Chapter

Gamma-Aminobutyric Acid (GABA) and the Endocannabinoids: Understanding the Risks and Opportunities

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Abstract

The Gamma-aminobutyric acid (GABA) system is the main inhibitory neurotransmitter system in the central nervous system (CNS) of vertebrates and is involved in critical cellular communication and brain function. The endocannabioid system (ECS) was only recenty discovered and quickly recognized to be abundantly expressed in GABA-rich areas of the brain. The strong relationship between the GABA system and ECS is supported both by studies of the neuraoanatomy of mammalian nervous systems and the chemical messaging between neurons. The ECS is currently known to consist of two endocannabinoids, Anandamide (AEA) and 2-Arachidonyl Glycerol (2-AG), that function as chemical messengers between neurons, at least two cannabinoid receptors (CB_1 and CB_2), and complex synthetic and degradative metabolic systems. The ECS differs from the GABA system and other neurotransmitter systems in multiple ways including retrograde communication from the activated post-synaptic neuron to the presynaptic cell. Together, this molecular conversation between the ECS and GABA systems regulate the homeostasis and the chemical messaging essential for higher cortical functions such as learning and memory and may play a role in several human pathologies. Phytocannabinoids are synthesized in the plant *Cannabis sativa* (*C. sativa*). Within the family of phytocannabinoids at least 100 different cannabinoid molecules or derivatives have been identified and share the properties of binding to the endogenous cannabinoid receptors CB_1 and CB_2. The well-known psychoactive phytocannabinoid Δ^9-tetrahydrocannabinol (THC) and the non-psychoactive cannabidiol (CBD) are just two of the many substances synthesized within *C. sativa* that act on the body. Although the phytocannabinoids THC and CBD bind to these endogenous receptors in the mammalian CNS, these plant derived molecules have little in common with the endocannabinoids in structure, distribution and metabolism. This overlap in receptor binding is likely coincidental since phytocannabinoids evolved within the plant kingdom and the ECS including the endocannabinoids developed within animals. The GABA and ECS networks communicate through carefully orchestrated activities at localized synaptic level. When phytocannabinoids become available, the receptor affinities for CB_1 and CB_2 may compete with the naturally occurring endocannabinoid ligands and influence the GABA-ECS communication. In some instances this addition of phytocannabinoids may provide some therapeutic benefit while in other circumstances the presence of these plant derived ligands for the CB_1 and CB_2 receptors binding site may lead to disruption of important

functions within the CNS. The regulatory approval of several THC products for nausea and vomiting and anorexia and CBD for rare pediatric seizure disorders are examples of some of the benefits of phytocannabinoids. Concerns regarding cannabis exposure in utero and in the child and adolescence are shrill warnings of the hazards associated with disrupting the normal maturation of the developing CNS.

Keywords: endocannabinoids, GABA, phytocannabinoids, homeostasis

1. Introduction to the GABA system

Gamma-aminobutyric acid (GABA), an amino acid, is the primary inhibitory neurotransmitter in the vertebrate central nervous system (CNS). Although it was first identified in plants in the late nineteenth century, only in 1950 was it first identified in fresh extracts of animal brain including reptiles, avian, mammals and man [1]. It is now accepted that GABA is present almost exclusively within the brain and retina of vertebrates and only in extremely limited amounts in the peripheral nervous system and other organs of the body. It has been estimated that within the CNS, GABA is the neurotransmitter for as many as one-third of the neurons with the majority of these cells as interneurons that modulate the activity of neural networks. GABA neurons are widely expressed throughout the CNS including the cerebral cortex, hippocampus, striatum, substantia nigra, globus pallidus, cerebellum and olfactory bulbs. Within the structures, GABA receptors are found not only on the cell membranes of neurons but on supporting glial tissue and astrocytes [2].

As an amino acid, GABA serves other biological roles in addition to that of a neurotransmitter. It also functions as a precursor for the assembly of proteins and as metabolic intermediary. Despite these multiple functions, GABA is also responsible for regulation of neuronal excitability and is the primary inhibitory messenger in the CNS. GABA is highly concentrated in the CNS and present in millimoles per gram in the brain compared to nanomoles per gram of the more more commonly recognized neurotransmitters including dopamine, 5-hydroxytryptamine (serotonin) and norepinephrine [3].

GABA is known to have affinity for two distinct families of receptors similar to the excitatory amino acid Glutamate. The first and most prevalent of the two in the brain is the ionotropic $GABA_A$ receptor, a large glycoprotein of ~275 kDa and consists of a pentameric transmembrane receptor typically including two α subunits, two β subunits and one γ. Variations frequently occur and may even include δ subunit substituted for γ that encircle a central, chloride-permeable pore. The $GABA_A$ is found on both presynaptic and postsynaptic neuronal cell membranes. Upon the binding of two GABA molecules to the extracellular site, the pore opens and allows the flow of chloride ions into the cell with hyperpolarization of the cell membrane and inhibition of action potentials [4].

The $GABA_A$ receptor was cloned in 1987 and multiple subunits have subsequently been identified and grouped within seven functionally unique families. These multiple isoforms result in a highly complex system of receptors with functions dependent upon the expression of subunits.

Two binding sites for GABA sit on the $GABA_A$ receptor along with other sites that include a benzodiazepine receptor, a barbiturate receptor, and alcohol. In every instance, these binding sites function independently of each other. As a result, each receptor does not compete with activation of other receptors and the overall effect is synergestic rather than competitive [5].

The $GABA_B$ receptor is a second type of receptor and is a metabotropic site that belongs to the G-Protein Coupled Receptor (GPCR) superfamily. Pretreatment

of isolated tissue from rodent atria and vas deferens with the $GABA_A$ antagonist bicucullin in 1979 first eslablished that two populations of receptors existed when the expected response to GABA was not found [6]. Twenty years passed before the $GABA_B$ receptor was finally cloned. As a GPCR, this receptor is broadly distributed throughout the CNS and mediates slow and prolonged inhibitory messaging through $G_{ai/o}$-type proteins. As a GPCR, $GABA_B$ contains seven transmembrane domains with an extracellular N-terminus tail and acts through a second messenger system by inhibition of adenylate cyclase and cAMP formation inactivating voltage-gated Ca^{2+} channels and K^+ channels [5].

Three receptor subunits are associated with $GABA_B$ site. A long, extracellular N-terminal called the Venus fly-trap (VFT) domain includes an orthosteric binding site, a seven transmembrane domain and the C-terminus tail within the cell comprise the $GABA_B$ receptor. Ligands to the $GABA_B$ receptor have been identified and include the selective $GABA_B$ agonist Baclofen, various investigational antagonists that poorly penetrate the blood- brain barrier (BB) and several allosteric modulators under study [7].

Because of the ubiquity of GABA in the CNS It is not surprising that disordered GABA signaling has been implicated in several human neurological and psychiatric diseases. Anxiety, sleep, seizure, Alzheimer's, Parkinson's and substance abuse are some of several disorders suspected to be linked to the GABA system. Already several medication classes that have affinity for the GABA receptor, including benzodiazepines, muscle relaxants, sedative-hypnotics and anticonvulsants, are now routinely used in clinical medicine.

The production, release and degradation of GABA is mediated through multiple processes. The main precursor of GABA is glutamic acid, an excitatory neurotransmitter itself. GABA is synthesized by the irreversible single-step α-decarboxylation of glutamic acid by the enzyme glutamic acid decarboxylase (GAD), found initially in bacteria and plants and then later in the mammalian CNS and retina. There are two isoforms of the decarboxylase GAD (GAD_{65} and GAD_{67}) that are involved in the synthesis of GABA with GAD_{65} closely associated with the presynaptic vesicles. This relationship strongly suggests that a coupled process is involved in the the conversion of cytosol glutamate to storage of intravesicular GABA. There are also vesicular transports systems termed VGAT for the sequestration of the neurotransmitter into the vesicle. VGAT is also the same vesicular transport for another inhibitory amino acid transmitter glycine in the spinal cord [8].

Similar to most decarboxylases, pyridoxine is required as a co-factor [1]. The localization of GAD in the brain generally correlates closely with the distribution of GABA. After synthesis, GABA is stored in vesicles in the presynaptic terminals in cells classified as "GABAergic" cells. When GABAergic cells receive a depolarizing stimulus, vesicular fusion and exocytosis occurs and GABA is released into the synaptic cleft. GABA signaling is primarily terminated by its reuptake into both neuronal and glial cells through membrane transporter systems. Through this uptake system the presynaptic cytosol and vesicles can reuse GABA. Astrocytes also express membrane transporters systems for GABA and play a significant role in GABA metabolism. When reuptake occurs in these non-neuronal cells or non-GABAergic cells, the availability of GABA as a neurotransmitter is reduced [8].

In addition to uptake through membrane transporters, GABA may also be broken down by the enzyme GABA Transaminase (GABA-T). GABA-T is, unlike GAD, widely expressed in both central and peripheral systems and possibly helps limit exogenous GABA from influencing CNS activities. In the CNS, this primary enzyzme is associated with GABA breakdown and is found both in GABA-ergic neurons and astrocytes. One product of GABA-T is glutamate which may be involved in the recycling of glutamate to form new GABA. GABA is also

metabolized extracellularly by GABA-transaminase (GABA-T) into succinate semialdehyde, which then enters the krebs cycle for further metabolism [9].

2. Introduction to the endocannabinoid system (ECS)

The identification of Δ^9-tetrahydrocannabinol (THC) as the psychoactive constituent of cannabis opened a door to unexpected discoveries in neuroscience. Cannabis is the generic name for *C. sativa* (*C. sativa*) or hemp and belongs to the botanical group *Cannabaceae* that also contains hop. Cannabis was found to contain numerous molecular structures similar to THC, including cannabidiol (CBD) and cannabinol (CBN) and others. These new structures were initially referred to as cannabinoids and led to the obvious question of how, and why these botanical compounds worked in animals.

It was initially believed that these plant-based cannabinoids like THC, now referred to as phytocannabinoids, probably influenced animal physiology through a nonspecific mechanism to alter cellular membranes. Soon after establishing the laboratory synthesis of THC, modifications of the structure were created and tested in the laboratory. The availability of these synthetic analogs of THC led to the unexpected finding that the psychoactive effect of THC was stereospecific and occurred through binding to an unknown endogenous receptor [10, 11]. Evidence of an endogenous receptor was discovered in 1988 that revealed affinity for the THC molecule in rodent brain [12]. This previously unknown receptor was named CB_1 and found to be a G-Protein Coupled Receptor (GPCR) with seven transmembrane helices. Within a few years, a second peripheral receptor was cloned and named CB_2. Both receptors in humans were found to have 44% of the amino acid residues identical and in the transmembrane crossings 68% were the same. Although CB_1 was the first receptor identified in the brain and was considered a central receptor, it is now known that it is widely distributed outside the CNS but at lower expression, including the respiratory, cardiovascular, skin, ophthalmic systems, and the adrenal glands. CB_2, originally discovered in the spleen and thought to be a peripheral receptor, was later found to be present in limited amounts within the CNS and widely available in immune tissue and skin [13].

Although only recently discovered in the late 20th century, it is now recognized that the CB_1 and CB_2 receptors are the most plentiful G-protein coupled receptors (GPCR) in the body. CB_1 is especially abundant in the brain and is more plentiful than all other receptors including GABA.

The presence of these two endogenous cannabinoid receptors led to the expectation that endogenous ligands must lay ahead. Several years earlier the opiate receptors had been discovered in the brain that had affinity for compounds obtained from the opium plant. This led to the isolation of a class of endogenous ligands termed the enkephalins that were bioactive neuropeptides.

Soon after the identification of the cannabinoid receptors, the endogenous ligand arachidonylethanolamine was isolated in 1993 and found to have agonist properties for CB_1. This ligand was found in rodent brain and was composed of elements from arachidonic acid and ethanolamine. This unexpected ligand was soon christened Anandamide (AEA), a Sanskrit word for 'bliss' [14].

Arachidonic acid is a polyunsaturated fatty acid found in membrane phospholipids in several body organs including the brain [15–17]. In addition to being a precursor for AEA, arachidonic acid is also an important precursor for eicosanoids including prostaglandins. Shortly after the discovery of AEA, a second bioactive lipid that also included arachidonic acid, 2-arachidonylglycerol (2-AG), was found with binding affinity for both cannabinoid receptors. Unlike AEA, 2-AG had been

known for over fifty years as an intermediary in metabolic pathways of triglycerides and other glyceride molecules and is far more available than AEA. 2-AG was found to be a full agonist of CB_1 and CB_2 and abundantly available throughout the body [18, 19]. In contrast, anandamide is a partial agonist of CB_1 and CB_2 and belongs to the family of N-acylethanolamines (NAE). NAEs consist of saturated and monounsaturated fatty acids that include palmitic and oleic acids and these other NAEs are more abundant than AEA but do not bind to cannabinoid receptors [20]. Although only recently discovered in the late 20th century, it is now established that the CB_1 and CB_2 receptors are the most plentiful G-protein coupled receptors (GPCR) in the body. CB_1 is especially abundant in the brain and is more plentiful than all other receptors including GABA. The observation that the ECS is so highly expressed within the brain and the finding that the system is highly conserved in the evolution of animals illustrate the importance of the system in the healthy function of man.

Together AEA and 2-AG are referred to as endocannabinoids. These two endogenous ligands are produced in multiple body systems and activate cannabinoid receptors. These endocannabinoid chemical structures are long-chain, polyunsaturated fatty acid chains and differ significantly from the ring structured phytocannabinoids present in cannabis, with different binding affinities to the cannabinoid receptors. The endogenous 2-AG, for example, is a full agonist to the CB_1 and CB_2 receptors while the plant-derived THC is only a partial agonist. In addition, another important phytocannabinoid, CBD, has even less affinity with only very limited binding to cannabinoid receptors. As endogenous lipids, although both bind to the cannabinoid receptors, the NAE molecule AEA and the monoacylglycerol (MAG 2-AG as) belong to two distinct families with different synthetic and degradative pathways. Both AEA and 2-AG appear unique among their separate families as they are the only molecules that bind to the cannabinoid receptors CB_1 and CB_2.although they share affinities with the several similar lipids for non-cannabinoid receptors. In addition, both endocannabinoids and other bioactive lipids have redundant pathways in the synthesis and breakdown of the lipid molecules. This diversity in metabolism and binding to multiple receptor families by the NAEs and MAG lead to a highly complex system that regulates many important functions [21].

Collectively, the cannabinoid receptors CB_1 and CB_2, the two endocannabinoid messengers AEA and 2-AG, and the associated and separate enzymatic systems are called the endocannabinoid system (ECS). The ECS is a major system in human and the CB_1 and CB_2 receptors are expressed within the CNS and several peripheral organs including heart, liver, fat, skin, eye and the intestines [22].

As details about the ECS emerged during the 1990s and into this century, it has become apparent that endocannabinoids interact with several neurotransmitter systems and play an important role in regulating physiological functions. Autoradiographic localization of cannabinoid receptors in the rat established the rich co-localization of cannabinoid receptors with GABA-containing neurons [23, 24]. It has been reported that GABA is produced and released by inhibitory interneurons comprising between 20–60% of neurons in some areas of the brain [25]. The CB_1 and CB_2 receptors have been found to be highly expressed in areas rich with GABA neurons including the cortex, basal ganglia, substantia nigra and cerebellum. Compared to classic neurotransmitters including GABA and Glutamate [24, 26], the ECS is far more abundant and widely distributed compared to these systems. Thus, activation of the CB_1 receptor (the most abundant GPCR in the CNS) interacts with adjacent neurons including GABA and regulates neurotransmitter function to express their central effects.

The ECS is also one of the most pleiotropic systems in mammals and differs from other neurotransmitter systems in several ways. Importantly, most intercellular transmission proceeds anterograde with the release of neurotransmitters

from presynaptic neurons that bind to receptors on the postsynaptic membranes. Neurotransmitters, stored in vesicles within the presynaptic cytosol, are released as chemical messengers upon activation of the presynaptic neuron. After release into the synapse, the chemical messengers are subsequently broken down in the synaptic cleft or taken up by transport systems into the neuron or adjacent supporting cells [27].

Endocannabinoids act in the opposite direction from a postsynaptic neuron to presynaptic neuron. This retrograde direction allows the ECS to neuromodulate the forward direction of chemical communication. Because of their highly lipophilic properties, endocannabinoids are not stored in vesicles but are synthesized from membrane lipids only when required. Once released, the endocannabinoid diffuses to its' receptor target on the presynaptic neuron and helps regulate overall neurotransmission. In the brain, the presynaptic receptor is predominantly CB_1 with limited CB_2 found in microglia and other tissue. Eventually the endocannabinoid is released by the receptor and taken up by either the pre- or postsynaptic neuron for final degradation [17].

The endocannabinoids are synthesized in the post-synaptic membrane only after the cell is activated and then rapidly degraded after binding to the presynaptic cannabinoid receptor, the effect of stimulation is localized and limited in duration similar to GABA and other neurotransmitters. In addition, although these actions occur binding of AEA and 2-AG primarily to the CB_1 receptor in the brain, other non-cannabinoid receptors have also been identified that directly bind and are activated by endocannabinoids [28].

3. The discovery of anandamide (AEA)

Anandamide (AEA) was isolated from pig brain in 1992 and found to be a derivative of the fatty acid arachidonic acid. As the first endocannabinoid to be discovered, the molecule was named anandamide after the Sanskrit word Ananda that means bliss [29]. As a member of the N-acylethanolamines, it was established that AEA shared multiple synthetic pathways with other glycophospholipids [17].

Typical of other neurotransmitters, AEA functions as a chemical messenger between neurons. However, there are significant differences between endocannabinoids and neurotransmitters including GABA. Soon after its discovery, the uniqueness of AEA was established with the observation that the messenger was synthesized only on demand and diffuse across the synaptic cleft in a retrograde direction to the presynaptic neuron [17].

Following the inflow of calcium^{2+} into the postsynaptic cell, AEA is synthesized from the precursor membrane lipid N-arachidonyl-phosphatidylethanolamine (NAPE). NAPE is present in brain only in small amounts and cannot sustain prolonged synthesis of AEA. As with 2-AG, AEA contains arachidonic acid and combines this membrane constituent with phosphatidylethanolamine (PE), utilizing a calcium^{2+} dependent enzyme N-acyltransferase (NAT). The primary pathway for synthesis of anandamide is conversion of NAPE to anandamide through the action of a NAPE-specific phospholipase D (PLD), although several other pathways are known to exist. Similar to other synthesis in the NAE family, the NAPE pathway is not exclusive for AEA. Although the importance of other pathways have yet to be established, it is known that in genetically modified mice without NAPE-PLD, no reduction of the production of AEA is found [30].

Since multiple pathways may be associated with the synthesis of AEA, the abundance of choices has been suggested to enhance the number of stimuli that may initiate the production of AEA. Lipopolysaccharide (LPS), for example, is an

endotoxin in the outer membrane of gram-negative bacteria that plays a critical role in the protection of the microbe. Exposure to macrophages activates LPS to defend the bacteria and numerous lipid mediators including AEA are released. The synthesis and release of AEA and the other bioactive lipids is not believed to occur through the intermediate NAPE but rather through the secondary pathways that lead to AEA [20].

The breakdown of AEA results in the release of arachidonic acid and ethanolamine. Within the post-synaptic cell, an intracellular serine amidase named fatty acid amide hydrolase (FAAH) cleaves the long-chain fatty acid of AEA although other available hydrolytic enzyme systems in the cytosol appear to have little effect on AEA. Numerous studies have used disruption of this serine hydrolase through genetic or pharmacological manipulation to increase AEA activity. Manipulation of the FAAH system has already become the target of new drug development in an attempt to increase AEA in the treatment of human pathology [31, 32].

Other non-hydrolytic enzymes also break down AEA including lipoxygenases and cyclooxygenases. These non-FAAH systems are very active at non-cannabinoid receptors although their importance in deactivation of AEA at cannabinoid receptors has yet to be determined [20].

AEA is not the only ethanolamide that can bind to cannabinoid receptors. Other bioactive lipids in this class include numerous compounds including palmitoylethanolamide (PEA) and oleoylethanolamide (OEA) bind to the CB_1 receptor. Each of these ligands has distinctive physiological effects associated with them. PEA is associated with several indications including use as an anti-inflammatory or analgesic, while OEA appears useful as an appetite suppressant to reduce body weight [33, 34].

Both PEA and OEA are polyunsaturated fatty acids with multiple double bonds within the long chain. Other polyunsaturated fatty acids have also been reported to have agonist activity for the cannabinoid receptors. Only AEA, among the saturated and monounsaturated fatty acids, has been found to have affinity for the cannabinoid receptors.

4. 2-Arachidonylglycerol (2-AG): the second endocannabinoid

2-arachidonylglycerol (2-AG) is a monoacylglycerol that incorporates arachidonic acid at the 2 position of the glycerol backbone. This molecule serves the dual function of a lipid intermediary while also functioning as a chemical messenger within the ECS. Although this endocannabinoid was discovered later than AEA, 2-AG is several hundred fold more common in the CNS compared to AEA and is a full agonist to both the CB_1 and CB_2 receptors.

There are two major pathways for the synthesis of 2-AG. Similar to AEA, initiation of the process to manufacture 2-AG requires an inflow of calcium^{2+} into the neuron. The primary pathway for synthesis involves a precursor, phosphatidylinositol, converted by phospholipaseβ or phospholipaseγ, to the intermediary lipid 1,2-diacylglycerol (1,2-DAG). The 1,2-DAG is then hydrolyzed by a DAG lipase to form the endocannabinoid 2-AG.

There is a secondary pathway also available that involves the production of the intermediary 2-arachidonyl lysophospholipid. Once 2-arachidonyl lysophospholipid is available, this lysophospholipid in the presence of the enzyme lysophosphotase-C (LYSOPLC) is rapidly converted to 2-AG.

The breakdown of 2-AG also occurs through a primary pathway but several minor alternatives are also present. Hydrolysis of 2-AG by monoacylglycerol lipase (MAGL) is the most common pathway and involves the cleavage of the ester bond within the 2-AG structure to form arachidonic acid and glycerol. There are at least

two forms of MAGL that have been found in rodent and rabbit models. In comparison to the small amounts of AEA and its associated degradative enzymes, 2-AG is widely distributed throughout the CNS along with its synthetic and degradative enzymes. Perhaps because of the breadth of distribution of 2-AG in the CNS, some overlap with AEA occurs. However, a more important distinction is that MAGL is found only in the presynaptic neuron and degradation of 2-AG occurs after release from the presynaptic cannabinoid receptor. AEA, in comparison, after its release from the presynaptic cannabinoid receptor must traverse the synaptic cleft and enter the postsynaptic neuron where it is broken down by the NAE degrading enzyme FAAH [17, 35, 36].

The development of genetically modified mice deficient in MAGL along with the synthesis of MAGL inhibitors have provided useful tools to study the properties of 2-AG. Use of these ligands that block the synthesis of MAGL have revealed elevations of this endocannabinoid, especially in the brain and to a lesser extent multiple organs in the body including the heart, liver, kidney, and brown adipose tissue. Although 2-AG is the major endocannabinoid that binds to the cannabinoid receptors in brain, it clearly also serves an important role in the the regulation of chemical signaling in other organ systems. When the breakdown of 2-AG appears is impaired due to these receptor anatagonists or genetic manipulations, arachidonic acid is significantly reduced in the brain. This suggests that the production of 2-AG serves an important role not just in the formation of an endocannabinoid but also in the in the production of proinflammatory molecules [37].

Other alternative routes for 2-AG degradation are also available. Cycloxygenase-2 (COX-2) and lipoxygenases are secondary enzyme systems that also reduce 2-AG. COX-2 serves an important role in the inflammatory process and converts arachidonic acid to prostaglandins. Lipoxygenases oxidizes polyunsaturated fatty acids and these are non-heme, iron-containing enzymes that are found in a broad range of eukaryotes. They are known to be involved in the metabolism of the eicosanoids including the prostaglandins [37].

5. Endocannabinoid-GABA regulation of chemical messaging

In the 1990s, the phenomenon of "depolarization-induced suppression of inhibition" (DSI) was first reported in the purkinje cells of the cerebellum [38] and later in hippocampal pyramidal cells [39]. DSI occurs through the activation of the CB_1 receptor and is considered the classic example how endocannabinoids regulate neuronal behavior through retrograde signaling and suppression of GABA release. The CB_1 receptor is densely expressed on the GABA presynaptic neurons that are abundantly found in the cerebral cortex, hippocampus and amygdala and are essential for higher cortical functions including learning and memory. Small interneurons release GABA and communicate with the larger purkinje cells and pyramidal neurons. This interaction moderated by the release of GABA results in hyperpolarization of the larger post-synaptic cell and subsequent inactivation. Activation of the CB_1 receptor located on the presynaptic interneuron inhibits the release of GABA and thus suppresses the inhibition of the larger cells. It is now well established that this inhibition of GABA release from the interneuron is the result of retrograde communication from the activated postsynaptic cell to the presynaptic GABA-containing interneurons through the release of endocannabinoids that facilitate an increase of intracellular calcium^{2+} and the initiation of the DSI. Other cannabinoid agonists in addition to endocannabinoids are also known to block interneuron release of GABA through depolarization-induced suppression of inhibition. Presynaptic CB_1 antagonists, such as rimonabant, have also been reported

to block the effect of CB_1 receptor activation further establishing the critical role of retrograde modulation of chemical signaling through the ECS [22]. Thus, inhibition of GABA release is governed through depolarization of the presynaptic neuron by endocannabinoid binding to the presynaptic CB_1 receptor [40, 41].

A few years after the discovery of DSI, presynaptic stimulation of CB_1 through retrograde transmission of endocannabinoids was found to also occur with excitatory neurons and the phenomenon was termed "depolarization induced suppression of excitation"(DSE). Unlike DSI and the inhibition of GABA release, DSE inhibits the release of excitatory neurotransmitters including glutamate through a similar retrograde release of endocannabinoids. Although initially discovered the inactivation of Purkinje cells, DSE has also been observed in other regions of the brain although the role of endocannabinoids in these areas is less well established [42].

Dependent upon the presynaptic neurotransmitter, stimulation of presynaptic CB_1 receptor through retrograde release of endocannabinoids moderates the communication between cells. This changing effect of the endocannabinoids on GABA and glutamate release and the shaping of synapses occrs through a process called synaptic plasticity. Activation of a single synapse is usually insufficient to activate the post-synaptic cell and multiple synapses must fire simultaneously. The coordination and magnitude of the synaptic communication determines the change of voltage in the post-synaptic cell and the strength of the signal. Reductions in the number of presynaptic cells or incoordination of firing results in weakening of the signal.

The strengthening of synapses over time is termed long term potentiation and requires coordination of firing of the pre and post synaptic cells within a window of 20 msec. Cellular firing outside the temporal window weakens the synapse and reduces the voltage difference over time and is referred to as long term depression.

There is a balance in the regulation of excitation and inhibition that allows the brain to physically adapt for learning and memory [43]. Generally these changes are incremental and occur continuously at the synaptic level through a process termed synaptic plasticity [44].

Although glutamate has received a great deal of attention in the process of neuroplasticity, GABA also plays an important, or perhaps equal, role in the adaptation of the nervous system. Changes in neuronal activity and excitation by glutamate release may initiate off-setting activation of inhibitory inputs through GABA interneurons. In both activation and inhibition of the synaptic signal, retrograde release of endocannabinoids through DSI and DSE likely mediates synaptic depression [43].

6. GABA and the tale of two cannabinoids

The endocannabinoid system maintains homeostatsis in the CNS primarily through activation of the CB_1 receptor. This receptor is also responsible for the well-known behavioral and physiological effects of the phytocannabinoids. The mechanism of how this modulation of the CNS occurs is by retrograde signaling through activation of the CB_1 receptor. As noted earlier, the ECS and GABA neurons are collocated in many areas of the brain and this close proximity may explain how CB_1 binding influences the GABA system. The cortex, hippocampus, hypothalamus and cerebellum are areas in the brain where this overlap of the ECS and GABA is especially prominent.

There are several preclinical studies that have examined the inhibition of GABA release in the presence of cannabinoid agonists. One early *in vitro* study employing

an investigational synthetic cannabinoid agonist (WIN 55,212–2) on hippocampal interneurons found a diminuition of GABA release from the neurons after exposure. In another *in vitro* study the same investigational agent plus a second experimental cannabinoid agonist (CP-55940) were evaluated in rodent corpus striatum and found a dose-dependent reduction in GABA release.

Acute administration of the phytocannabinoid THC has also been studied. In an *in vivo* electrophysiological project after treatment, extracellular GABA in the prefrontal cortex was found to be significantly reduced compared to baseline. Different areas of the rodent brain were studied including the corpus striatum, and prefrontal cortex. One study reported different findings that THC and a synthetic cannabinoid failed to have effects on GABA synthesis and uptake in the globus pallidus in substantia nigrae of the rodent brain [45, 46].

Two other studies also evaluated the effect of THC on GABA release in rodent models. One evaluated THC alone and reported a dose-dependent reduction in GABA uptake in the rat globus pallidus [47, 48].

The abundance of CB_1 receptors on presynaptic neurons and their relationship to the strength of inhibition was assessed in a study of cholecystokinin (CCK) expressing GABA interneurons in the hippocampus. Earlier studies had demonstrated that the number of ion-channel-forming AMPA receptors could predict the magnitude of the postsynaptic response [49, 50] and that more GABA receptors were associated with greater inhibition. However, CB_1 receptors are GPCR and operate through different mechanisms including modulation of voltage-gated Ca^{2+} and K^+ channels and second messenger systems. Using the CB_1 receptor antagonist AM251, the effect of activation was measured in basket cells and dendritic-layer innervating (DLI) cells. Basket cells have a significant higher expression of CB_1 receptors and DLI have significantly less receptor density. The CB_1 receptor antagonist AM251 increased the action-potential inflow of Ca^2 by 54% in basket cells but not in DLI. However, this increase was significantly reduced from the expected effect of the large number of receptors. A CB_1 agonist decreased Ca^{2+} independent from the CB1 receptor expression. Collectively this suggests that only a subpopulation of CB_1 receptors in close proximity to the Ca^{2+} channel participate in the endocannabinoid modulation of GABA release [51].

Another study evaluated the effect of exposure to cannabinoids in adolescent rats. Using electrophysiological and immunohistochemical techniques, early-, mid- and late adolescent rats were treated with a CB_1 agonist (WIN). Early and middle adolescent rats were found to exhibit significant disinhibition of prefrontal cortex (PFC) behaviors at the later adult stage. This result was reversed when the adolescent rat was infused with the positive allosteric modulator GABAA agonist Indiplon. This response suggests that at certain stages of development exposure to cannabinoid agonists may be critical in the downregulation of GABA in the PFC and expressed in the adult stage of maturation [52].

A recent review summarized the literature on the interaction of endocannabinoids and neurotransmitters [22] although only a few have been reported for GABA. Administration orally or intravenously of the endogenous cannabinoid agonists including the endocannabinoids is technically difficult and their interpretation limited. On the other hand, phytocannabinoids can be smoked, ingested or applied as a topical with significant absorption and physiological effects mediated through cannabinoid receptors. In one report of adolescents, thirteen habitual users of cannabis were compared to sixteen non-canabis normal controls in a study using standard ^1H MRS techniques performed on a MAGNETOM trio whole body MRI/MRS system to determine GABA metabolism in the anterior cingulate cortex (ACC) [53]. reported reduced levels of GABA in the anterior cingulate cortex (ACC) of adolescents that were habitual users of marijuana when compared to match controls. The ACC

surrounds the anterior area of the corpus callosum and communicates with the prefrontal cortex and parietal lobe in addition to deeper limbic structures including the amygdala, nucleus accumbens and hippocampus. It is well established that GABA plays an important role in the maturation of these area in the adolescent brain and disruption of this process may result in neuropsychiatric and substance abuse issues later in life.

Results of the MRS scans revealed significantly lower levels of ACC GABA activity in adolescents that habitually used cannabis. Reduced ACC glutamate levels in adolescents that habitually used cannabis had been reported in an earlier study [54] with MRS imaging and in this follow-up report these findings paralleled the reduction in glutamate with a similar reduction of GABA.

Enhancement of GABA activity has been proposed as a therapeutic approach to the treatment of cannabis use. In one randomized clinical trial (RCT) fifty patients with cannabis dependency were treated with Gabapentin 1200 mg/day or placebo for twelve weeks. Compared to placebo, the study reported significant reduced use of cannabis measured by several assessments including urine drug screens. Gabapentin is a structural analog of GABA and was initially thought to act on the GABA system. Later studies demonstrated that Gabapentin does not alter GABA activity or receptors although it may increase GABA synthesis and non-synaptic GABA release [55].

In the first of two studies, the GABA reuptake inhibitor Tiagabine (Gabitril), was assessed in eight cannabis users and compared when combined with oral THC. THC was dosed at 30 mg p.o. and tiagabine at 6 and 12 mg p.o.. Subjects were trained to use established drug-discriminationprocedures to identify placebo and drug conditions, blinded to the study condition and were informed they would receive placebo, THC and tiagabine, alone or in combination during the study. Tiagabine was found to enhance the discriminative-stimulus, self-report and performance results when given with THC and to produce similar outcomes when administered alone [56].

In a subsequent study the investigators replaced tiagabine with baclofen and repeated the trial. In contrast to tiagabine, baclofen is a selective $GABA_B$ agonist but has not effect on the $GABA_A$. Results of both studies were similar suggesting that $GABA_B$ receptors are involved at least in part with the effect of elevated GABA on cannabinoid-related behaviors [57].

The authors commented that although $GABA_B$ enhanced the effects of THC, they could not rule out that accentuation of GABA at $GABA_A$ receptors could also contribute to the outcome.

In addition to evaluation of the ECS and GABA through pharmacological enhancement of GABA, an interesting clinical study reporting that pharmacological-induced deficiency of GABA increased the effects of THC in several psychiatric assessments. Using normal subjects, this double-blind, placebo-controlled study evaluated flumazenil, an antagonist and partial inverse agonist of the $GABA_a$ receptor, against intravenous THC or placebo. Blocking the $GABA_a$ receptor with flumazenil accentuated the psychological effects of THC including psychoses and anxiety and a decrease in the THC-induced P300 amplitude [58].

Through imaging studies of the ECS, manipulation of the synthesis and degradation of endocannabinoids, and pharmacological interventions much has been learned about the cannabinoids since the initial discovery of of the first cannabinoid receptor CB_1 in 1988 [59]. The ECS plays a major role in the maturation and homeostatsis of the CNS and activation of the CB_1 receptor is the primary initiating event. Modulation of other neurotransmitter systems including GABA can then occur through retrograde transmission [60].

Ligands other than the endocannabinoids also bind to CB_1 and CB_2 receptors and much can be learned through observation of the effects of these non-endocannabinoids. Although phytocannabinoids, evolved through time in the plant kingdom and differ significantly from endocannabinoids, the overlap in affinity for cannabinoid receptors offer additional means to study the modulation by the ECS and neurotransmitter systems.

Phytocannabinoids are produced in the plant *C. sativa* (cannabis) and are C_{21} terpenophenolic molecular ring structures grouped into eleven classes. Currently about 120 different phytocannabinoids have been identified in cannabis and comprise approximately 24% of the weight of the plant. The first class of phytocannabinoids is the most common (approximately 17%) and contains the psychoactive THC. Variations in the growth of the plant *C. sativa* including growing conditions and sunlight, geography, processing and storage, and plant variety can all significantly alter the proportion of each chemical class. For this reason, cannabis is constantly in change and this variation can influence the pharmacological properties of different cannabis extracts [61].

There are several large epidemiological studies of phytocannabinoid effects on the ECS. Although banned in many areas, Cannabis is the most used illicit drug globally with an estimated 3.8% (182.5 million) of the global population exposed to cannabis [62, 63]. Within the United States, the estimated exposure is even higher with 8.4% (22.2 million) of the population reported to have used cannabis in one year. With relaxation of laws and greater duration of use combined with the change in composition and potency of cannabis, real world studies can provide us important information in understanding the function of the ECS system and the effects of disruption of normal processes.

Among the most important epidemiological studies are reports of exposure to cannabis of pregnant women and the effects on their offspring. In a recent study it was estimated that 5.2% (115,000) of pregnant women are exposed during their preganancy. Some of these women likely use cannabis unaware of their pregnancy and inadvertently expose the first trimester fetus to THC when the nervous system is first initiated. Others may choose to use THC later in pregnancy believing it is a safe remedy for pregnancy-associated nausea and vomiting while neurotransmitter systems are evolving. Others may just believe that cannabis use is safe and be unaware of the potential hazard to the unborn [64].

As with many drugs, however, cannabinoids carry significant safety concerns for pregnant women and as a lipophilic molecule easily traverse the placenta into the fetal bloodstream. Animal studies have shown a clear association between cannabinoids and lower birth weight. In humans, several large, well-conducted studies have explored the short- and long-term effects on fetal, child and adolescents and possible teratogenicity of prenatal cannabis exposure on fetal development (Hurd et al. 2005).

The Ottawa Prenatal Prospective Study (OPPS) was a large, epidemiological study of 291 expectant, middle class Canadian women. Within this group of expectant mothers, 20% used cannabis sometime during their pregnancy. All subjects were evaluated during their pregnancy and for the first six years using standardiazed neuropsychological tools.

At birth, there were observations made of increased startle reflex in children exposed in utero to cannabis, but no significant change in weight or increased presence of congenital malformations. By age four, however, behavioral changes including decreased visual performance, attention, and memory were apparent. In older children, impaired executive function was reported [65, 66].

In 1991 a second longitudinal study named the Maternal Health Practices and Child Development Study (MHPCD) was reported on 519 expectant mothers

and live born infants. Unlike the earlier study in Ottawa, expectant mothers were largely lower class economically with poorer prenatal care. Expectant mothers were evaluated at 4 and 7-month gestation offspring evaluated until young adulthood. Growth parameters including birth weight, head or chest circumference, and gestational age were analyzed at birth with no statistical differences noted between newborns with non-exposure in utero and in newborns with maternal use of cannabis. There was a small effect on decreased birth length in exposure the first two months and a positive effect on body weight with usage in the third trimester [67]. In a follow-up of the offspring in this study up to two decades later, prenatal maternal exposure to cannabis was found to result in a greater risk of cannabis use in their children at adolescence (38% before age 15). By age 22 in-utero cannabis-exposed children were more apt to not complete high school (54.4% vs. 37.2% in controls), be unemployed (67.6% vs. 52.1%) and more likely to have been arrested (56% vs. 27.3%) [68].

The Dunedin study was a third, and more controversial, project conducted in New Zealand on 1037 individuals followed from birth to 38 years. One measurement obtained over the course of the study was the evaluation of the association between cannabis use and neuropsychological outcomes. Neuropsychological assessments were obtained before the age when cannabis use occurred and changes studied. Cannabis use was obtained at age 13 and then at age 38 after a pattern of consistent use. It was found that there was an associated decline in IQ related to the frequency and length of exposure to cannabis. The greatest vulnerability appeared to occur with adolescent exposure. The authors found that persistent cannabis use was associated with neuropsychological decline broadly across domains of functioning, most significantly in the domains of executive functioning and processing speed. Study participants with more persistent cannabis dependence also showed greater IQ decline over the years, along with greater overall cognitive decline. Greater cognitive impairment was observed in those who began cannabis use in adolescence. The investigators also pointed out that cessation of cannabis use did not fully restore neuropsychological functioning in these adolescence-onset users [69, 70].

Another recent large, retrospective, cohort study of 661,617 pregnant women study conducted over six years in Ontario, Canada examined the association between self-reported cannabis use in pregnancy and any adverse maternal or perinatal outcomes. The investigators accounted for known confounding factors, such as tobacco use, in one of two cohorts by the use of a matched design analysis. The results showed that preterm birth rate, at less than 37 weeks' gestation, for both the matched and unmatched cohorts were significantly higher in the women who reported cannabis use. The rate of preterm birth rate in the unmatched cohort was 12.0% in cannabis users, compared to 6.1% in nonusers. In the matched cohort, the rate of preterm birth was 10.2% in cannabis users versus 7.2% in nonusers. A continuous increase in relative risk of preterm birth from cannabis exposure was observed between 34 to 36 6/7 weeks' and 28 to 31 6/7 weeks' gestation, respectively. Because this type of increase was not observed for very preterm birth at less than 28 weeks' gestation, it was conjectured that cannabis exposure may be more strongly associated with early and moderate preterm births versus very preterm births. Cannabis use in the subjects was also significantly associated with the following secondary outcomes: small for gestational age, placental abruption, transfer to neonatal intensive care, and 5-minute Apgar score of less than 4 [71].

Both the OPPS and MHPCD studies were consistent in demonstrating behavioral and cognitive impairment years after exposure to cannabis in-utero. The Dunedin study also reported decline in IQ related to cannabis exposure beginning in adolescence. Collectively, all three studies report important deficits that

emerge over time in child and adolescent maturation. A limitation of these studies, however, is the continuing social acceptance of cannabis use and increasing potency of THC.

To provide more current information, an NIH-initiative, the Adolescent Brain Cognitive Development (ABCD) Study is ongoing. This is a national, multisite, longitudinal cohort study that is prospectively following subjects from childhood through adolescence to explore the effects of substance use such as cannabinoids, among other experiences, on neurocognitive development. There are, of course, many challenges associated with long epidemiologic studies. Aside from participant loss and difficulty maintaining controls, the constant flux in the content of cannabinoid products over the years, namely the significant increases in the ratio of THC to CBD, presents significant inconsistency in comparing these long studies or predicting current risk.

7. Final comments

GABA is an amino acid concentrated within the CNS and is recognized as the major inhibitory neurotransmitter in the brain [1]. With the exception of a second, excitatory amino acid neurotransmitter glutamate, GABA is present in millimoles/gm in brain tissue compared to nanomolar/gm concentrations of the other classic neurotransmitters [72].

The physiological effects of GABA do not occur in isolation. The functional relationship beween the two systems begins after the release of GABA from an activated presynaptic neuron and stimulation of the postsynaptic cell. Endocannabinoids are then manufactured on-demand and released to bind to cannabinoid receptors on the presynaptic membrane terminating the release of GABA.

The CB_1 receptor is highly expressed in several regions of the brain including the forebrain, amygdala, hippocampus, substantia nigra and cerebellum. This receptor is frequently in GABA containing neurons and this overlap allows for close coordination and interaction between the two systems. As a result, the ECS provides an important feedback to the GABA system and participates in the maturation of the CNS and the function of the adult brain [72, 73].

The GABA system and the ECS, similar to all neurotransmitters, are limited to brief synaptic activity at discrete locations and are quickly terminated through either enzymatic breakdown or reuptake mechanisms. GABA is stored in presynaptic vesicles and released after excitation by an action potential into the synapse to stimulate the postsynaptic cell. The endocannabinoids, in contrast, are synthesized in the postsynaptic membrane on demand only after the cell is stimulated. Upon release, the endocannabinoid moves in a retrograde direction across the synapse and binds to the CB_1 receptor on the presynaptic neuron. Once the endocannabinoid is bound to the CB_1 receptor, the release of neurotransmitters from the presynaptic neuron is terminated.

How endocannabinoids work in moderating GABA is introduced in the discussion of depolarization induced suppression of inhibition (DSI). This is a critical concept on how the chemical signal with GABA release is moderated by the activation of the CB_1 receptor. Although less established, activation of this cannabinoid receptor may also activate another amino acid transmitter glutamate through a similar mechanism termed depolarization induced suppression of excitation (DSE).

Several preclinical studies of ECS and GABA in this chapter followed the initial papers on DSI and DSE and the concept of CB_1 receptor activation influencing the release of GABA (and potentially glutamate). Although for technical reasons it has not been possible to study the effect of AEA and 2-AG directly, these studies chose

to utilize several laboratory-created CB_1 agonists under investigation or the phytocannabinoid THC. No matter the source of the agonist, the findings consistently found that stimulation of the CB_1 receptor reduced the release of GABA.

From these studies it is apparent that activation of the CB_1 receptor is not exclusive to endocannabinoids. As discussed earlier, the plant *C. sativa* produces phytocannabinoids including THC that also are agonists and partially bind to the CB_1 receptor [74]. These molecules evolved in the plant kingdom for evolutionary imperatives that are incongruent with the evolution of the ECS in animals. Although they differ significantly from the endocannabinoids in chemical structure, synthesis, degradation, phytocannabinoids including THC and CBD are of great interest since they have CB_1 receptor activity and similarly influence the release of GABA. This affinity is likely coincidential yet provides additional information on the interplay between the physiological functions regulated by GABA and activation of CB_1.

Earlier in this chapter several large epidemiological studies were reviewed reporting the effects of cannabis on the development of the nervous system in utero to maturity. These studies are informative because they describe the effects of cannabinoids on the developing nervous system and adult where GABA plays an important role. From these reports it is likely that early maternal exposure to phytocannabinoids results in impairment in the offspring through disruption of the development of the nervous system with behavioral abnormalities appearing later in life [65, 68, 75, 76].

There are obvious limitations in large scale studies since In normal circumstances ECS and GABA collaborate in limited and localized coordination in development. Phytocannabinoids act systemically throughout the body and are not limited to discrete synapses. In addition, since phytocannabinoids are lipid soluble, sequestered in fat tissue, and broken down by hepatic enzymes, the location and duration of exposure to phytocannabinoids differs from the brief, focused synaptic interaction between GABA and the endocannabinoids. Nevertheless, these large studies of cannabis use provide important information on how phytocannabinoids may disrupt GABA function that may be reflected in the abnormalities reported in these larg scale studies. Cannabis is regarded by many as relatively 'safe' and is becoming 'legal' in many areas. However, other 'safe' and 'legal' drugs including nicotine and alcohol are associated with serious public health concerns. These studies give us insight into the possible risks associated with using phytocannabinoids and influencing the communication between GABA and the endocannabinoids.

The interaction of GABA and the ECS is important for normal physiological function. As our knowledge of this modulation of the CNS advances, additional knowledge and treatments will likely emerge that will provide unexpected benefits to patients. However, epidemiological studies of exposure to cannabis also provide important information they reveal the disadvantages and risks of disruption of the GABA-ECS systems. As increased access and duration of usage evolve, we will learn more of the benefits, and risks, of cannabiods.

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