrocannabinol also known as Δ^9-THC. Both Cannabidiol as well as THC are naturally present

as acids; however, they are decarboxylated when cannabis is heated. Both these compounds are naturally present as (-) enantiomers. Later both (+) and (-) enantiomers were synthesized chemically. The structures of these compounds are elucidated in **Figure 2**.

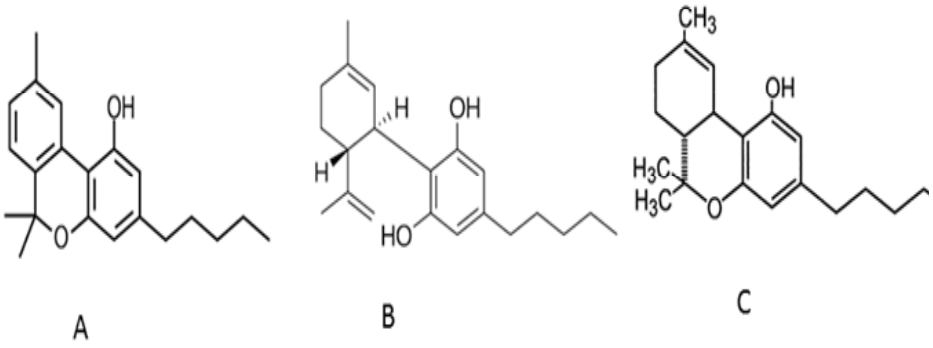

Figure 2: Structures of cannabinoids (A) Cannabinol (B) Cannabidol (C) Δ^9- THC

In mid 1960s and 1970s, several research were performed to understand the pharmacology of cannabinoids. At that time, therapeutic value of these drugs was unknown and recreational value of the drug was widespread. Many experiments were performed in animals and human beings to understand if the psychotropic properties of cannabis could be attributed to Δ^9-THC and the results obtained from those studies were positive in this regard. In one of such studies, it was observed that Δ^9-THC caused "static ataxia" when introduced in dogs. In rodents, cannabis and Δ^9-THC caused immobility index to rise.

Initially the term "Cannabinoids" was used to suggest the C21 compound present in Canabis Sativa. Later the term was used for any compounds that showed pharmacological activity similar to Δ^9 -THC. It was proposed that their membrane fluidity makes them to interfere with the membranes and not to specific receptors. However various groups suggested the binding of the cannabinoids to be stereoselective which kept the search for cannabinoid receptors to be active [25] in the past years. In addition, the binding of THC stereoisomers was studied in different experimental animals and it was concluded that their potencies differed across animal background [26,27]. It took long time to understand the binding site of cannabinoids. Allyn Howlett provided the proof for binding site of cannabinoids as cannabinoid receptors [28]. Her work suggested that the cannabinoids activate G protein coupled receptors which in turn inhibit adenyl cyclase. Studies relating the cellular effects of the synthetic cannabinoids, revealed that they inhibit cAMP production and that they mediate via cell membrane [29].This result is also concluded from numerous studies on the role of cannabinoids in the modulation of cAMP levels in cell cultures, brain homogenates and in *in-vivo*. Initial work indicated that cAMP is altered in biphasic manner in brain. This was because, at lower dose of cannabinoids there was an increase in the level of cAMP while at higher dose there was decrease in the cAMP levels. This study correlated with the initial stimulatory effect of low doses of cannabinoids but depressant effect at the high levels of the same [30]. A second major advance in this field was again made by Allyn Howlett in collaboration with Bill Devane. This was possible

because of the presence of the technique that can detect the binding site of the receptor using radiolabeled ligand and labelled Tritium cannabinoid, CP55940. There was evidence of high affinity binding sites for this in rat brain. Moreover, the ability to displace the labelled by the unlabeled cannabinoids from the binding sites in addition to inhibition of adenyl cyclase was also concluded from their work. It was thus certain that cannabinoids acted via receptor and the receptor was G protein coupled receptor. The cannabinoid receptors were first cloned from rat brain. Later they were cloned from humans, fish, mouse etc. The CB1 receptors are also known to activate mitogen activated protein kinase (MAPK), inhibit voltage activated Ca^{2+} channels and activate K^{+} channels.

2.2 Endocannabinoid System

The endocannabinoid system consists of endocannabinoids, cannabinoid receptors and the enzymes responsible for their degradation [28] (**Figure 3**). This system has been preserved across the species and is selected by the evolution to maximize energy intake and conservation [31,32].

Two more splice variants of CB1 (Cb1b and Cb1a) have been identified in human brain [33,34]. Although these receptors are located in the brain, they are also known to be present in various peripheral tissues like pancreas [35], liver [19] and skeletal muscles [36]. The predominant expression of cannabinoids are the full length CB1 with low expression of CB1a and CB1b in brain [34]. Contrary to this, Cb1b is the major isoform in the liver with 10 fold more expression [34]. The isoforms were different in their N terminal sequence and hence are different in their pharmacological behavior. The isoforms could be detected in hepatocytes and beta cells using modern proteomic approach [37]. Several research also focused in the search of endogenous cannabinoid agonist. Such compound was first isolated from rat brain mem-

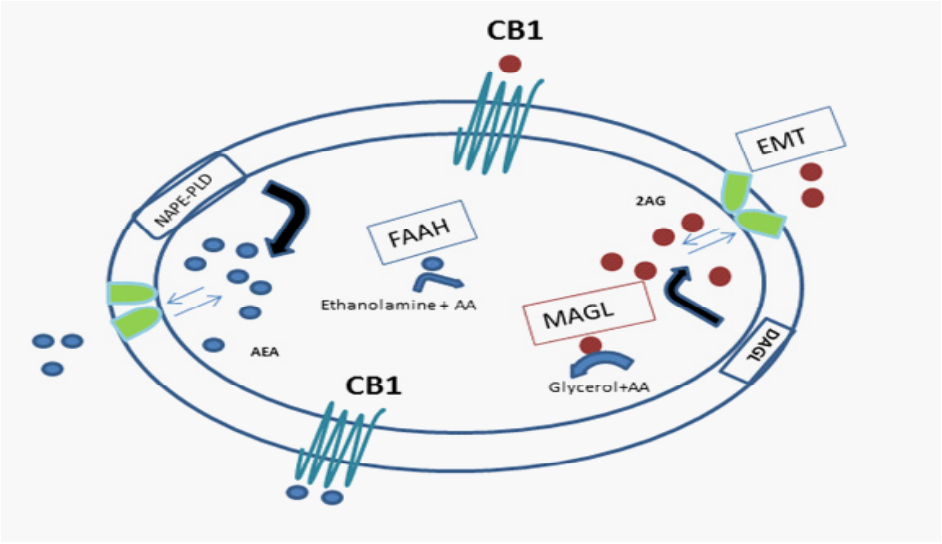

Figure 3: Components of Endocannabinoid system. The biosynthesis of AEA (blue circles) is catalyzed in presence of NAPE-PLD. The biosynthesis of 2AG (orange circles) is catalyzed by MAGL. Endocannabinoids are transported by EMTS on both the direction of cell membrane (EMT stand for Endocannabinoid membrane transporter). FAAH hydrolyze AEA while MAGL hydrolyze 2AG. Adapted from [38].

branes. The molecule was lipophilic and also displaced 3H-HU243. The first endocannabinoid to be discovered was Anadamide (AEA) which was derived from Sanskrit word "Ananda" meaning bliss. Later Anandamide and 2-Arachidonoyl Glycerol (2AG) were identified as the ligands for cannabinoid receptors [39,40] (Fig 4). In the brain, endocannabinoids function to decrease neurotransmitter release at CB1 terminal.Unlike CB1,CB2 is present in the immune cells [41], NK cells. However they are recently also found in brainstem [42] and cerebellar granule cells.

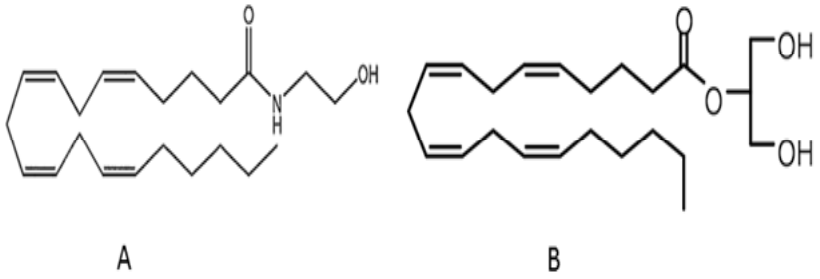

Figure 4: Chemical Structure of Endocannabinoids (A) AEA (B) 2AG

2.3 Endocannabinoids

The term "endocannabinoids" was derived from the name endogenous cannabinoids [43]. They can work as autocrine and paracrine manner on the cannabinoid receptors. The synthesis, transport and degradation of the endocannabinoids are performed on demand. They are not stored in advance for the future use [44]. Thus, the concentration of endocannabinoids varies with respect to energy requirements in the body. It increases during fasting and decreases after refeeding [45]. The synthesis of ECs depends on intracellular Ca^{2+} ions [46,47] and they are derived from arachidonic acid. AEA elicit neuroprotective and immunosuppressive role both by cannabinoid receptor dependent and independent manner. AEA is formed by the hydrolysis of NAPE (N arachidonoyl phosphatidyl ethanolamine) [48] in presence of Phsopholipase D (NAPE-PLD). The hydrolysis of phsopho diester bond of NAPE is brought about in presence of NAPE-PLD which was uncharacterized till recently. It is a member of zinc –metallo hydrolase enzymes. The NAPE precursors of AEA is produced in presence of trans acylase enzyme. This enzyme, catalyzes the transfer of acyl group from sn-1 position of phospholipids to nitrogen of phosphatidylethanolamine. A second pathway is also operative in the brain in which Phospholipase C catalyzes NAPE to phosphorylated AEA which further undergoes de-phosphorylation to form AEA [49]. There are two other pathways for the formation of AEA. The pathways are summarized in Figure 5. 2 AG (**Figure 4**) is mainly formed by the catalytic action of diacylglycerol lipase (DAGL) from arachidonate containing diacylglycerol. Two different isoforms of DAGL are present namely DAGL α and DAGL β. However, another route of synthesis of 2AG is also known. The pathways are summarized in **Figure 6**.

Both AEA and 2AG are removed from the extracellular space by cellular uptake. The

transport of AEA to intracellular space may be facilitated by FAAH like AEA transporter (FLAT), however this finding is controversial. The degrading enzymes for AEA and 2AG are also characterized. They major ones are Fatty acid amide hydrolase (FAAH) and Monoacylglycerol lipase (MAGL) respectively. AEA is also degraded by other enzymes like 12-Lipoxygenase, 15-Lipoxygenase, CYP2B, CYP2D, CYP4F and COX2. AEA and 2AG can also bind to non-cannabinoids such as "Transient receptor potential vanilloid 1" (TRPV1), the activation of which opposes CB1 receptor [51].

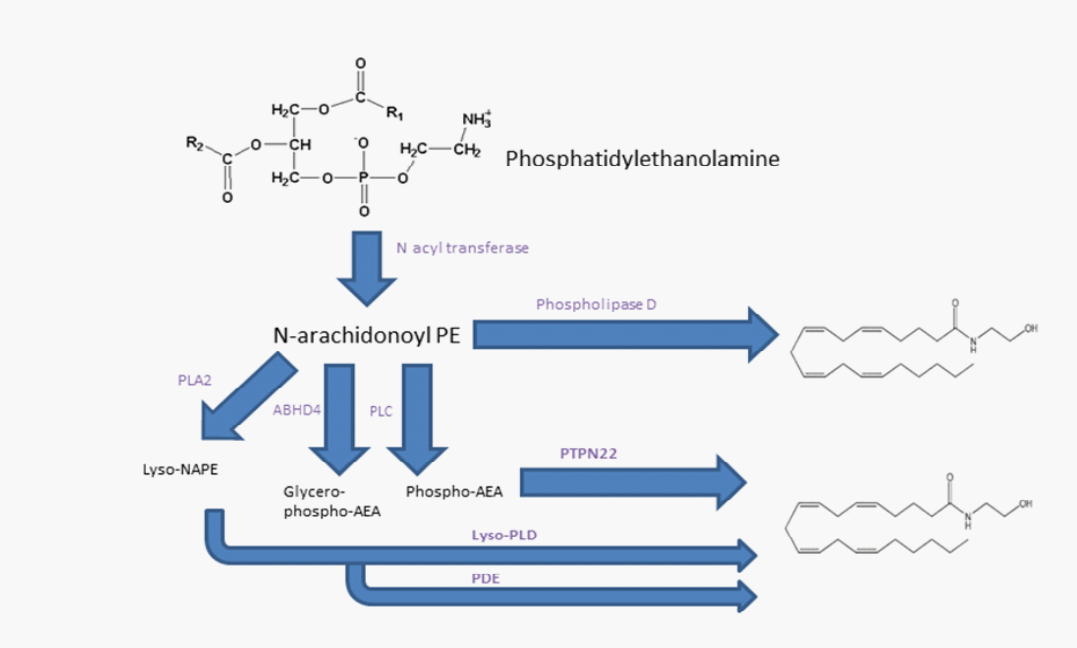

Figure 5: Anandamide biosynthesis pathway. There are four different pathways for the formation of AEA. PlA2 is abbreviated from Phospholipase A2 while PDE is abbreviated from Phosphodiesterase. This is adapted from [50]

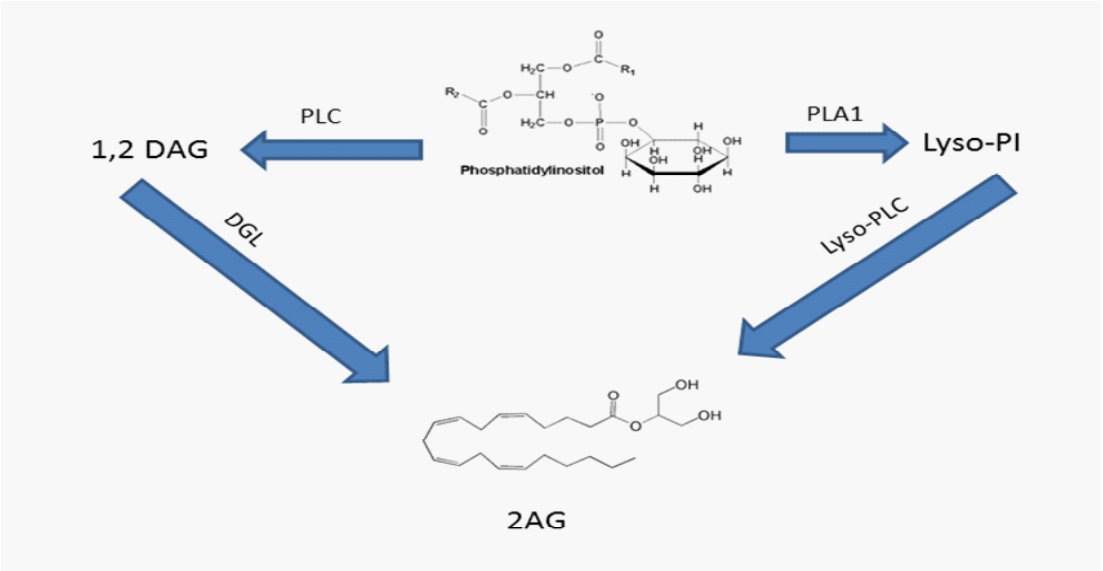

Figure 6: Biosynthetic pathway of 2AG. The abbreviations are used as follows. PLC- Phospholipase C, DGL –Diacylglycerol Lipase, PLA1- Phospholipase A1, Lyso-PI-Lysophospholipid, Lyso-PLC- Lisophospholipase C. This is adapted from [52].

In addition to AEA and 2AG, many EC like molecules have been discovered, but their functions are not completely known yet. For example anti-inflammatory lipid, lipoxin A4 could be the endogenous allosteric enhancer of CB1 [53,54]. More studies are required in this direction.

Within CNS, the endocannabinoids work in the retrograde manner to the cannabinoid receptor in the presynaptic neurons leading to suppression of release of neurotransmitter [55]. Other than CB1, the next prevalent cannabinoid receptor that exists is CB2. Although there exists 44% homology between CB1 and CB2 [56] the ligands for CB1 and CB2 are similar. This could be because of 68% identity in the binding domain of CB1 and CB2. However, the affinity of the endocannabinoids towards the cannabinoid receptors are not similar. While AEA exhibits higher affinity towards CB1R compared to CB2R, 2AG has similar affinity towards both the receptors. One of the challenges in the study of endocannabinoids is their measurement. This is because of rapid degradation and isomerization of 2AG. The sampling treatments are crucial for the assay of endocannabinoids because their half - life is in the order of minutes. Thus, for the measurement of endocannabinoids in blood, blood collection should be performed in ice, centrifuged immediately and kept in -80^0C until the analysis.

2.4. Role of age and sex in the modulation of ECs

Endocannabinoid level exhibit variations in sex and age. A study in 2006, suggested AEA level to be different in men and in women, but no difference was exhibited in the level of 2AG, however it was correlated to visceral fat. Moreover only 2AG was correlated to age [57]. In a different study no difference was observed in AEA levels in different sex while 2AG was shown to be different [58]. In addition, 2AG was also shown to be correlated with age only in women. Also, AEA correlated with BMI, waist circumference and fasting insulin. 2AG exhibited correlation with triglycerides irrespective of sex. The discrepancy of the results in two studies could be due to the variations, selection of the cohort. While in the first study obese individuals were recruited, in the second one, normal individuals are studied. Although no concrete conclusions could be drawn from these studies, it pointed out that age, as well as sex might have potential role to play in ECs levels which could be due to role of gonadal hormones in the modulation of ECS [59]. However more investigations are required in this front.

3. Endocannabinoids and Eating Disorder

Cannabis can induce weight gain and is known to occur by the stimulation of central nervous system(CNS). The first report of increase in appetite was reported in AD 300. Smoking cannabis in patients with the history of HIV was shown to modulate leptin and ghrelin but not insulin levels demonstrating their effect on hormones [60]. They were also prescribed as appetite enhancing medicine in patients with AIDS and cancer [61,18].

Endocannabinoids mediate eating disorder by the activation of the cannabinoid receptors, CB1 which are present in central [57] as well as in peripheral nervous systems [62]. Elevated levels of AEA and 2AG have been reported in different studies in obesity. Administration of AEA in ventromedial hypothalamus or systemically, induces hyperphagia. Like AEA, 2AG also evoke increase in feeding behavior when injected systemically or to lateral

hypothalamus. It is known that both endogenous and exogenous administration of cannabinoids in rats increases feeding [63,64]. The key area in brain responsible for the motivation of feeding is hypothalamus. CB1 receptors present in hypothalamus are responsible for food intake behavior. In one of the studies, it has been shown that administration of endocannabinoids in nucleus accumbens (NAc) increases intake of sucrose solution in rats [65]. In addition, administration of Δ^9 tetrahydrocannabidol increases hedonic taste response after sucrose administration by releasing dopamine in NAc. Administration of one of the selective CB1 antagonist, Rimonabant reduces food intake suggesting a role of CB1 in the energy intake because dopamine release was prevented in NAc [66]. Thus, intake of palatable food is associated with increase in dopamine in the brain. This mechanism has been attributed to the activation of dopaminergic neurons in ventral tegmental area in the brain. This is achieved by the activation of CB1 receptors by endocannabinoids in glutamatergic neurons which in turn inhibit GABA-ergic neurons that project from NAc to ventral tegmental area thus disinhibiting dopaminergic neurons in ventral tegmental area [67]. Besides, modulation of ECS leads to change in level of neuropeptide hormones that are responsible for the signaling of appetite. Administration of Rimonabant has reduced expression of neuropeptide Y and increased in the expression of other anorexigenic peptides such as CART and αMSH levels in hypothalamus. Thus, increase in the food consumption is brought by increasing the motivation associated with the food intake. Several experiments by different groups were performed on role of CB1 in food intake. It can be further noted that the mice with CB1 receptor knocked out ate less than their wild type littermates. While food intake in the CB1 knock out (CB1KO) mice is independent of Rimonabant dose, it reduced food intake in the wild type mice. Apart from cannabinoid receptors, ECS are also known to exhibit their properties by other G protein coupled receptors like GPR55, GPR18, CB2 etc. The role of CB2 in this context has also been studied by two different groups. It has been shown that mice deficient in CB2 exhibited hyperphagia and administration of CB2 selective agonists increased food intake [68]. Moreover, selective over expression of CB2 in brain led to decrease in feeding that is induced by fasting and hence lean phenotype [69,70]. These receptors have opposing effects to CB1 in the chemically induced liver damage. It has been demonstrated that CB1 antagonist and CB2 agonist protect against liver injury [71]. The role of CB1 and CB2 could be opposing in nature and more experiments are required to understand the role of each receptors in context of energy metabolism. Both the cannabinoid receptors (CB1 and CB2) are present in pancreas although there remain discrepancies about the exact location of CB1 and CB2. While CB1 receptors are mostly present in α and β cells, CB2 is present mostly in δ cells. High fat diet increase in the concentration of AEA and 2AG in the whole pancreas, however loss of islets in diabetic mice did not alter the endocannabinoids level in pancreas. *In vitro* experiments conclude that stimulation of CB1R enhance secretion of insulin and glucagon but stimulation of CB2R lowers glucose dependent insulin secretion [72,73]. In a different study it was demonstarted that Rimonabant was useful to prevent the islet loss as well as weight of pancreas in obese Zucker rats along with proper

renal function and hence led to decreased mortality [74]. Under hyperglycemia, AEA and 2AG are dysregulated in pancreas. RIN-m5F β cells, known as a model of pancreatic islets β cells when kept in low glucose medium show low ECs. In addition, it did not show glucose induced rise in ECs when co-stimulated with insulin [75,73]. Thus, pancreatic ECs also play a role in energy metabolism.

Along with food intake, ECs also modulate smell and taste that guide the organism towards the food [76]. In this context, it is important to note that malfunctioning of olfaction is known to occur in obesity in various organisms. Hunger is known to stimulate 2AG in olfactory epithelium. In mice, olfactory neuronal circuits are modulated by ECS [77]. Also, CB1R in the mouse taste cells co-localize with sweet receptor component, T1r3 and the neural response to the sweet taste is enhanced by endocannabinoid signaling. Taste related signal is processed in the hindbrain specially in parabrachial nucleus and nucleus of solitary tract which also receive information from the gastrointestenial tract, and thus affect the meal size [78,79]. Endocannabinoids in parabrachial nucleus thus enhance the intake of palatable food by acting through CB1. An interesting fact regarding olfaction and fat ingestion is that, the oral cavity leads to the production of endocannabinoids in the gastrointestinal tract by efferent vagal signaling which in turn also leads to further fat intake [80,31]. Endocannabinoids in the gut are known to modulate food intake and hunger signals and are known to vary under fasting and satiety [81]. Their concentration rises during fasting and fall after feeding. CB1R expression in gut is modulated by cholecystokinin, a hormone secreted by gut to induce satiating effect [82]. Some studies suggest that orosensory properties of AEA and 2AG lead to increase in fat intake when present in high concentration while their reduction lead to meal termination. In addition, food intake is also modulated by CB1 signaling in olfactory bulb. Besides, in recent times, a link between ECS and cephalic phase response has been established. The role of ECS in cephalic phase response is well established in sham feeding model. In this model it has been established that gut derived endocannabinoids are responsible for fat intake based on its orosensory properties [80,83]. Gut endocannabinoids have been identified to participate in positive feedback mechanism of fat ingestion. It has been suggested by other studies that oral exposure to fat cause the release of dopamine in ventral striatum [84] which is a hub for evaluating rewarding sensory stimuli [85]. This also prove the cephalic phase response to the fat rich foods. Blockade of CB1 in gut just before sham feeding lowers food intake [80]. CB1 in the gut cells also induces ghrelin which in turn can increase fat taste perception [86]. In addition to this, there exists literature that gastrointestinal tone is controlled by gut microbiota. However the connection between microbiota and ECS level is yet to be studied. Apart from digestion, gut also functions to convey satiety function.

Besides, CB1 receptor is also found in the fundus of stomach, although cellular localizations are not particularly known. A small dose of Rimonabant was able to reduce the effect of

ghrelin [87], the production of which takes place in gastric endocrine (X-) cells [87,88]. Thus, stomach has also a role to play in energy metabolism.

Another molecule known to regulate glucose homeostasis is adiponectin [89]. In obese animals it exhibits improvement in hyperglycemia, insulin resistance [90] etc. It has been suggested by various studies that Rimonabant exhibit its effect on adiponectin. *In- vitro* studies have suggested that Rimonabant increases adiponectin, while activation of CB1 inhibits adiponectin concentration [91]. This is further supported by *in-vivo* experiments in Zucker rats. Further in CB1KO mice, Rimonabant had no effect in adiponectin mRNA levels in adipose tissue. This suggests that Rimonabant affects adiponectin in a CB1 dependent manner.

Although endocannabinoids are well studied for their role as neuromodulators very less is known about their biological functions. In different studies, the concentration of endocannabinoids is shown to be positively correlated to BMI, waist circumference etc. However, whether that results from the spillover from the tissues or if its bears a biological relevance to obesity is yet to be understood [92].

4. Dietary Long Chain PUFA Disrupts ECS Tone in Centrally and Peripherally and Results in Obesity

In the modern time, diet induced obesity (DIO) is prevalent in western countries and is a good model to study obesity in humans. Some of the constituents in the high fat diet is known to modulate ECS system and hence play a role in obesity. High brain concentration of AEA was observed in piglets fed with arachidonic acid (20:4n-6) [93]. It has been proposed in an epidemiological study that an increase in the prevalence in obesity was due to increase in arachidonic acid pool due to intake in linoleic acid which in turn lead to increase in 2AG concentration. A study showed that obesity is directly linked to consumption of soybean oil which has high content of linoleic acid [94]. Moreover, increase in dietary linoleic acid from 1% to 8% cause increase in weight gain, arachidonic acid phospholipid (ARA-PL); AEA and 2AG. Also, ARA-PL pool is decreased by increase in Eicosapentanoic acid (EPA) and Docosahexanoic acid (DHA) which also reduced the obesogenic effect of linoleic acid in rodents by reducing the stimulation of endocannabinoid system. In addition to this, in a separate study, it has been shown that a low fat diet could be made obesogenic by increasing the concentration of linoleic acid [95]. Thus, excess in the EC activity is associated with obesity. In a separate study, by a different group, DIO was shown to lower CB1 density in the extrahypothalamic regions such as hippocampus, nucleus accumbens etc. It was hypothesized that increase in endocannabinoids has resulted in lowering density of CB1 [96] in these regions.

CB1KO mice were resistant to DIO and have decreased weight gain [19]. The mice with this phenotype also have reduced feed efficiency [97] and have reduced leptin, triglycerides, insulin and increased adiponectin, suggesting improved lipid metabolism and hormone

sensitivities [98,99]. Mice with selective CB1 knock out in forebrain and sympathetic nervous systems are also resistant to DIO because they display thermogenesis, lipid oxidation and decrease in energy absorption [100]. Besides, virally mediated CB1R mRNA knockout in mice led to decrease in body weight gain and increase in energy expenditure [101]. It is also been noted that deletion of CB1 receptor from Sim-1 expressing neurons protect mice against DIO by increasing the expression of thermogenic genes in white adipose tissue [102,45].

The peripheral EC system is also stimulated in obesity in humans. In a study, DIO was associated with increase in the size of adipocytes. It is important to note that adipocytes from humans as well as rodents are known to express all the components of ECS including CB1 and CB2 [57]. Also human adipocytes metabolize and bind to 2AG and AEA [103,104]. Lipogenesis is mediated by AEA and stimulation of CB1 in adipocytes. AEA is also known to activate peroxisome proliferator activated receptor γ (PPARγ) inducing differentiation of adipocytes [105]. It has been shown that activation of CB1R in obese mice lead to decrease in mitochondrial biogenesis in white adipose tissue [106]. The study also demonstrate the positive effect of Rimonabant in lipolysis and hence reduction in fat mass [107,69]. In addition, administration of Rimonabant in wild type mice has led to induction of certain genes which are involved in β oxidation and mitochondrial biogenesis. The study suggests that blocking CB1R, improves mitochondrial oxidative capacity and hydrolysis of triglycerides in adipocyte. Several other studies also indicate that obesity induced inflammation in adipose tissues. It has been shown by a research group that inhibiting CB1R attenuate LPS induced pro-inflammatory cytokines like Interleukin-6(IL-6), Tumor necrosis factor-α, (TNFα), in human adipocytes [108]. Similar result is obtained by a different group which showed decrease in circulatory cytokines in obese Zucker rats by Rimonabant [109]. CB1 protein expression in adipocytes was seen to increase while exercise training reduced the effects of DIO in subcutaneous and visceral adipose tissues. The authors found that high fat feeding decreased while exercise increased the protein expression of PPARδ which in turn inhibited CB1 expressions. This study suggested a new regulatory pathways towards expression of CB1 [110]. Along with CB1, CB2 also play an important role in inflammation in adipocytes. *In vivo* administration of selective agonist of CB2; JWH-133 led to increase in inflammatory genes in mice under both normal and high fat diet. As an extension, CB2R KO mice were resistant to inflammation. Also administration of CB2R antagonists; AM630 in ob/ob mice led to reduced adipose tissue inflammation [111]. It must be noted that the role of CB2R in adipocytes are quite contrary with respect to other tissues, where stimulation of this receptor in other tissues led to attenuate inflammation [112,69].

High fat diet is also associated with increased expression of CB1 in liver. There is an elevation of AEA in liver after high fat diet along with decrease in activity of FAAH [13]. Both increase in synthesis as well decrease in degradation resulted in activation of ECs. In rodents, stimulation of CB1R in the hepatocytes results in expression of lipogenic transcription fac-

tor SREBP-1c [113], Acetyl coenzyme carboxylase-1(ACC1), and Fatty acid synthase (FAS) which in turn leads to de novo lipogenesis [19]. Lipogenesis pathway can be activated by AEA which could result in DIO. Treatment of isolated primary hepatocytes with 2AG cause increase in gluconeogenic gene expression as well as hepatic glucose. This effect was attenuated by application of Rimonabant thus explaining the role of CB1 in hepatic gluconeogenesis [114]. Mice deficient in FAAH (AEA degrading enzyme) have elevated fasting glucose although having elevated fasting plasma insulin levels [115]. This result suggests the unfavorable effect of ECs activation in host under high fat diet.

Both AEA and 2AG levels are increased in diabetic patients [116]. Some studies have elucidated whether ECS is correlated to visceral fats as it is regarded as hallmark of obesity. Studies show that there is negative correlation between plasma 2AG levels and insulin sensitivity independent of body mass. In humans, plasma 2AG concentrations is correlated to visceral fat but there is no difference in 2AG level between the lean and the obese subjects [57,117]. However, in both these studies it was shown that 2AG level was correlated to decrease in insulin sensitivity, increase in free fatty acids, triglycerides, cholesterol etc. CB1 receptor is also known to be dysregulated in white adipose tissue (WAT) in humans in DIO. However, the nature of dysregulation is controversial as one group report CB1 to increase in WAT in obese condition, other suggested the opposite. FAAH is known to be lowered in subcutaneous WAT in obese individuals [57]. Also in visceral fat mass, FAAH expression was negatively correlated to circulating 2AG [57,73]. An interesting data from morbid obese subjects indicate increased level of 2AG in visceral fat (and not subcutaneous) compared to controls. The enzyme levels associated with the formation and degradation of 2AG was not seen to be different in the adipose tissue. The authors predicted that elevated fatty acids in the diet to be the reason for increased bioavailability of 2AG [118].

It has also been argued in literature that cannabinoid receptors and the related enzymes undergo site specific perturbation. In a study, while the enzymes involved in the endocannabinoid pathway were shown to be decreased in gluteal subcutaneous adipose tissue in the obese individuals, the same individuals exhibited elevated abdominal fat [119]. This results shed light on the role of peripheral endocannabinoids in obesity. An important aspect to note in this regard is that, although ECs are correlated to weight gain, a decrease in weight gain by exercise and diet does not lead to decrease in EC concentration. In two independent studies it was shown that loss in weight in the obese individuals did not result in decrease in circulating endocannabinoids, although the metabolic parameters were improved [20,120]. This study was followed by a different study in which there was intervention in the physical activity and healthy eating which led to decrease in plasma AEA and 2AG in men after one year. The decrease in 2AG levels was correlated to decrease in visceral fats, decrease in triglycerides and increase in HDL cholesterol levels [121]. The different results obtained from different studies

could be because of different diet being used in different studies to reach the weight loss goal and also different time frame being used. In addition to liver, fats and plasma, perturbation of endocannabinoids also exists in other biofluids and tissues. In a study it was shown that intervention in the lifestyle leads to change in salivary content of AEA while no change in 2AG [122]. Apart from liver, pancreas, CB1 is also present in skeletal muscle [123]. Impaired glucose utilization by skeletal muscle led to insulin resistance. It is known that ob/ob mice exhibited insulin resistance along with hyperglycemia and hyperinsulemia however treatment with Rimonabant leads to proper glucose uptake in isolated soleus muscle preparation [124]. An *in-vitro* model of skeletal muscle, E6 cells are known to modulate glucose uptake by ECS at the level of PI3 Kinase leading to change in the activity of downstream PI3Kinse; like Protein kinase B, Pyruvate dehydrogenase, Protein kinase C, although protein level expression of glucose transporter like Glut1 and Glut4 were not affected by ECs [125,73]. *In-vitro* studies have also suggested that CB1 antagonist AM251elevated the level of AMP activated protein kinase (AMPKα1) in myotubes of both lean and obese individuals which in turn lead to fatty acid oxidation [123]. CB2R deficient obese mice, exhibited elevated insulin mediated glucose uptake in skeletal muscle relative to wild type mice indicating that CB2R also has role to play in insulin sensitivity in skeletal muscle [126].

5. Leptin and Endocannabinoids

One of the key regulators of endocannabinoids in the hypothalamus is serum leptin. Leptin is known to reduce endocannabinoids in brain. In obese mice the dysregulation of leptin signaling give rise to higher endocannabinoid level in hypothalamus and is known to interfere with ECS signaling [127]. It prevents the ECS synthesis by lowering the levels of calcium ions. While in the normal rats, injection of leptin reduce endocannabinoids in the brain, EC levels increase in db/db, ob/ob as well as fa/fa mice [128]. All these three categories of mice have leptin deficiency or defective leptin receptor signaling. These results were specific to endocannabinoids in hypothalamus [127]. In addition to this, endocannabinoids in the uterus of ob/ob mice were elevated and could be reversed by using leptin treatment. Leptin administration was effective to restore all the enzyme activity of the endocannabinoid pathways; both the synthesis as well as degrading process related to endocannabinoids [129].

Leptin requires hypothalamic CB1 to exert its anorexic effects. It has been noted that partial deletion of CB1 from the hypothalamus has resulted in stopping the ability of leptin to reduce food intake [101]. In addition, leptin interacts with glucocorticoids for the regulation of endocannabinoids in paraventricular nucleus (PVN). Glucocorticoid can lead to repression of synaptic excitation in PVN through endocannabinoids. Leptin can cause a decrease in glucocorticoid mediated ECS synthesis and hence the excitation in PVN neuron [130]. Increase in EC in hypothalamus not only interfere with leptin signaling but also lead to insulin resistance in periphery [131]. In some other studies it has been noted that when CB1 is deleted from ste-

roidogenic factor-1 expressing neurons, of ventromedial hypothalamus, it increases the sensitivity of leptin to function as anorexigenic agent during consumption of normal chow, however leptin resistance is caused during the consumption of high fat diet [132].

In addition, Ghrelin is also known to be associated with the endocannabinoid level in the brain. Endocannabinoid is known to mediate the orexigenic effect of ghrelin when the hormone is administered to PVN [133]. Ghrelin require CB1 machinery to be active which in turn recruit AMP activated protein kinase, and which is required for ghrelin to function in hypothalamus. Ghrelin and 2AG are both elevated in human plasma when food for pleasure is ingested. This indicates that endocannabinoids and ghrelin are closely associated in reward related actions [134].

ECS have been studied in various animal models. It has been observed that the mice developed obesity due to mutation in leptin or leptin receptor gene. Dysregulation of ECS and leptin deficiency is also referred to be confounders of obesity.

6. CB1 Blockade Improves Obesity

One of the many causes of obesity is the presence of dysregulated and overactive ECS system. Several cannabinoid antagonists were developed. Both plant derived as well as synthetic compounds are known to suppress the food intake. Chronic treatments lead to improvement in weight loss in genetic and DIO.

Synthetic compound like Rimonabant also referred as SR141716A acts as an inverse agonist to CB1 has shown potential therapeutic use in obesity both in animals as well as in humans [135,136]. It has been noted that weight reducing effect of Rimonabant in mice can be enhanced by blocking μ type opoid G protein coupled receptor or by co-treatment with the gut hormones like oxyntomodulin or YY3-36 [137,138].

Peripheral administration of Rimonabant decrease the synthesis of Stearoyl Coenzyme A Desaturase 1(SCD1) in DIO induced obese mice. This suggests that CB1 blockade reduce synthesis of monounsaturated fats in WAT, independent of food intake however central administration gives same result as pair fed group [139]. In addition, other groups have reported that Rimonabant results in enhanced expression of Acetyltransferase, Palmitoyltransferase2 which are involved in fatty acid oxidation [107]. Due to psychiatric side effects Rimonabant was withdrawn from the market [140]. However, it has been shown that co-administration of melanin concentrating hormone receptor (MCHR) antagonist can augment the effect of CB1R, while normalizing the behavior changes [141].

The neutral CB1 antagonists are AM4113 and VCHSR1. VCHSR1 has lower affinity to CB1 and decreases milk ingestion in mice [142] and AM4113 reduce food intake in mice

under high fat, high carbohydrate and lab chow [143]. A lot of research was dedicated to find the antagonist of peripheral CB1in past years as it was shown to be an efficient way to suppress appetite, increase energy expenditure and reduce lipogenesis in both liver and adipose tissues. Several factors are studied and evaluated. JD 2144 as well as JD-5006 have been shown to be very effective to reduce weight and improve metabolic parameters in obese mice. AM6545 shows promising results as it reduces food intake in mice under high fat and high carbohydrate diet, however fails for normal chow diet. In addition, URB447, CB1 antagonist and CB2 agonist is known to decrease weight gain and also reduced brain penetration [144]. Recently LH-21 which also has low brain penetrability was used as potential drug in rodents. It is reported to have reduced high fat diet induced weight gain in obese rats modulating the lipogenic pathway [145]. One of the other approaches was to reduce the formation of 2AG. The mice lacking DAGLα were lean. Thus inhibiting DAGL-α using O-7460 was actually shown to be effective in reducing high fat diet intake in mice [146]. In addition to this, nonsteroidal anti-inflammatory drugs (NSAIDS) is shown to alter cannabinoid receptor induced response [147]. This is because these drugs can inhibit cyclooxygenase2 (COX2) which is also the degrading enzymes for AEA and 2AG. Another class of compounds known as "allosteric modulators" are developed which are known to decrease activity of CB1 in presence of their ligands [31]. Some of the examples being homopressin, pepcans and pregnenolone. Hemopressin [148] is known to modulate circuits in mediobasal hypothalamus and not the reward related areas. Pregnenolone is neurosteroid which restricts weight gain and adiposity in DIO. Another approach of reducing ECs would be to reduce the ω-6 pool which is the precursors of ECs. This could be achieved by increasing the ω-3 fatty acids pool which in turn could be achieved by introducing of docosahexaenoic acid and eicosapentanoic acid in the diet. This technique is known to reduce fat in adipose tissue, heart in Zucker rats [149]. More research is ongoing worldwide in search for a suitable compound to reduce obesity, with no side effects. Since different isoforms are present for CB1, with varied pharmacological properties, researchers should take into account the localizations of different isoforms in tissues when designing for drugs.

7. References

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