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The Endocannabinoid System Handbook

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Media: Handbook

Topic: Endocannabinoid System

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Target Audience:

Clinical cardiologists, endocrinologists, family/general practice physicians, internists, neurologists, scientists and researchers

Statement of Need/Program Objective:

Progress has been made in understanding the endocannabinoid system (ECS). However, there are still gaps in the biology and scientific data at the molecular/cellular level, as well as a lack of understanding at the integrative, whole body/clinical level. It is essential that the complex, biologic and neurological foundation of the ECS is translated from the realm and language of preclinical science to the clinical world.

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Educational Objectives:

Upon completion of Chapters, the participant will be able to:

Chapter 1: Introduction to the Endocannabinoid System

- Describe the components of the ECS system to evaluate published data
- Differentiate between cannabinoids, endocannabinoids, and peptide or aminergic neurotransmitters to be able to differentiate between the effects of cannabis and the endogenous system
- Identify the main targets for endocannabinoid action, centrally and peripherally, to better understand emerging therapeutic targets
- Identify the biological pathways that might be modulated by the ECS, which will increase awareness of how deregulation can destruct specific biological pathways relevant to certain disease states

Chapter 2: Cell Biology of the Endocannabinoid System

- Identify the most-studied endocannabinoids and describe their mode of synthesis and action to be able to understand that endocannabinoids are made on demand, unlike neurotransmitters and hormones
- Describe the endocannabinoid receptors and their signaling mechanisms to be able to explain which signaling pathways are affected upon cell activation
- Identify cellular signaling pathways that interact with the ECS in human pathophysiology

Chapter 3: Normal Function of the Endocannabinoid System

- Identify the key areas involved in the central regulation of appetite and feeding behavior, to understand the rationale for a multimodal approach to treating obesity
- Describe the role of the ECS in the modulation of food intake and behavior to understand how therapeutic targets can be used to control appetite to get patients to weight goals
- Describe the role of the ECS in the modulation of energy expenditure and fuel partitioning, to understand emerging therapies targeting ECS regulation for patients with cardiometabolic risk factors

Chapter 4: The Endocannabinoid System and Obesity

- Assess the preclinical evidence for the role of the ECS in the development of obesity
- Evaluate the findings suggesting an aberrant regulation of the ECS in human obesity
- Explain how integration of leptin and endocannabinoid signaling might regulate appetite

Chapter 5: The Endocannabinoid System: Effects on Lipid and Glucose Homeostasis

- Describe the effects of CB_1 receptor blockade on hepatic lipid metabolism in animal models of obesity
- Describe the effects of CB_1 receptor blockade on plasma lipids in animal models of obesity
- Discuss the evidence for the involvement of the ECS in modulating glucose homeostasis as it relates to the management of patients with obesity and diabetes

Chapter 6: The Role of the Endocannabinoid System in the Central Nervous System

- Describe the effects of central CB_1 receptor activation on neurotransmitter release, to understand the design of therapeutic agents that modulate synaptic activity
- Describe how the ECS might modulate cognition, memory, and emotionality
- Describe the effects of exogenous cannabinoids and CB_1 receptor antagonists on nausea and emesis
- Explain how cannabinoid-based drugs might influence the pathogenesis and symptoms of various disorders of the central nervous system
- Explain the role of the ECS in modulating pain and reinforcement

Table of Contents

Chapter 1

Introduction to the Endocannabinoid System

The endocannabinoid system (ECS), a complex endogenous signaling system, influences multiple metabolic pathways.[1] The ECS is composed of transmembrane endocannabinoid receptors (the cannabinoid [CB] receptors), their endogenous ligands (the endocannabinoids), and the proteins involved in endocannabinoid synthesis and inactivation, as well as the intracellular signaling pathways affected by endocannabinoids.[2]

The ECS takes its name from the cannabis plant (*Cannabis sativa*), an annual herb also known as hemp. Not until the modern era did scientists discover the first types of cannabinoids, the active chemical compounds found in the cannabis plant that are responsible for the plant's psychoactive and physiological effects. To date, three types of cannabimimetic compounds have been described: herbal cannabinoids, which occur uniquely in the cannabis plant; endogenous cannabinoids (or endocannabinoids),[3] which are produced in the brain and peripheral tissues; and synthetic cannabinoids, which have been developed by drug companies as potential pain medications.[1]

Researchers identified Δ^9-tetrahydrocannabinol (THC) as the main psychoactive component in marijuana in 1964.[4] In 1988 Howlett and coworkers[5] described the presence of high-affinity binding sites for cannabinoids in rat brain membranes that correlated pharmacologically with antinociception. The cannabinoid receptors CB_1 and CB_2 were cloned in the early 1990s. Shortly thereafter, the first endocannabinoids, anandamide (N-arachidonylethanolamine) and 2-arachidonoylglycerol (2-AG), were discovered.[6-8] More recently, the field of endocannabinoid biology has advanced markedly with the characterization of CB_1 receptors in a variety of tissues including adipose tissue, liver, and skeletal muscle and the discovery of peripheral metabolic effects of the ECS.[1,9]

Cannabinoid Receptors and Endocannabinoids

The CB receptors belong to Class A of the superfamily of G protein-coupled receptors (GPCRs [Figure 1]).[10] The CB_1 receptor is the most abundant GPCR expressed in the brain.[9] It is also found in a variety of peripheral tissues such as adipose tissue, liver, the gastrointestinal tract, and pancreas (Table 1).[1,9,11,12] The neuronal circuitry involved in regulating energy balance is concentrated in the hypothalamus and brain stem.[1] The CB_1 receptor is expressed in the pituitary gland and hypothalamus where it appears to be an integrated component of the networks controlling appetite and food intake.

Cannabinoid Receptors

- G protein-coupled receptors (GPCRs)
- Expressed in central and peripheral tissues
- Appear to modulate metabolic functions

A role for the CB_1 receptor in the stress response is supported by data showing that its activation modulates the hypothalamic-pituitary-adrenal axis.[9] The CB_1 receptor is also highly expressed in mesolimbic dopamine reward circuits within the brain, where perceptions associated with pleasure/palatability and appetite/incentive stimuli are processed.[1] Stimulation of CB_1 receptors in appetite-modulating reward centers is believed to drive the preference for palatable food items. The identification, characterization, and localization of specific membrane receptors mediating the effects

Table 1. Human Tissues and Organs Expressing the CB_1 Receptor Gene[9,37]

Central Nervous System	Genitourinary/ Reproductive	Gastrointestinal	Other
Brain	Kidney	Ileum	Adipose
Spinal cord	Placenta	Liver	Lung
	Prostate	Stomach	Skeletal muscle
	Testis and sperm	Pancreas	Spleen
	Uterus		

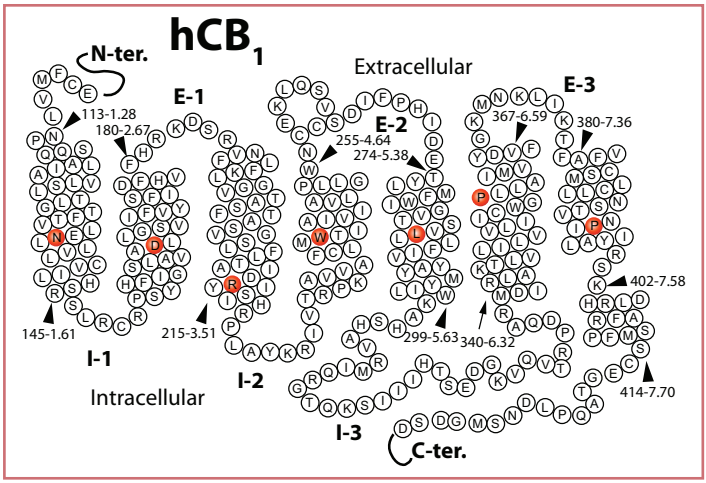

Figure 1. G protein-coupled receptors (GPCRs), such as the CB_1 receptor, are the largest family of cell-surface receptors.[41] A helix net representation of the human CB_1 receptor sequence is provided here. This helix net uses the Ballesteros and Weinstein GPCR numbering system.[42] The most highly conserved residue in each transmembrane helix across Class A GPCRs is highlighted in red. Based upon the crystal structure of the Class A GPCR, rhodopsin, GPCR topology has been shown to include an extracellular N terminus, seven transmembrane helix regions arranged to form a closed bundle, with intervening loops extending intra- and extracellularly, and a cytoplasmic C terminus.[43] The three extracellular (E-1, E-2, and E-3) and intracellular (I-1, I-2, and I-3) regions are labeled here. The structure also includes a short eighth helix (from residues D7.59[403] to P7.69[413]) which is an intracellular helix that occurs at the beginning of the C terminus. N-ter, N terminus; C-ter, C terminus.

of THC and synthetic CB_1 receptor agonists suggested the presence of endogenous ligands to which these receptors must respond.[13] When the first two endocannabinoids, anandamide and 2-AG, were identified they were shown to be synthesized from membrane-derived phospholipids,[14,15] and their biologic effects were found to be mediated through coupling with CB_1 and CB_2 receptors as well as other cellular mechanisms.[14] The endocannabinoids (endogenous cannabinoids) are derivatives of arachidonic acid.[9,16] Because endocannabinoids are lipophilic compounds derived from membrane

phospholipids, they are not stored in synaptic vesicles like peptide or aminergic neurotransmitters.[1,9,17] In the brain, they are produced by neurons at their sites of action and, when released, generate a transient, rapid effect before being hydrolyzed and inactivated.[1,13,17] Because of their lipophilic nature and the mechanism of their synthesis and release, endocannabinoids are considered to be local neuromodulators.[1]

Physiologic Actions of the ECS

The ECS appears to be present in all vertebrate phyla, which implies a role in vital biological functions.[1,2,18] The ECS is hypothesized to play a role in a wide variety of physiologic processes, including nociception (pain sense), motor control, memory and learning, appetite, food intake, and energy balance (see Chapter 3).[9,13,14] Other functions of the ECS in normal physiology may be related to endocrine functions, vascular responses, immune modulation, neuroprotection, and bone turnover.[19-26] The ECS is postulated to connect the physical and emotional responses to appetite and energy regulation. Other findings support the role of the ECS in modulating the rewarding properties of palatable high-sugar, high-fat food. For example, stimulation of the ECS may possibly occur as part of the pathogenesis of obesity, as a function to modulate certain feedback mechanisms involved in energy balance.[1,27,28]

Endocannabinoids

- Lipophilic compounds derived from membrane phospholipids
- Act locally
- Generate transient and rapid modulatory effects

The ECS has been shown to modulate energy balance and metabolic homeostasis, as well as behaviors such as food intake (Figure 2).[28-32] Studies in rodents and cultured cells suggest that glucose homeostasis and lipid metabolism can be modulated by the ECS through CB_1 receptors located in metabolically active tissues throughout the body, such as the adipose tissue, the liver, and possibly the pancreas and skeletal muscle (see Chapters 3-5).

Pathophysiological Responses Modulated by the ECS

Preclinical studies on obesity suggest that the ECS is associated with impaired lipid and glucose homeostasis.[33,34] ECS activation enhances lipogenesis in adipose tissue and liver.[29,35,36] These studies are reviewed in the subsequent chapters.

The ECS has also been shown to play a role in conditions in which cardiovascular disorders are comorbid.[37-39] Administration of a CB_1 receptor antagonist (AM281) prevented changes in systemic hemodynamic and internal carotid artery blood flow in experimentally induced septic shock in rats, implying that CB_1 receptor antagonism might improve survival in this rodent model.[39] In those studies, the endocannabinoids were believed

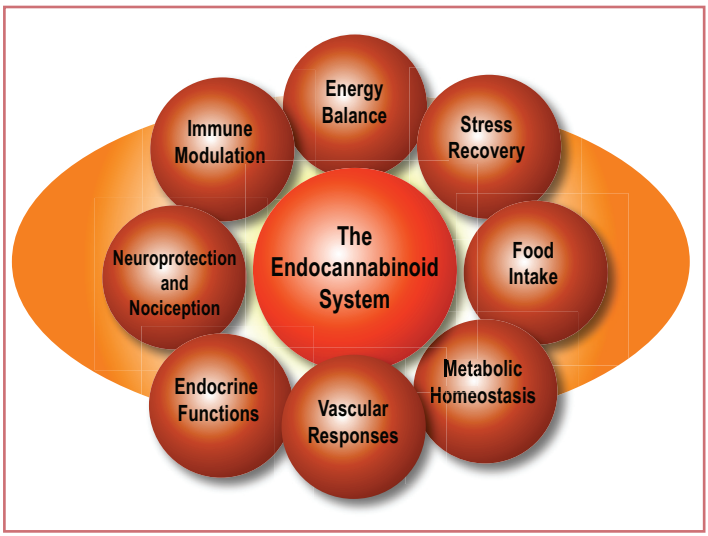

Figure 2. Normal functions of the ECS. The ECS has been shown to modulate energy balance and metabolic homeostasis, as well as behaviors such as food intake.[28-32] Other functions of the ECS in normal physiology may be related to endocrine functions, vascular responses, immune modulation (including implantation), nociception, neuroprotection, and bone turnover.[19-26]

to be released from macrophages and platelets. The ECS may also be involved in cirrhotic cardiomyopathy (cardiac dysfunction associated with liver cirrhosis). During cirrhosis, treatment of rats with a CB_1 receptor antagonist (AM251) reversed the blunted response of the cardiac muscle to isoproterenol.[38] Perturbations in endocannabinoid signaling are also associated with neurological, psychiatric, and gastrointestinal disorders, as well as some forms of cancer.[37,40]

Summary

The ECS is a complex physiological system that affects multiple metabolic pathways. It is composed of cannabinoid receptors, their endogenous ligands (the endocannabinoids), and the proteins involved in endocannabinoid synthesis and inactivation, as well as the intracellular signaling pathways affected by endocannabinoids.[2] In the hypothalamus, the CB_1 receptor is a component of the integrated central networks controlling appetite and food intake.[1] In the periphery, CB_1 receptors appear to modulate metabolic functions through effects in adipose tissue, liver, the gastrointestinal tract, and skeletal muscle.[1] Pharmacological intervention at the level of the ECS may improve a wide range of morbidities. The next chapter provides details of the cell biology of the ECS.

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Chapter 1:

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Chapter 2

Cell Biology of the Endocannabinoid System

The first-discovered and most extensively studied endocannabinoids are anandamide (N-arachidonylethanolamine) and 2-arachidonoylglycerol (2-AG).[1-3] Membrane depolarization of neurons or activation of certain receptors in different cell types leads to the formation of anandamide and 2-AG from phospholipid precursors through multiple pathways.[2,4] Elucidating these enzymatic pathways is an area of active investigation. Following their synthesis, anandamide and 2-AG are released into the extracellular milieu where they can bind to cannabinoid receptors.[2,4] Both anandamide and 2-AG appear to be synthesized on demand in a tightly regulated fashion, ideally whenever and wherever they are needed.[3] While most thoroughly studied in the central nervous system, endocannabinoids also appear to be produced on demand and act on cells in a paracrine or autocrine manner in peripheral tissues.[5] For example, preclinical studies have found anandamide and 2-AG in the uterus, pancreas, and liver.[3,6,7] Both anandamide and 2-AG have been detected in human plasma[8,9] and adipose tissue (see Chapter 4).[3,10]

- Anandamide and 2-AG are the most extensively studied endocannabinoids
- They are present in brain, adipose, uterus, pancreas, and liver

The Cannabinoid Receptors

The CB_1 receptor was cloned first from the rat cerebral cortex, then from the human brain and testis, and then from the mouse brain.[11] Over the past 16 years, CB_1 receptors have been found in a wide range of peripheral tissues and organs (see Chapter 1, Table 1).[11] In the brain, the density of CB_1 receptors is very high, and is as abundant as γ-aminobutyric acid (GABA)- and glutamate-gated ion channels.[1] A second endocannabinoid receptor, the CB_2 receptor, is expressed in the spleen and tonsils as well as on immune cells (B-cells, monocytes, and T-cells), indicating a role in immune function. The CB_2 receptor may be expressed in nervous tissue as well, particularly

following injury.[11] Data from CB_2 receptor knockout mice indicate that CB_2 receptors play a role in macrophage-mediated helper T-cell activation[12]; THC inhibited helper T-cell activation through macrophages derived from wild-type mice, but not from mice lacking functional CB_2 receptors (CB_2 receptor knockout mice).[13] CB_2 receptor expression is induced in brain microglial cells during inflammation,[14] and recent studies using human neutrophils indicate that the CB_2 receptor may suppress neutrophil migration during inflammation.[15]

- CB_2 receptors appear to play a role in immune function

The family of endogenous agonists for CB_1 receptors is larger than initially thought.[1,2] By definition, endocannabinoids are endogenous compounds that bind to and activate CB_1 receptors, CB_2 receptors, or both.[10] Pharmacologically, 2-AG binds both CB_1 and CB_2 receptors with similar affinity and activates them with similar efficacy. In contrast, anandamide has a lower affinity for CB_2 than CB_1 and is a low-efficacy agonist at both receptors. Thus, anandamide often acts as a partial agonist at CB_1 and CB_2 receptors, while 2-AG usually shows full agonism at both receptors.[16] Other newly proposed endocannabinoids are 2-arachidonyl-glyceryl ether (2-AGE, noladin ether), *O*-arachidonoyl-ethanolamine (virodhamine), and *N*-arachidonoyl-dopamine (NADA), among others. The physiological importance of noladin ether, virodhamine, NADA, and other emerging endocannabinoids is currently being investigated.[2,17]

Data from studies on CB_1 and CB_2 receptor knockout mice suggest that there may be several additional endocannabinoid receptors.[4,18] For example, several cannabinoid agonists bind to and activate the orphan G protein-coupled receptor (GPCR) GPR55, which is expressed in brain and various peripheral tissues in humans and rats.[19] There is also evidence for a vascular endocannabinoid receptor distinct from GPR55, CB_1 or CB_2. In addition, endocannabinoids can produce effects that are not mediated by G protein-coupled receptors.[20,21] These non-cannabinoid receptor–mediated mechanisms are currently under investigation.[11]

CB_1 Receptor Signal Transduction

- CB_1 receptors are G protein-coupled receptors

The CB_1 receptor is a member of the superfamily of GPCRs and consists of seven transmembrane-spanning domains (see Chapter 1, Figure 1).[11,22] CB_1 receptors typically couple to G proteins of the G_i/G_o class. Major effects of their activation will include inhibition of adenylyl cyclase, modulation of ion channels, and activation of mitogen-activated protein kinases (MAPK). However, in some systems and under some conditions, CB_1 receptors may occasionally be coupled to G_s, and/or $G_{q/11}$ in addition to $G_{i/o}$ proteins; ongoing studies will determine the physiological significance of this promiscuous coupling.[11]

Endocannabinoid-mediated activation of CB_1 receptors on nerve terminals inhibits neurotransmission in many brain regions, including striatum, hippocampus, cerebellum, cortex, hypothalamus, and nucleus accumbens, among others.[18] Inhibition of Ca^{2+} channels and stimulation of K^+ channels both contribute to inhibition of neuronal excitability and suppression of neurotransmitter release.[23] CB_1 receptor activation inhibits GABA and glutamate release, depending on which class of neuron is expressing CB_1, and also inhibits release of neuropeptides from CB_1 receptor-containing nerve terminals.[24] Although the density of CB_1 receptors varies between neuronal subpopulations and brain regions, there is little correlation between levels of expression and receptor functionality.[17]

CB_1 receptor activation

- Inhibits neurotransmission in many brain regions
- Is directly coupled to inhibition of voltage-activated Ca^{2+} channels
- Modulates multiple intracellular signaling pathways in peripheral tissues and neurons
- Inhibits neurotransmission in many brain regions

Activation of CB_1 receptors affects several major signaling pathways.[23] In neurons, CB_1 receptor stimulation is directly coupled to inhibition of voltage-activated Ca^{2+} channels[23] and mediates many forms of retrograde signaling (Figure 1).[4] CB_1 receptor stimulation is also linked to activation of inwardly

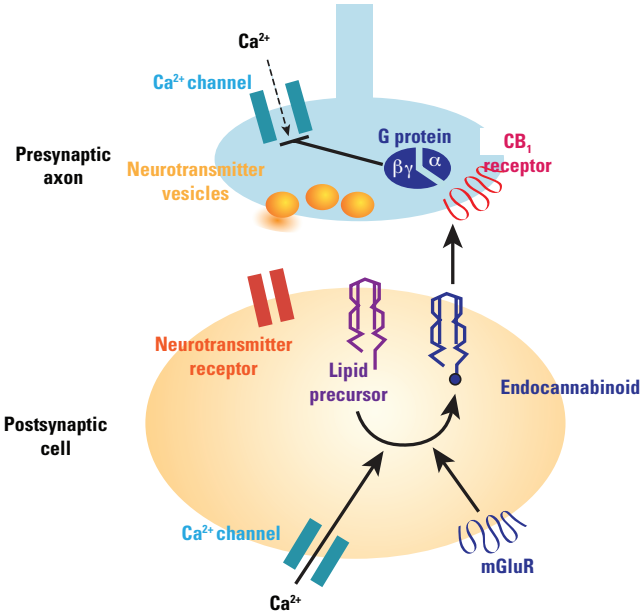

Figure 1. Retrograde signaling by endocannabinoids. Endocannabinoid synthesis in response to direct depolarization is strongly dependent on increased intracellular Ca^{2+},[5,41,42] which may synergize with the activation of group 1 metabotropic glutamate receptors (mGluRs) as well as other phospholipase C-linked GPCRs, to further stimulate endocannabinoid production.[42] Postsynaptic depolarization opens voltage-dependent Ca^{2+} channels; postsynaptic Ca^{2+} then activates enzymes that synthesize endocannabinoids from lipid precursors. Activation of postsynaptic mGluRs can also generate endocannabinoids, likely by activation of phospholipase C. Endocannabinoids leave the postsynaptic cells and activate presynaptic CB_1 receptors. G protein activation liberates $G\beta\gamma$, which then directly inhibits presynaptic Ca^{2+} channels, decreasing intracellular calcium, which decreases the probability of neurotransmitter release. In general, the ECS is characterized by rapid endocannabinoid synthesis, receptor activation, and endocannabinoid degradation, with tightly regulated spatial and temporal selectivity.[17] From Wilson and Nicoll.[4]

rectifying K^+ channels, which decreases neuronal excitability. In peripheral tissues and neurons, activation of CB_1 receptors triggers intracellular signaling events including inhibition of adenylyl cyclase with corresponding

attenuation of the protein kinase A signaling, and stimulation of MAP protein kinases (Figure 2).[17,23] The type of signaling pathway modulated by CB_1 receptor activation will vary depending on the type of agonist used as well as the tissue or organ involved.[23] For example, 2-AG was recently shown to stimulate adenosine monophosphate (AMP) kinase activity in the hypothalamus and to inhibit AMP kinase activity in the liver and adipose tissue of rats.[25]

- The degradation of anandamide is mediated by fatty acid amide hydrolase (FAAH)

Endocannabinoids are rapidly cleared from the extracellular milieu (terminal half-life [$t_{½}$] is seconds to minutes).[2] Studies suggest that following CB_1 receptor activation, anandamide and 2-AG are taken up by a putative facilitated transport mechanism known as the anandamide membrane transporter (AMT) (reviewed in Bari et al).[26] Although data from several biochemical studies support the existence of an AMT, this is a controversial topic and an AMT protein remains to be identified.[2] Regardless of the mechanism of entry into cells, degradation of endocannabinoids plays a major role in the termination of their action. Fatty acid amide hydrolase (FAAH) catalyzes the hydrolysis of anandamide in vivo (Figure 3).[5, 27-29] In addition, a monoacylglycerol lipase is believed to play a key role in the enzymatic hydrolysis of 2-AG.[3] Additional hydrolytic pathways for the endocannabinoids have also been described.[30,31] These characteristics of on-demand synthesis and rapid degradation suggest that endocannabinoids act close to their site of synthesis.[32]

Interactions with Other Physiological Systems

The ECS appears to influence other physiological systems through interactions with their receptors, intracellular signaling pathways, hormones, and neurotransmitters. Thus, some or many of the biological effects of the ECS may occur through a complex interplay with other systems. Some of the ligand-gated receptor systems implicated include the transient receptor potential vanilloid type 1 (TRPV1) receptor, serotonin ($5\text{-}HT_3$) receptor, *N*-methyl-D-aspartate (NMDA) receptor, and nicotinic acetylcholine receptors (nAChRs).

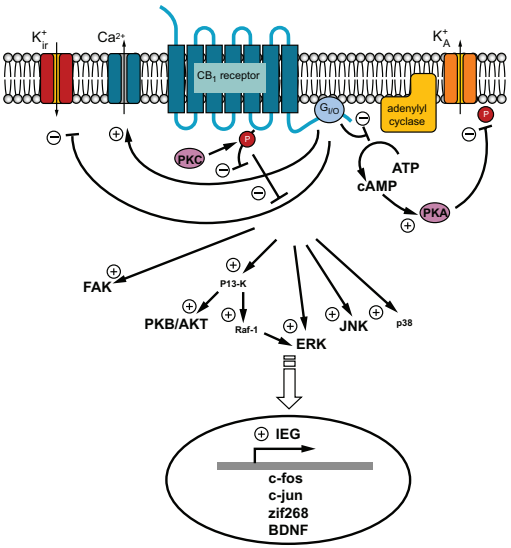

Figure 2. CB_1 receptor intracellular signaling cascades.

- Activation of the CB_1 receptor leads to the stimulation of $G_{i/o}$ proteins that, in turn, inhibit the adenylyl cyclase–mediated conversion of adenosine triphosphate (ATP) to cyclic AMP (cAMP). cAMP molecules can bind the regulatory subunits of protein kinase A (PKA) and cause the liberation of the catalytic subunits.
- Activated PKA can phosphorylate A-type potassium (K^+_A) channels, causing a decrease in current. Since CB_1 receptor activation inhibits adenylyl cyclase, the final result is a stimulation of K^+_A channels. $G_{i/o}$ activated by the CB_1 receptor can also directly inhibit N- or P/Q-type Ca^{2+} channels and activate inwardly rectifying potassium (K^+_{ir}) channels.
- These latter two actions are subject to modulation by protein kinase C (PKC), which, after activation, can phosphorylate the CB_1 receptor in the third cytoplasmic loop and prevent the receptor from modulating ion channels.
- Activation of the CB_1 receptor can also stimulate several intracellular kinases, such as focal adhesion kinase (FAK), phosphatidyl inositol-3-kinase (PI3-K), and its downstream effector protein kinase B (PKB/AKT), extracellular signal-regulated kinases (ERKs), c-jun N-terminal kinase (c-JNK), and p38 MAPK (p38).
- Stimulation of these or other protein kinases likely mediates the CB_1 receptor–induced expression of immediate early genes (IEG), such as the transcription factors c-fos, c-jun, and zif268, and the brain-derived neurotropic factor (BDNF).
- Note that these events were described in different cellular systems and, therefore, they might not all occur in the same cell type. Reproduced with permission from Pagotto et al.[17]

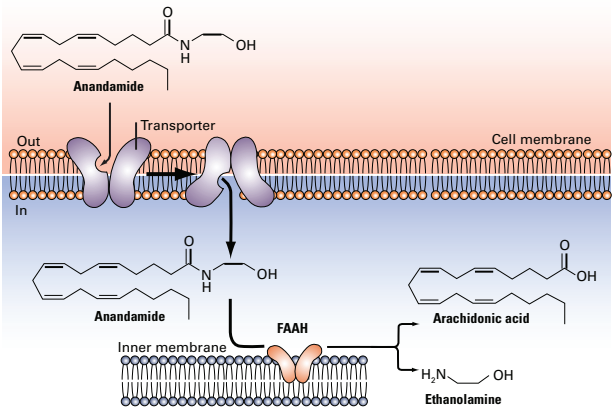

Figure 3. Mechanisms of endocannabinoid inactivation in neurons. Anandamide and 2-AG can be taken up by neurons, possibly through a high-affinity transport mechanism, the "endocannabinoid transporter." Two possible routes of degradation shown here both involve hydrolysis. Once inside cells, anandamide and 2-AG can be hydrolyzed by distinct serine hydrolases—anandamide by FAAH and 2-AG by monoglyceride lipase (not shown)—to yield breakdown products. From Piomelli.[5]

The endocannabinoid anandamide activates the TRPV1 receptor, an ion channel found on sensory neurons, to cause Ca^{2+} influx, which can lead to neurotransmitter release.[11] Recent data indicate that signaling by anandamide and TRPV1 may converge on the regulation of spontaneous and L-3,4-dihydroxyphenylalanine (L-DOPA)–induced locomotion in rats.[21] Multiple lines of evidence support the notion that the ECS may play an important role in brain reward processes by interacting with the mesolimbic dopaminergic system.[33] Stimulation of nucleus accumbens CB_1 receptors may suppress glutamatergic activity, with consequent inhibition of GABAergic neurons that normally inhibit ventral tegmental area dopamine neurons.[20] Moreover, there is evidence for interactions between CB_1 and dopamine receptors in the rat and monkey

- The ECS may play an important role in brain reward processes

striatum.[34] Endocannabinoids can increase extracellular levels of the neurotransmitter, dopamine. As an example, Solinas et al[33] demonstrated that intravenous administration of anandamide was associated with increased levels of extracellular dopamine in the nucleus accumbens shell of awake, freely moving rats. This effect was dependent on CB_1 receptors, but not on TRPV1 receptors.

- CB_1 receptors can form heterodimers with dopamine D2, orexin and opioid receptors

The ECS can interact with other receptor systems through the formation of CB_1 receptor heterodimers.[35] Results from cell-culture studies demonstrate that CB_1 receptors and dopamine D2 receptors form heterodimers.[36] HEK293 cells stably expressing dopamine D2 and CB_1 receptors were treated with subsaturating concentrations of agonists for both receptors. Coimmunoprecipitation experiments revealed heterodimer formation that was dependent on the concentration of the respective agonists. When stimulated individually, each receptor inhibited the intracellular signaling enzyme adenylyl cyclase. In contrast, activation of the CB_1 receptor/dopamine D2 receptor complex resulted in stimulation of adenylyl cyclase and enhanced mitogen-activated protein kinase (MAPK) activity, an effect attributed to differential coupling to $G\alpha_s$ proteins compared with $G\alpha_{i/o}$ proteins.[36] An interaction between the ECS and the opioid system is supported by studies showing that the opioid-receptor antagonist naloxone and the CB_1 receptor antagonist SR141716 synergistically depress food intake at doses that do not affect food intake when these agents are administered alone.[17] In the enteric nervous system, the actions of opioids, like those of endocannabinoids, are mediated by modulating transmitter release from nerves that decrease motility, peristalsis, and secretion.[37] Opioid receptors (μ, δ, and κ) and CB_1 receptors are coexpressed in the same subcellular compartments and are coupled to similar intracellular signaling pathways.[11,35] CB_1 receptors are colocalized with δ- and κ-opioid receptors in cultured porcine myenteric neurons[38] and both opioid and CB_1 receptors inhibit adenylyl cyclase activity through the activation of G proteins.[11] Recently, Rios et al[39] showed that the simultaneous activation of μ-opioid and CB_1 receptors

attenuates the response observed upon activation of individual receptors and inhibits neuritogenesis in vitro.

Summary

Considerable progress has been made in elucidating the cellular mechanisms involved in endocannabinoid signaling. In addition to direct signaling of cannabinoid receptors, substantial evidence supports functional crosstalk between the various components of the ECS and other signaling systems. The ECS modulates adenylyl cyclase, Ca^{2+} and K^{+} ion channels, and MAP kinases. Through multiple levels of interactions, this leads to effects on other cellular receptors, neuropeptides, hormones, and their intracellular signal-transduction pathways.[40] A more thorough understanding of cannabinoid receptor signal-transduction pathways will allow for innovative therapeutic strategies for pathological conditions associated with aberrant ECS signaling.[40]

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Chapter 3

Normal Function of the Endocannabinoid System

Expression of components of the ECS throughout the body, as well as its presence in lower-level organisms, indicates a vital role for this system in normal physiology.[1,2] Various experimental strategies—genetically disrupting ECS signaling via cannabinoid receptor deletion or inhibiting the system via cannabinoid antagonists, and stimulating CB_1 receptors by administering agonist compounds—have helped to elucidate the functions of the ECS. The ECS plays a role in the regulation of energy balance and behaviors such as food intake, fear, and anxiety, as well as in the modulation of lipid and glucose metabolism.[3-6] The ECS also participates in numerous other physiologic processes including those related to immune response, neuroprotection, memory and learning, nociception (pain sense), fertility, and bone turnover.[7-14]

Food Intake and Stress Recovery

The ability of cannabis to stimulate hunger and increase appetite, especially for sweet and palatable food, has been recognized since as early as 300 AD.[15] The ECS is postulated to connect the physical and emotional responses to stress with appetite and energy regulation. As such, the ECS is hypothesized to function as a general stress-recovery system.[15,16]

Indeed, endocannabinoids have been described as being stress-recovery factors that are produced in response to stressful stimuli to help protect the homeostatic activity of neuropeptides, hormones, and neurotransmitters.[15] Therefore, on a broad level, the ECS appears to achieve its goal by stimulating relaxation, rest, and eating, and by extinguishing aversive memories (Table 1).[16-18]

Table 1. The ECS as a Stress-Recovery System[15-18,56]	
Transient Activation of the ECS	**Stress-Recovery Effect***
• Reduced pain and anxiety	Relaxation
• Inhibition of motor behavior	Rest
• Extinction of aversive memories	Forgetting
• Induced appetite	Eating
*Stress-recovery effects were first linked to ECS in 1998.[16]	

Appetite, Satiety, and Feeding Behavior

Central Regulation

Appetite, satiety, and feeding behavior are complex physiologic processes involving interactions among multiple neuromodulatory systems in the brain.[19] The hypothalamus and hindbrain are the key areas of the brain that regulate food intake, energy homeostasis, and body weight, while the limbic system is believed to contain the neuronal circuitry that determines perceptions of food palatability and the "wanting and liking" of food.[15,20] Several signals arising from the periphery influence the regulation of energy homeostasis by the brain. These signals can be divided into two categories. The first category includes factors with a short-term action that are released concomitantly with the meal and help limit further ingestion of food. These "satiety signals" are mainly represented by hormones released by the gastrointestinal (GI) tract, like the hormone cholecystokinin (CCK). The second category of signals includes hormones such as leptin and insulin, which inform the brain about the amount and distribution of fat in the body.[21] Importantly, these "adiposity signals" have direct access to neuronal circuits within the hypothalamus and other brain regions, and thereby directly influence energy-balance regulation.[21,22] As described below, the ECS seems to have a modulatory action on virtually every key point affecting energy homeostasis.

- The hypothalamus and hindbrain regulate energy balance
- The limbic system regulates perceptions of food palatability and the "wanting and liking" of food

- CCK is a satiety signal
- Leptin and insulin are adiposity signals

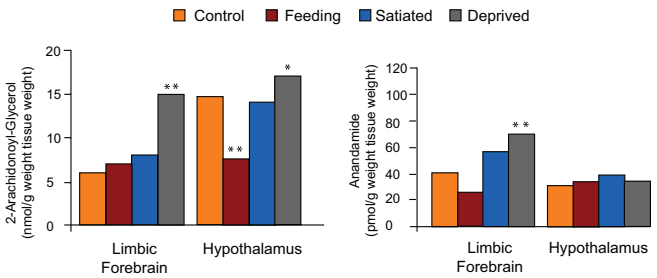

Figure 1. Food deprivation increases endocannabinoid levels in the limbic forebrain and hypothalamus. *P <0.05, **P <0.01. From Kirkham et al.[26]

Numerous pharmacologic studies have demonstrated the ability of the ECS to modulate feeding using either CB_1 receptor agonists or antagonists (able to increase or decrease food intake, respectively).[3,15,23-26] Phenotypical studies conducted on CB_1 receptor knockout mice have further clarified that the CB_1 receptor is the CB receptor subtype most involved in the modulation of energy balance.[3,27]

Nucleus accumbens

- Located in the limbic forebrain
- Implicated in eating motivation

It is now clear that the ECS directly modulates the activity of neuronal circuits regulating food intake, both within the hypothalamus and the limbic system.[15] For example, administration of the endocannabinoid anandamide within the ventromedial nucleus of the hypothalamus stimulated eating in presatiated rats; an effect that was inhibited by pretreating the animals with a CB_1 receptor antagonist.[24] Anandamide and synthetic compounds that inhibit its deactivation also cause hyperphagia when injected into the nucleus accumbens (a limbic forebrain area implicated in eating motivation).[25] In the same fashion, administration of the endocannabinoid 2-AG into the shell subregion of the nucleus accumbens also induced short-term hyperphagia.[26] As reported before, CB_1 receptor antagonism reversed the 2-AG effect on food intake, suggesting that CB_1 receptor activity in both the hypothalamus and the limbic system is

- Injection of 2-AG or anandamide into the nucleus accumbens of rats induces hyperphagia

essential to induce feeding in response to cannabinoids.[26] Interestingly, endocannabinoid levels within the brain are directly modulated by nutritional status. In the rat limbic forebrain and hypothalamus, levels of anandamide and 2-AG were elevated with fasting and declined with feeding (Figure 1).[19,26] In contrast, endocannabinoid levels in the cerebellum—a region not directly involved in food intake—were unaffected by fasting or feeding.[26]

ECS Interactions with Anorexigenic and Orexigenic Signals

Within the brain, the CB_1 receptor has been colocalized with several neuropeptides and receptors known to have a role in the modulation of feeding.[3,23] This implies that the ECS may interact with both anorexigenic and orexigenic neuronal circuits within both the hypothalamus and the limbic system.

The relationship between endocannabinoids and leptin, the adipocyte-derived hormone that inhibits orexigenic signaling in the hypothalamus, is of particular interest.[28,29] Hypothalamic endocannabinoid levels appear to be under negative control by leptin.[29] Moreover, integration of endocannabinoid and leptin signaling may regulate the excitability of appetite-related neural circuits in several hypothalamic nuclei (see Chapter 4, Figure 1).[28,30]

The melanocortin system, which includes melanocortins (such as the potent anorexigenic peptide alpha-melanocyte stimulating hormone [α-MSH]) and the melanocortin receptor type 4 (MCR4), plays an essential role in the central regulation of energy balance and is a target of leptin's effects within the brain.[31] Several studies have demonstrated the anorectic action of melanocortins, while genetic or pharmacologic inhibition of the MCR4 is associated with obesity.[32,33] Recent findings have revealed a synergistic interaction between the cannabinoid and melanocortin systems in feeding behavior.[33] This functional interaction has been supported by the ability of subanorectic doses of the CB_1 receptor

Melanocortin System

- Produces anorexigenic signals
- Interacts synergistically with the ECS

antagonist SR141716 and a MCR4 agonist to synergistically reduce food intake when administered together.[33] A very recent study shows that α-MSH does not influence the levels of anandamide or 2-AG in the hypothalamus, whereas blockade of MCR4 is accompanied by elevation of endocannabinoid levels 6 hours after administration. This suggests that melanocortins do not directly act via inhibition of the ECS, and that late elevation of endocannabinoid levels may contribute to the stimulatory effect of MCR4 antagonists on food intake.[34]

Neuropeptide Y (NPY) is a very potent orexigenic neuropeptide that stimulates feeding when administered to wild-type mice.[22] However, NPY fails to induce food intake when administered to CB_1 receptor knockout mice.[35] Conversely, administration of the CB_1 receptor antagonist SR141716 to NPY knockout mice reduces food intake.[29] Therefore, whereas NPY does not seem to be essential for the endocannabinoid modulation of food intake, the CB_1 receptor is necessary for the orexigenic action of NPY.

Another potent orexigenic signal that interacts with the ECS is the gastric peptide ghrelin. Unlike GI hormones classified as satiety signals, such as CCK, ghrelin levels in the blood tend to be increased before a meal and decrease after the consumption of food.[36] Furthermore, exogenous administration of ghrelin increases food intake and body adiposity.[37] CB_1 receptors appear to be involved in modulating the orexigenic effect of ghrelin.[38] In rats, the feeding-stimulatory effect of intracerebroventricular ghrelin infusion was blocked by pretreatment with a subanorectic dose of the CB_1 receptor antagonist SR141716. Moreover, systemic administration of a CB_1 receptor antagonist suppressed circulating ghrelin levels.[39]

Ghrelin

- Gastric peptide with orexigenic function
- CB_1 receptor antagonism blocks ghrelin-induced feeding in rats

Besides the above-described interactions, it is also known that the ECS modulates the serotoninergic, dopaminergic and opioidergic pathways, all neuronal circuits involved in the regulation of feeding behavior.[23,40]

Appetite, Satiety, and Feeding Behavior

Peripheral Regulation

The intestine and associated organs of the GI tract play a well-defined role in the regulation of energy balance, predominantly by communicating with centers in the brain through neural and endocrine pathways.[41] As discussed earlier, signals arising from the gut act in concert with central mechanisms to influence eating behavior.[15]

The vagus nerve may be a target through which the ECS modulates food intake.[5] The vagus nerve connects the GI tract with medulla and brainstem nuclei that are intimately involved in the control of satiety.[5] The gut hormone CCK is secreted during a meal, and interacts with specific CCK receptors located on the afferent terminals of the vagus nerve.[15] From there, information is transmitted via vagal axons and ultimately relayed to the hypothalamus, where it is integrated with other signals to decrease food intake.[15] Recent studies have shown that the expression of CB_1 receptor mRNA in vagal afferent neurons projecting into the duodenum is decreased in rats fed ad libitum, while its expression is increased when rats are food deprived.[42] Importantly, renewed feeding in previously fasted rats or the administration of CCK leads to decreased levels of CB_1 receptor mRNA in the same vagal afferents.[42] Thus, the induction of satiety by CCK is associated with reduced ECS activity.[5] Moreover, CB_1 receptors on neurons innervating the GI tract are also known to affect gastric emptying, gut motility and peristalsis.[43]

- Induction of satiety by CCK is associated with reduced ECS activity
- This effect may involve peripheral CB_1 receptors

Endocannabinoids are synthesized in the GI tract and, similar to what happens in the brain, their levels are modulated by the intake of food.[44] For example, anandamide levels were shown to increase 7-fold in the small intestines of rats in response to 24 hours of food deprivation, whereas levels normalized when feeding was resumed. In presatiated rats, peripheral (intraperitoneal) administration of either anandamide or a synthetic CB_1 receptor

agonist induced hyperphagia, while intracerebroventricular administration had no effect on food intake.[44] Similarly, peripheral, but not central administration of the CB_1 antagonist SR141716A, resulted in reduced food intake in this animal model.[44] This study also showed that chemical destruction of sensory terminals innervating the gut—the same nerves that express CCK receptors and mediate CCK-induced satiety—abolished these CB_1 receptor-mediated effects, thus implying a peripheral mode of action for the ECS in affecting feeding behavior.[5,44]

Regulation of Body Weight

It is absolutely predictable that changes in food intake in the absence of changes in energy expenditure lead to changes in body weight. However, the ECS appears to modulate body weight by not only affecting feeding, but also by modulating metabolic processes involved in the regulation of energy expenditure and storage. Pair-feeding experiments using CB_1 receptor knockout mice and their wild-type littermates have shown that while body weight reduction results from a reduction in caloric intake in young CB_1 receptor knockout mice, an increase in energy expenditure likely contributes to the decreased body weight of the adult CB_1 receptor knockout mice.[3] The difference in body weight was entirely accounted for by decreased fat mass since the CB_1 receptor knockout animals had a slightly increased proportion of lean body mass despite the reduction in body weight. Moreover, heterozygous mice carrying one disrupted allele of the CB_1 receptor gene exhibited a degree of reduced food intake and decreased fat mass that was intermediate between the wild-type mice and CB_1 receptor knockout mice.[45] Thus, the intermediate phenotype of the heterozygous mice further confirms the role of endogenous CB_1 receptors in the regulation of body weight.

- Adult mice lacking a functional CB_1 receptor have a lower fat mass than pair-fed, wild-type mice

Preclinical and clinical data suggest that CB_1 receptor blockade may increase energy expenditure. Kunz et al[46] showed that, compared with control

animals, rats treated with 3 and 10 mg/kg rimonabant had an increase in O_2 consumption of 18% and 49%, respectively, after 3 hours. There did not appear to be changes in the rate of carbohydrate and fat oxidation, and factors other than physical activity appeared to contribute to the increase in O_2 consumption.[46] Addy et al[47] measured the effect of the CB_1 receptor antagonist taranabant on resting energy expenditure in 17 overweight or obese subjects. Compared with placebo, the peak resting energy expenditure 2-5 hours post-treatment with 12 mg taranabant was increased significantly.[47] Moreover, the 12 mg dose of taranabant appeared to increase the rate of fat metabolism, as evidenced by a significant decrease in the mean respiratory quotient compared with placebo.[47]

CB_1 receptors have been localized in white adipose tissue as well as in organs and tissues such as pancreas, liver, and skeletal muscle that are closely involved in the regulation of energy balance.[48,49] A growing number of studies point to the ability of the ECS to directly modulate peripheral metabolism, and dysregulation of this system might have a role in obesity and other metabolic disorders. Thus, the ECS may regulate body weight (energy balance) independently of food (energy) intake (ie, by reducing energy expenditure and at the same time maximizing lipid accumulation into the adipose tissue). Indeed, recent data indicate that the ECS may stimulate pre-adipocytes to differentiation, as well as lipid accumulation into mature adipocytes, resulting in increased adipocyte size.[49] This physiological role of the ECS becomes dysregulated during obesity, as will be reviewed in detail in the next chapter.

Other Functions of the ECS

The ECS appears to play an important role in the regulation of hormonal balance through actions on endocrine axes. The CB_1 receptor is expressed in the hypothalamus and the pituitary gland, and CB_1 receptor activation can modulate all of the endocrine hypothalamic-peripheral endocrine axes (reviewed in Pagotto et al[50]). Conversely, the endocrine system may

modulate the ECS. Preclinical ex vivo data indicate that endocannabinoid synthesis in the hypothalamus can be stimulated by glucocorticoids,[51] although it is not yet known whether there is a link between the ECS and steroid-induced changes in feeding behavior. In addition, FAAH activity is regulated by several hormones, including progesterone and follicle-stimulating hormone. These findings suggest a potential role for the ECS in fertility and pregnancy.[50] In general, the ECS has been shown to inhibit hypothalamic-pituitary-adrenal functions and to modulate fertility.[50] Recent data demonstrate that FAAH protein expression and activity varies as a function of the mouse estrus cycle[52] and that CB_1 receptor expression and function correlate with larval organogenesis of *Xenopus laevis*.[53] In rats, the CB_1 receptor has been shown to mediate cardiodepressor and vasodilator effects of endogenous anandamide,[54] and in mice, anandamide was shown to modulate cough sensitivity.[55] Other functions of the ECS in normal physiology may be related to immune modulation, neuroprotection, and bone turnover.[7-14] Taken together, these data indicate that the ECS has pleiotropic functions with important implications for human physiology.

- There appears to be reciprocal regulation between the ECS and the endocrine system

Summary

The ECS is an endogenous signaling system that interconnects food intake, energy balance, stress recovery, and metabolic homeostasis. Transient activation of the ECS, its modulation of appetitive behavior, and the ensuing metabolic sequelae may be viewed in the context of normal physiology. Endocannabinoids are produced on demand to act as local mediators, not as hormones, and they intervene in the sequence of physiological events going from food intake following brief food deprivation, to nutrient digestion and accumulation of energy into adipocytes. Since these events need to be coordinated in time and space, and endocannabinoids act locally, their levels and

Endocannabinoids

- Act as local mediators, not hormones
- Levels and activity are regulated by leptin, glucocorticoids, possibly ghrelin and NPY, dopamine, CCK, and estrogens

actions are under the control of several hormones, including leptin and glucocorticoids, as well as possibly ghrelin and NPY in the hypothalamus, dopamine in the nucleus accumbens, CCK in the brainstem termination of the vagus nerve, and estrogens in both peripheral and central tissues. There is a growing body of evidence to support the notion that obesity, insulin resistance, type 2 diabetes, and dyslipidemia may involve perturbations in one or more components of the ECS. This is discussed in Chapters 4 and 5. Malfunctioning of any of the aforementioned hormones, as well as dietary and genetic factors affecting endocannabinoid biosynthesis and degradation, might underlie ECS overactivity in obesity.

ECS

- Interconnects food intake, energy balance, stress recovery, and metabolic homeostasis

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Chapter 4

The Endocannabinoid System and Obesity

The high prevalence of obesity has resulted in great interest in the study of the biological mechanisms involved in feeding behavior and metabolic regulation.[1] Interactions between the human thrifty genotype and reduced physical activity and increased food intake have been suggested as the root cause of the rising prevalence of obesity and its associated complications.[2] This chapter reviews preclinical as well as clinical data that support a role for the ECS in obesity.

Preclinical Data

The neuronal circuitry involved in regulating caloric intake and energy expenditure is concentrated in the hypothalamus and hindbrain. The CB_1 receptor is an integral component of the hypothalamic networks controlling appetite and food intake.[3] The CB_1 receptor is also highly expressed in mesolimbic dopamine reward circuits within the brain,[3] suggesting that CB_1 receptor activation modulates both caloric and hedonic aspects of eating.

- In rodents CB_1 receptor antagonism reduces food intake and body weight

Studies in obese Zucker rats show that treatment with CB_1 receptor antagonists reduced food intake and body weight gain.[4-6] Selective blockade of the CB_1 receptor also decreased food consumption in genetically normal (wild-type) mice but had no effect on food intake in mice lacking functional CB_1 receptors (CB_1 receptor knockout mice),[7] thereby confirming that the effects of CB_1 receptor antagonists are attributed specifically to endogenous CB_1 receptors. Moreover, heterozygous mice carrying one disrupted allele of the CB_1 receptor gene exhibited a degree of reduced food intake and decreased fat mass that was intermediate between the wild-type mice and CB_1 receptor

- Integrated signals from both the ECS and leptin may regulate appetite
- Rodents lacking normal leptin signals have increased endocannabinoid levels in the hypothalamus

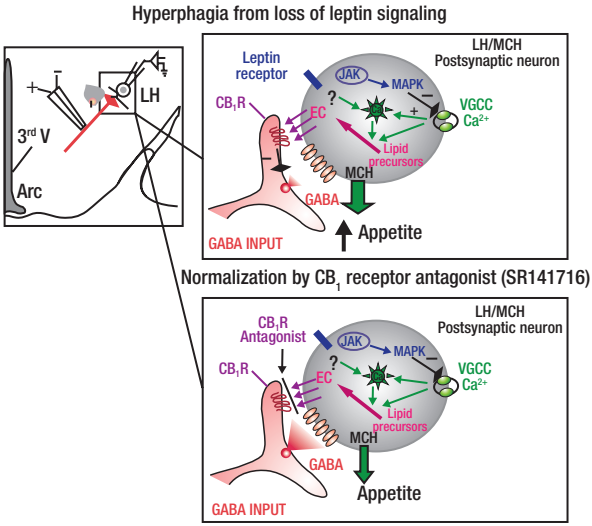

Figure 1. Integration of central CB_1 receptor and leptin signaling in appetite regulation.

- Left: schematic of lateral hypothalamus (LH) illustrating perifornical LH neurons. Melanin-concentrating hormone (MCH) neurons receive GABAergic inputs from diverse brain areas, including the nucleus accumbens/ventral striatum and the arcuate nucleus. The regulation of these GABAergic inhibitory tones to MCH neurons appears to be an important factor for controlling food intake and appetite.
- Right top: proposed model for mechanisms of endocannabinoid signaling and modulation of GABAergic transmission in the perifornical LH neurons of the LH. The activation of presynaptic CB_1 receptors located on the GABA terminal decreases GABA release, thereby enhancing the net excitability of perifornical LH neurons, consistent with increased feeding behavior. The activation of leptin receptors on perifornical LH neurons inhibits voltage-gated calcium currents (VGCC) via activation of janus kinase 2 (JAK2) and MAPK. The consequent decrease in intracellular Ca^{2+} results in less synthesis and release of endocannabinoids, and decreases depolarization-induced suppression of inhibition. Perifornical LH neurons in leptin-deficient, obese mice (*ob/ob*) have larger VGCCs, consistent with increased endocannabinoid signaling, enhanced excitability, and consequent hyperphagia.
- Right bottom: proposed model for mechanisms in which CB_1 receptor antagonist SR141716 decreases body weight and food intake. SR141716 would inhibit CB_1 receptors, antagonizing the elevated endocannabinoids from the MCH neuron. This would potentially normalize GABA release and inhibit MCH release, leading to decreased appetite. From Jo et al.[8]

knockout mice.[9] Thus, the intermediate phenotype of the heterozygous mice further confirms the role of endogenous CB_1 receptors in the regulation of body weight.

Animal and cell culture studies suggest that hypothalamic endocannabinoids may be under negative control by leptin, an adipocyte-derived hormone that inhibits orexigenic signaling in the hypothalamus. Jo et al[8] showed that leptin attenuated the CB_1 receptor-mediated suppression of inhibitory postsynaptic currents in perifornical lateral hypothalamic neurons. These data indicate that integration of endocannabinoid and leptin signaling may regulate the excitability of appetite-related neural circuits (Figure 1). Previously, Di Marzo et al[10] had found that genetically obese rats and mice with disrupted leptin signaling (Zucker *fa/fa* rats and *db/db* mice), as well as mice lacking leptin (*ob/ob* mice), have higher levels of endocannabinoids in the hypothalamus compared with wild-type animals. The influence of leptin on endocannabinoids appears to occur specifically in the hypothalamus, as levels of endocannabinoids in the cerebellum did not differ between *db/db* mice and wild-type mice.[10] In addition, CB_1 receptor blockade reduced food intake in mice lacking leptin (*ob/ob* mice).

- Obese rodents have increased endocannabinoid levels

In rodents, increased endocannabinoid levels have been found in several genetically obese strains, as well as in diet-induced obesity.[6,10-12] Bensaid et al[6] showed that the level of expression of CB_1 receptor mRNA is increased in adipocytes of genetically obese Zucker fa/fa rats. A similar increase in CB_1 receptor mRNA expression was observed in differentiated mouse 3T3 F442A adipocytes compared with undifferentiated adipocyte precursors.[6] In addition, levels of the endocannabinoid 2-AG are substantially increased in differentiated and hypertrophic mouse 3T3 F442A adipocytes (Figure 2).[12] Gary-Bobo et al[13] demonstrated that CB_1 receptor blockade inhibited cell proliferation of cultured 3T3 F442A preadipocytes in a concentration-dependent manner, an effect that may play a role in the reduced fat mass

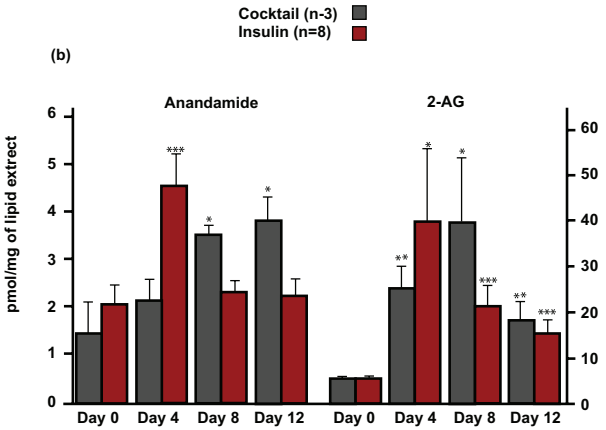

Figure 2. 2-AG levels are increased in differentiated and hypertrophic mouse 3T3 F442A adipocytes. Levels of endocannabinoids are shown during adipocyte differentiation induced with insulin alone (0.9 µm) or in a mixture with 3-isobutyl-1-methylxanthine (0.5 mM) and dexamethasone (1.0 mM). Endocannabinoid levels were measured by isotope-dilution liquid chromatography-mass spectrometry. Data are represented as mean ± SE of 3 to 6 separate experiments. $*P<0.05$, $**P<0.01$, $***P<0.005$ vs control, as assessed by analysis of variance (ANOVA) followed by Bonferroni's test. From Matias et al.[12]

associated with CB_1 receptor blockade in animals.[13] Additional studies are needed to confirm if CB_1 receptor stimulation or blockade affects adipocyte metabolism.

- Mice lacking a functional CB_1 receptor are resistant to diet-induced obesity
- Reduced food intake does not fully account for their lean phenotype

Compelling evidence for a link between the ECS and obesity was also observed in pair-feeding studies with CB_1 receptor knockout mice. Notably, these mice have a lean phenotype and are resistant to diet-induced obesity.[14,15] Despite consuming the same amount of food as the CB_1 receptor knockout mice, the pair-fed wild-type mice became significantly heavier.[14,15] Pair-feeding studies are important, because they demonstrate that factors other than reduced food intake contribute to the lean phenotype of CB_1 receptor knockout mice. Indeed, recent studies in animals and in human subjects

suggest that CB_1 receptor blockade may increase energy expenditure. Kunz et al[16] showed that, compared with control animals, rats treated with 3 and 10 mg/kg rimonabant had an increase in O_2 consumption of 18% and 49%, respectively, after 3 hours. There did not appear to be changes in the rate of carbohydrate and fat oxidation, and factors other than physical activity appeared to contribute to the increase in O_2 consumption.[16] Addy et al[17] measured the effect of the CB_1 receptor antagonist taranabant on resting energy expenditure in 17 overweight or obese subjects. Compared with placebo, the peak resting energy expenditure 2-5 hours post-treatment with 12 mg taranabant was increased significantly.[17] The 12 mg dose of taranabant appeared to increase the rate of fat metabolism, as evidenced by a significant decrease in the mean respiratory quotient compared with placebo.[17]

Clinical Data

Results from clinical studies indicate that the ECS is present in human adipose tissue and is up-regulated in women who are obese. Circulating levels of both anandamide and 2-AG were significantly increased in obese, compared with lean, postmenopausal women.[18] Plasma levels of anandamide were 2-fold higher in obese women with a binge-eating disorder than in normoweight healthy women or normoweight bulimic women.[19] Although the clinical significance of this alteration requires further investigation, it suggests a possible involvement of anandamide in the mediation of the rewarding aspects of some aberrant eating behaviors.[19] Matias et al[12] found that visceral, but not subcutaneous, adipose tissue from obese patients also contains significantly higher levels of the endocannabinoid 2-AG than the visceral fat from nonobese volunteers, thus paralleling the findings in mice with diet-induced obesity (Figure 3).[12] Moreover, circulating 2-AG levels were significantly correlated with body fat and visceral fat mass in lean and obese men and women (Figure 4).[12]

Recently, Bluher et al[20] and Côté et al[21] reported similar data indicating that, in obese men and women, higher 2-AG, but not anandamide, levels in the

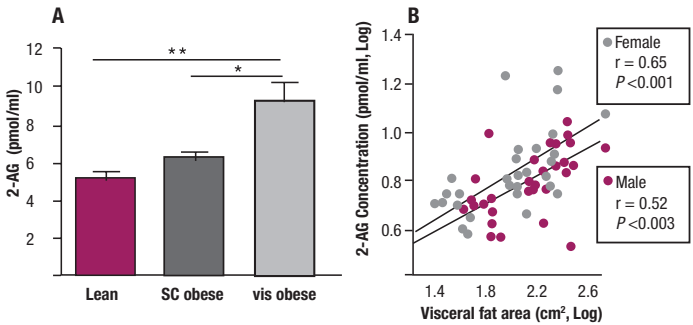

Figure 3. Circulating 2-AG concentrations in lean and obese subjects. Subjects were lean (BMI <25 kg/m^2, n = 20) or obese (BMI >30 kg/m^2). Obese subjects were divided into visceral (vis) obese (n = 20) or subcutaneous (SC) obese (n = 20) groups based on computed tomography measurements of abdominal adipose areas. **A.** 2-AG is increased in subjects with visceral obesity. No significant difference in circulating 2-AG levels in men vs women was found in the three groups. **B.** Circulating 2-AG concentrations correlated with abdominal visceral fat area as measured by computed tomography scans. Data are means ± SE. *P <0.05, **P<0.01 by analysis of variance (ANOVA). From Bluher et al.[20]

blood are specifically correlated with intra-abdominal, but not subcutaneous, adiposity, as well as with several cardiometabolic risk factors normally associated with obesity. The two studies differed in the number of patients sampled, and in the study by Côté et al,[21] the cohort of male obese patients investigated was under no pharmacological treatment. In this study, a negative correlation between elevated blood 2-AG levels and adiponectin was also observed.[21]

- Obese humans have increased 2-AG levels in the plasma and in visceral adipose tissue

A population study in humans provided crucial practical insights into how increased activity of the ECS is associated with overweight and obesity (Figure 5).[22] This study investigated the relationship between a relatively common missense polymorphism for the gene encoding FAAH and overweight/obesity in subjects of multiple ethnic backgrounds attending a medical screening clinic.[22] The variant may result in a functionally deficient protein (subjects with this polymorphism have approximately half the FAAH

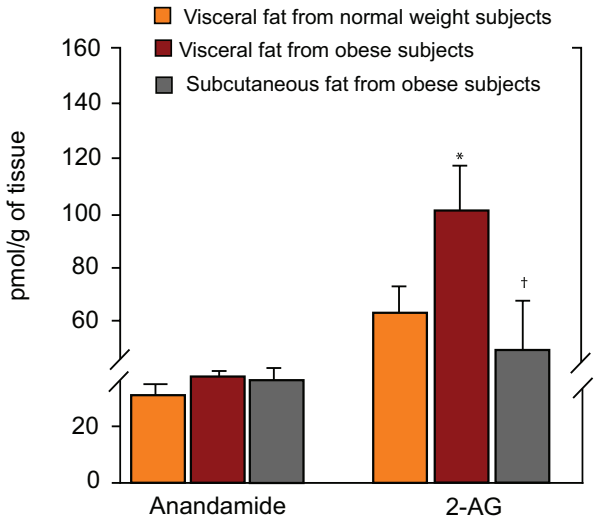

Figure 4. Endocannabinoid levels in visceral adipose tissue of normoweight (4 male, 6 female) and obese (9 male, 11 female) subjects.[11] *$P \leq 0.01$ vs visceral fat from normoweight subjects; †$P < 0.01$ vs visceral fat from obese subjects as assessed by the Kruskal-Wallis nonparametric test. From Matias et al.[12]

enzymatic activity of those subjects without the polymorphism).[22] Results showed that significantly more Caucasian and African-American subjects with this FAAH genotype were overweight or obese than of normal weight.[22] The median body mass index (BMI) for all subjects was significantly greater in the homozygous FAAH polymorphism genotype group compared with subjects with a heterozygous or normal genotype.[22] However, these data have been recently questioned in part by Jensen et al,[23] who could not confirm the association between FAAH polymorphism and obesity in a population of German obese patients.

- FAAH catalyzes the hydrolysis of anandamide
- A polymorphism for the FAAH human gene may be associated with higher body weight

Summary

The role of the ECS in body-weight regulation is now being studied with great interest. Preclinical studies support a role for CB_1 receptors in the regulation

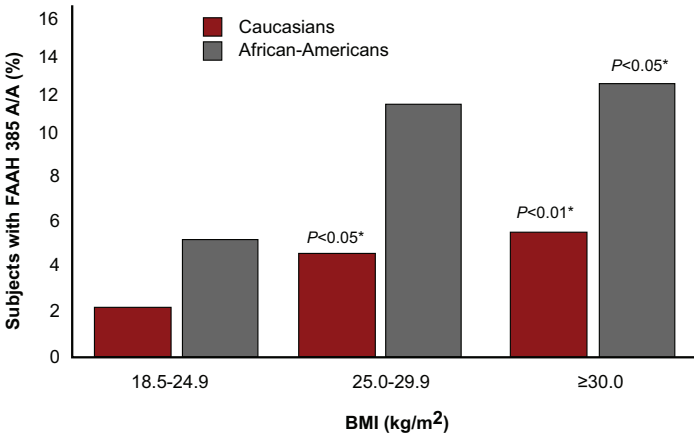

Figure 5. A mutation in the enzyme that degrades endocannabinoids is associated with increased BMI. This study investigated the relationship between a common missense polymorphism for the FAAH gene and overweight/obesity in subjects of multiple ethnic backgrounds. The polymorphism occurs at chromosomal DNA encoding for FAAH, and involves a single substitution of the nucleotide adenine for cytosine. Subjects with this polymorphism have approximately half the FAAH enzymatic activity of those subjects without the polymorphism. Bar graph shows that the median BMI for all subjects was significantly greater in the homozygous FAAH polymorphism genotype group compared with subjects with a heterozygous or normal genotype. In Caucasian subjects, there was an increasing frequency of the homozygous FAAH polymorphism genotype with increasing BMI categories of overweight and obesity; this was also seen in African-American subjects but it was significant only in the obese group. Versus normal BMI. Adapted from Sipe et al.[22]

of fat storage and indicate that the ECS may be activated in obesity. Thus, pharmacologic agents that selectively block the metabolic actions of this system may be a beneficial treatment strategy for obesity.

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Chapter 5

The Endocannabinoid System: Effects on Lipid and Glucose Homeostasis

The ECS and Hepatic Lipid Homeostasis

Multiple studies confirm the link between the ECS and liver biology. Osei-Hyiaman et al[1] demonstrated that hepatic anandamide levels are elevated in diet-induced obesity. The levels of endocannabinoids (anandamide and 2-AG) were measured in the liver of CB_1 receptor knockout mice and wild-type mice fed standard or high-fat diets. After 3 weeks, anandamide levels were significantly increased in both CB_1 receptor knockout and wild-type mice receiving the high-fat diet compared with mice receiving the standard diet. However, the increase was much smaller in the CB_1 receptor knockout mice. There were no significant changes in 2-AG levels in either wild-type or knockout mice. In addition, hepatic levels of CB_1 receptor expression were increased following the high-fat diet in wild-type mice. Wild-type mice developed fatty liver after only 3 weeks on the high-fat diet. In contrast, the CB_1 receptor knockout mice were resistant to the development of fatty liver (Figure 1).[1] A recent study by Gary-Bobo et al[2] further supports a hepatoprotective role of CB_1 receptor blockade. In this study, hepatomegaly in genetically obese Zucker (*fa/fa*) rats was characterized by a higher liver/body weight ratio (4.98% ± 0.15%) compared with that of their lean littermates (3.50% ± 0.18%). Obese (*fa/fa*) rats treated with the CB_1 receptor antagonist SR141716 for 8 weeks (once-daily, orally) had a liver/body weight ratio of 3.85% ± 0.09%, which was comparable to that observed in lean rats. Notably, obese (*fa/fa*) pair-fed rats had only slight reduction in the liver/body weight ratio (4.51% ± 0.17%). Similar results were found when histological analysis of hepatic steatosis was performed; liver slices from obese (*fa/fa*) rats treated with SR141716 were histologically comparable to those from lean rats, whereas there was severe hepatic steatosis in the pair-fed obese (*fa/fa*) rats.[2]

- CB_1 receptor blockade appears to inhibit the development of fatty liver

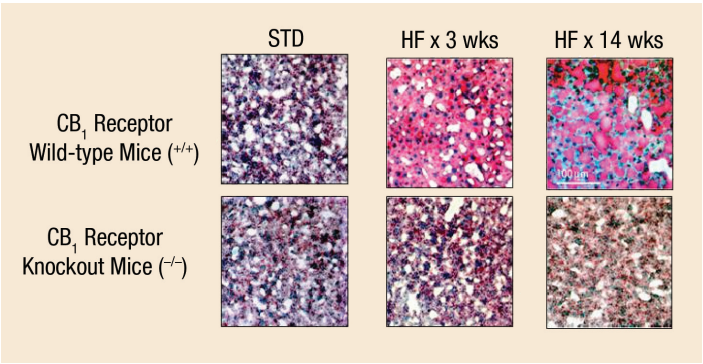

Figure 1. High-fat diet induces fatty liver in obese wild-type mice (*top panels*) but not CB_1 receptor knockout mice (*bottom panels*). Caloric intake did not differ significantly between obese wild-type mice and CB_1 receptor knockout mice. Fat deposition is visualized by Oil Red O staining in liver sections from mice on a standard diet or on a high-fat diet for 3 weeks or 14 weeks. STD, standard diet; HF, high-fat diet. Adapted from Osei-Hyiaman et al.[1]

In other animal studies, investigators have examined whether the ECS acts directly in the liver to regulate hepatic lipogenesis, affecting fatty-acid synthesis and oxidation. CB_1 receptor activation has been shown to enhance de novo lipogenesis in hepatocytes.[1,3] A primary molecular pathway for hepatic lipogenesis involves activation of the transcription factor sterol regulatory element-binding protein-1c (SREBP-1c) and its associated enzymes, acetyl-CoA carboxylase-1 (ACC1) and fatty acid synthase (FAS). Osei-Hyiaman et al[1] demonstrated the role of the ECS in this pathway using rodent models of diet-induced obesity and CB_1 receptor knockout mice. Activation of the CB_1 receptor with a potent receptor agonist (HU210) increased hepatic mRNA expression of SREBP-1c and its target enzymes, ACC1 and FAS, in genetically normal (wild-type) mice fed standard chow (Figure 2).[1] This increase in the expression of lipogenic enzymes was associated with a 2-fold increase in the rate of fatty-acid synthesis in the liver.[1] The role of the CB_1 receptor in mediating this effect was proposed because no increase in fatty-acid synthesis was seen in CB_1 receptor knockout

- The ECS may be involved in obesity-induced nonalcoholic fatty liver disease

mice or in mice pretreated with the CB_1 receptor antagonist SR141716.[1] Hepatic CB_1 receptor stimulation in vivo contributes to activation of the FAS lipogenic pathway, while CB_1 receptor blockade inhibits this effect.[1] As noted earlier, wild-type mice, but not CB_1 receptor knockout mice, became obese and developed fatty liver when they were fed a high-fat diet, despite similar levels of caloric intake between the two groups of mice.[1] The ECS also affects the activity of hepatic AMP kinase, which stimulates fatty-acid oxidation.[4,5] Kola et al[5] demonstrated cannabinoid-induced inhibition of AMP kinase activity in rat liver, although the involvement of CB_1 receptors was not demonstrated.[4,5] These data suggest that the ECS is an important regulator of hepatic fat metabolism and that ECS activation inhibits fatty-acid oxidation and stimulates de novo lipogenesis in the liver. Taken together, these studies suggest that the ECS may play a role in the metabolic mechanisms responsible for obesity-induced nonalcoholic fatty liver disease.

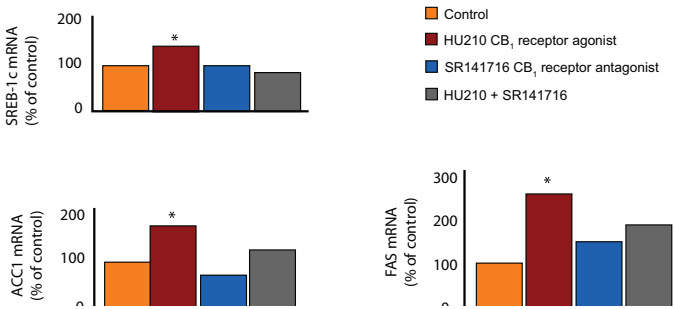

Figure 2. The CB_1 receptor modulates expression of lipogenic enzymes in the liver. Mice were injected intraperitoneally with vehicle, 20 ng/g HU210, 3 µg/g SR141716, or 20 ng/g HU210 plus 3 µg/g SR141716 1 hour prior to sacrifice and removal of the liver. Relative mRNA levels were quantified by densitometry, corrected for 18S ribosomal RNA levels used as a loading control, and expressed as a percentage of the value measured in vehicle-treated controls. ACC1, acetyl-CoA carboxylase-1; FAS, fatty acid synthase; SREBP-1c, sterol regulatory element-binding protein-1c. *P <0.05 vs control. From Osei-Hyiaman et al.[1]

Although nonalcoholic steatohepatitis is an important cause of liver disease, the mechanisms by which hepatic steatosis progresses to steatohepatitis and

hepatic fibrosis are only partially understood.[6] The role of the ECS in hepatic fibrosis, based on several recent studies, appears to be complex; both CB_1 and CB_2 receptors may be involved as well as nonreceptor-mediated effects of endocannabinoids.[7] Teixeira-Clerc et al[8] found that the CB_1 receptor antagonist SR141716 inhibited progression of fibrosis in mouse models of chronic liver injury. On the other hand, that same group had previously demonstrated that the CB_2 receptor was involved in antifibrogenic pathways in the liver.[9] Most recently, a study by Siegmund et al[10] suggested that 2-AG may produce antifibrogenic effects in the liver by inducing cell death in activated hepatic stellate cells, but not hepatocytes. This study demonstrated that 2-AG–induced cell death of rat and human hepatic stellate cells is mediated by mitochondrial reactive oxygen species. Of note, the 2-AG–induced cell death was independent of CB_1 and CB_2 receptors.[10] Thus, while it is clear that the ECS plays a role in hepatic fibrosis, significant complexity appears to exist, and whether activation or inhibition of CB_1 receptors will be beneficial remains to be clarified.

- The ECS plays a role in hepatic fibrosis
- It is unclear if ECS activity has causal or protective effects

The ECS and Dyslipidemia

Animal studies suggest that CB_1 receptor blockade can reverse the lipid abnormalities associated with diet-induced obesity. Poirier et al[11] investigated the effects of chronic treatment with SR141716 10 mg/kg per day orally for 10 weeks in mice with obesity induced by 5 months of a high-fat diet. Diet-induced obesity in mice led to abnormal serum lipid profiles. Treatment with SR141716 significantly reduced triglyceride (TG) and low-density lipoprotein cholesterol (LDL-C) levels and notably increased the high-density lipoprotein cholesterol (HDL-C)/LDL-C ratio (12.4 vs 7.9 in the high-fat control group, $P < 0.001$).[11] However, the body weight of mice treated with SR141716 was significantly lower than the body weight of mice in the high-fat control group, underscoring the importance of a pair-feeding protocol in these types of studies. A recent study in genetically obese Zucker (*fa/fa*) rats suggests that improvements in obesity-associated dyslipidemia in

animals treated with a CB_1 receptor antagonist are independent of weight loss (Figure 3).[2] Thus, CB_1 receptor blockade may produce liver-specific effects associated with changes in hepatic lipogenesis and/or lipoprotein secretion. Additional studies are needed to determine whether the ECS specifically affects hepatic lipoprotein production, and to confirm the weight-loss–dependent and weight-loss–independent effects of SR141716 on serum lipid profiles.

- CB_1 receptor blockade can reverse dyslipidemia in mice with diet-induced obesity

The ECS and Glucose Homeostasis

Glucose homeostasis is mediated in part by metabolic and hormonal interactions among the pancreas, liver, adipose tissue, skeletal muscle,

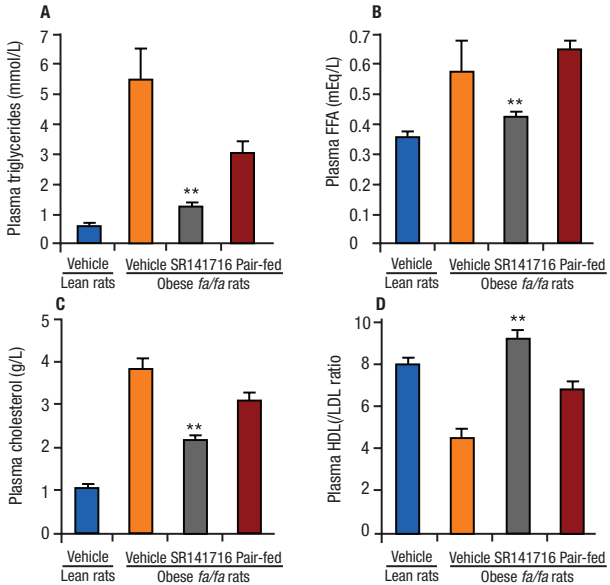

Figure 3. CB_1 receptor blockade attenuates dyslipidemia in obese (*fa/fa*) rats. (**A**) Plasma level of triglycerides; (**B**) Plasma level of free fatty acids; (**C**) Plasma level of total cholesterol; and (**D**) HDL-C/LDL-C ratio. FFA, free fatty acids; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol. **P* <0.01 compared with obese rats treated with vehicle. From Gary-Bobo et al.[2]

and GI tract; all of which express the CB_1 receptor. Understanding the role of the ECS on pancreatic β-cell function may provide additional insights into the effects of this system on glucose metabolism. Both CB_1 and CB_2 receptors are expressed in intact pancreatic islets of Langerhans isolated from mice.[12] CB_1 receptors were most abundant in the glucagon-producing α-cells, whereas CB_2 receptors were present in both β-cells and the insulin-producing α-cells.[12] Another study showed that CB_1 receptor mRNA and protein are expressed in rat islet cells and in the exocrine pancreas.[13] More recently, CB_1 and CB_2 receptor protein was detected in rat pancreatic β- and non–β-cells.[14] The meaning of these differences between mice and rats for human islet and β-cell CB_1 and CB_2 receptor expression is unclear. Pharmacological activation of CB_1 receptors in vitro appears to stimulate insulin secretion from rat-derived pancreatic β-cells.[13,15] Thus, when MIN6 or RIN-m5F insulin-secreting cells were stimulated with CB_1 receptor agonists and CB_1 receptor antagonists, the CB_1 receptor agonists caused a significant potentiation of glucose-stimulated insulin secretion from the cells. Although the CB_1 receptor antagonists did not inhibit insulin secretion, they blocked the effect of the agonists.

- CB_1 and CB_2 receptors are expressed in rodent pancreas

Endocannabinoid levels are increased in cells when cultured in high-glucose containing media. Levels of both anandamide and 2-AG were increased when RIN-m5F rat insulinoma β-pancreatic cells were cultured in media containing low (13 mM) and high (25 mM) concentrations of glucose and then stimulated with 33 mM glucose.[15] In cells cultured in 13 mM glucose medium, the effect of 33 mM glucose was abolished when co-administered with 100 nM insulin. However, insulin failed to inhibit the 33 mM glucose-induced endocannabinoid elevation in RIN-m5F cells cultured in 25 mM glucose.[15] This study suggests that chronic hyperglycemia may lead to increased ECS activity (as reflected by elevated levels of anandamide and 2-AG), and that the increased ECS activity occurring with severe hyperglycemia is insensitive to inhibition by insulin.

Animal models of obesity have provided important evidence supporting the clinical potential for CB_1 receptor blockade.[16] Ravinet Trillou et al[16] studied wild-type and CB_1 receptor knockout mice fed a high-fat diet. Both groups demonstrated an increase in fasting glycemia. The glucose-lowering effect of an intraperitoneal insulin injection was reduced in the wild-type mice fed a high-fat diet, but this effect was maintained in the CB_1 receptor knockout mice at the same level as control mice that were fed standard chow. Thus, when fed a high-fat diet, the CB_1 receptor knockout mice did not develop the insulin resistance that was observed in the wild-type mice. In addition, the CB_1 receptor knockout mice maintained their lean phenotype despite consuming a high-fat diet. However, it is unclear whether protection from insulin resistance is independent of protection from obesity in the CB_1 receptor knockout mouse fed a high-fat diet.

- CB_1 receptor blockade improves glucose homeostasis in mice with diet-induced obesity

Animal studies also demonstrate that selective blockade of the CB_1 receptor with the antagonist SR141716 ameliorates abnormalities in glucose metabolism associated with diet-induced obesity.[17] This response was studied in diet-induced obese mice. Treatment with SR141716 (10 mg/kg/day) for 5 weeks led to a transient reduction of food intake (–48% on week 1) and a marked but sustained reduction of body weight (–20%) in obese mice fed a high-fat diet. The fasting insulin and glucose levels of the high-fat–fed mice treated with SR141716 were reduced to the levels observed in the mice fed standard chow. In contrast, the nontreated mice fed the high-fat diet had elevated fasting insulin and glucose levels (Figure 4).[16] It should be noted that pair-fed controls were not included in this study. It is therefore not possible to determine how much the induced weight loss contributed to the improvement of glucose homeostasis compared with the direct effect of treatment with SR141716.

Studies in mice suggest that modulation of glycemic control by the ECS may be mediated in part by effects on skeletal muscle. Liu et al[18] showed that the rate of glucose uptake by isolated soleus muscle was significantly increased

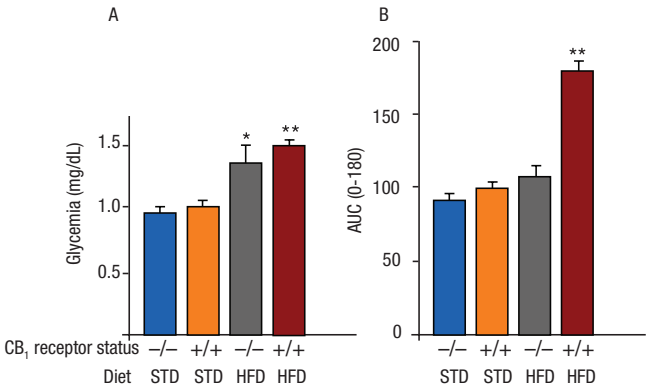

Figure 4. CB_1 receptor blockade is associated with decreased fasting glycemia (A) and enhanced insulin sensitivity (B) in obese mice on a high-fat diet. Wild-type (+/+) and CB_1 receptor knockout (–/–) mice were fed a standard diet (STD) or a high-fat diet (HFD) for 12 weeks. Insulin sensitivity was assessed by calculating the area under the curve (AUC) of glycemia during the 3 hours after intraperitoneal insulin (0.6 U/kg) injection in fasted mice. Values are means ± standard error of the mean (SEM). $*P<0.05$, $**P<0.01$. From Ravinet Trillou et al.[16]

in mice treated with SR141716 for 7 days compared with vehicle-treated mice. However, it is unclear whether this effect was independent of changes in body weight.

Gene expression studies also support a role of the ECS in glucose homeostasis. For example, CB_1 receptor blockade is associated with increased expression of genes involved in glucose metabolism. Oral treatment with the CB_1 receptor antagonist SR141716 enhanced the expression of genes that are critical regulators of glucose metabolism in mice with diet-induced obesity and significantly increased levels of 2-AG in epididymal fat.[15,19] Moreover, global gene expression analyses demonstrated that SR141716 enhanced the expression of 4 of the 8 glycolytic enzymes found in adipose tissue: phosphofructokinase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate mutase, and β enolase.[19] Samples from pair-fed controls were not analyzed in this study. However, the transcriptional patterns observed in adipose tissue of SR141716-treated obese mice were similar

to those seen in CB_1 receptor knockout mice fed a high-fat diet, and are resistant to obesity, supporting a CB_1 receptor-mediated process.

Overweight subjects with type 2 diabetes and hyperglycemia exhibited blood endocannabinoid levels significantly higher than those of age- and BMI-matched normoglycemic subjects (Figure 5).[15] These results indicate that the normal regulation of blood endocannabinoid levels may be disrupted in persons with hyperglycemia,[15] although additional studies are needed to determine if the higher visceral fat endocannabinoid levels observed in obese individuals are related to the insulin resistance associated with overweight and obesity.

- Adiponectin is an adipocyte-derived hormone
- ECS effects on glucose metabolism may involve changes in adiponectin levels

Although speculative, changes in adiponectin may be related to ECS effects on glucose metabolism. Adiponectin, a hormone that is derived primarily from adipocytes,[20,21] inhibits both the expression of hepatic gluconeogenic enzymes and the rate of endogenous glucose production.[22] Clinically, adiponectin levels rise in situations where insulin sensitivity improves, such as after weight loss or treatment with insulin-sensitizing drugs.[20] Studies in rodents suggest that adiponectin facilitates the actions of insulin in muscle and liver, and may also act in the central nervous system to facilitate glucose disposal and increase total energy oxidation.[20,23] Moreover, polymorphisms in the gene coding for adiponectin are associated with obesity and insulin resistance.[20] Studies in mice have shown that central administration (intracerebroventricular) of adiponectin leads to reductions in body weight and improvements in glucose metabolism.[23] CB_1 receptor stimulation may decrease adiponectin expression in adipocytes,[15] and CB_1 receptor blockade with SR141716 increased adiponectin levels in both diet-induced obese mice[11] and genetically obese rats.[24] Importantly, these changes in adiponectin levels were associated with favorable changes in serum insulin and glucose levels.[11,24] It is unclear whether the increased adiponectin levels were independent of weight loss in the animals treated with SR141716. However, in vitro treatment of mouse

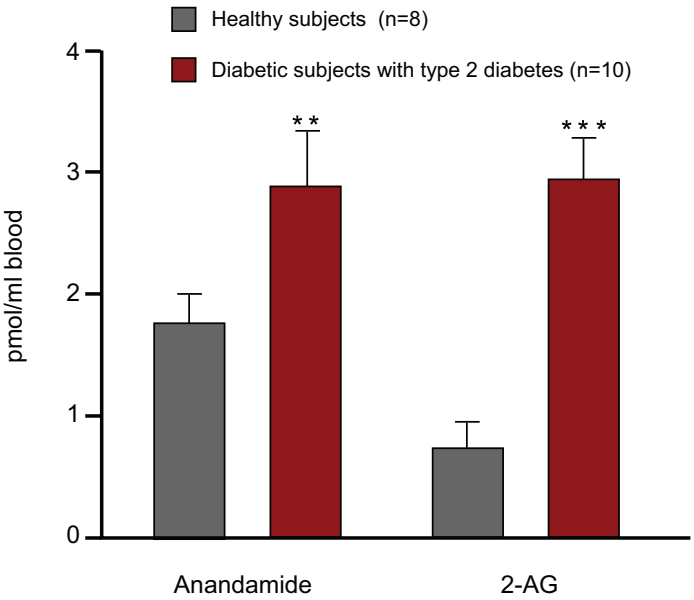

Figure 5. Dysregulation of blood endocannabinoid levels in hyperglycemia. This figure shows serum endocannabinoid levels in overweight subjects with type 2 diabetes compared with healthy volunteers. The study was designed uniquely to assess whether a noncorrected hyperglycemia, due to a pathological condition, results in increased serum endocannabinoid levels. For this reason, the study included male and female subjects with type 2 diabetes receiving randomized pharmacological treatments and whose only common clinical features were hyperglycemia (approximately 1.85 g/L), age (approximately 65 years), and BMI (approximately 30 kg/m^2). $^{**}P < 0.01$, $^{***}P = 0.005$ (vs controls, as assessed by the Kruskal-Wallis nonparametric test). From Matias et al.[15]

adipocyte cells with SR141716 was associated with significantly increased levels of adiponectin mRNA compared with control cells.[24]

Summary

Animal and cell culture studies suggest that selective pharmacologic antagonism of the CB_1 receptor improves lipid abnormalities associated with obesity, specifically, increased serum TG and decreased serum HDL-C

concentration. In addition, the CB_1 receptor appears to be an important component in the regulation of hepatic fat metabolism; ECS activation simultaneously inhibits fatty-acid oxidation and stimulates de novo lipogenesis in the liver.

Preclinical data indicate that the ECS may play a role in glucose homeostasis. Normal regulation of blood endocannabinoid levels may be disrupted in persons with hyperglycemia[15] and CB_1 receptor blockade in animals appears to enhance glucose uptake.[13,25] Additional studies are needed to determine whether there is a relationship between CB_1 receptors and insulin sensitivity in adipose tissue and to determine the effects of CB_1 receptor blockade on hepatic insulin clearance and glucose production.

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Chapter 6

The Role of the Endocannabinoid System in the Central Nervous System

Pivotal studies over the past 2 decades firmly established the presence and distribution of cannabinoid CB_1 receptors, endocannabinoids, and their metabolic enzymes in the brain,[1-7] thereby confirming the presence of the endocannabinoid system (ECS) in the central nervous system (CNS). In 1988, Howlett et al[8] described the presence of high-affinity binding sites for cannabinoids in rat brain membranes. Shortly afterwards, Herkenham et al[9] performed autoradiographic mapping studies of cannabinoid binding sites in rat, human, rhesus monkey, dog, and guinea pig brain.[9] Matsuda et al cloned the CB_1 receptor[10] and determined its distribution by in situ hybridization studies in rat brain.[11] Determining the location of CB_1 receptors in the brain has provided significant insight into the function of the ECS in the CNS (Table 1).[12]

Table 1. CB_1 Receptors in the Central Nervous System

Structure	Function
Hippocampus	Cognition and encoding memory
Cerebellum	Coordination of motor function, posture, balance
Basal ganglia	Motor function, reinforcement behaviors
Hypothalamus	Thermal regulation, neuroendocrine regulation, appetite
Spinal cord	Nociception
Brain stem	Nausea and emesis, appetite
Cerebral cortex	Cognition, emesis
Prefrontal cortex	Executive function, reinforcement

Adapted from Croxford, 2003.[12]

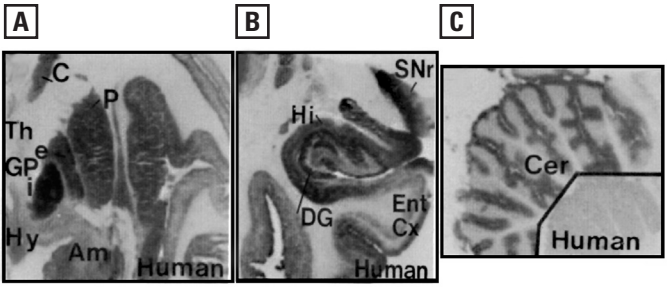

Figure 1. Coronal sections of human brain showing autoradiography of [^{3}H]CP-55940 binding. [^{3}H]CP-55940 is a radiolabeled synthetic cannabinoid used to visualize the distribution of cannabinoid receptors (predominantly CB_1 receptors in the brain). Gray levels represent relative levels of receptor densities. (A) C, caudate; P, putamen; TH, thalamus; GP, caudate-putamen (e, external; i, internal); Hy, hypothalamus; Am, amygdala (x 1.3 magnification). (B) SNr, substantia nigra pars reticulata; Hi, hippocampus; DG, dentate gyrus; Ent Cx, entorhinal cortex (x 1.7 magnification). (C) Cer, cerebellum (x 2.6 magnification). Reproduced with permission.[9]

Cell Biology

CB_1 receptors are among the most abundant receptors in the brain.[13] In the brain areas with the highest levels of CB_1 receptors, their density is similar to levels of γ-aminobutyric acid- (GABA) and glutamate-gated ion channels.[14] [^{35}S]GTPγS autoradiography studies in rat brain demonstrate that the distribution of cannabinoid-activated G proteins, in general, parallels CB_1 receptor mRNA expression and binding.[14] [^{3}H]CP-55940 autoradiography studies demonstrate the presence of CB_1 receptors in human brain (Figure 1). The extent of CB_2 expression in brain is controversial. However, expression of CB_2 receptor mRNA and protein were recently demonstrated in rat and ferret brainstem neurons.[15] Determining the extent of CB_2 receptors in the brain is an active area of investigation. An interesting aspect of CB_2 receptors is that in many cases their levels strongly increase following a pathological insult. For example, CB_2 receptor expression is induced in brain microglial cells during inflammation.[16]

An important role for CB_1 receptors in the brain is to mediate retrograde signaling. This is defined as the communication by signaling molecules (in this case, endocannabinoids) derived from postsynaptic and delivered to presynaptic structures (opposite to the direction of travel of conventional neurotransmitters) (Figure 2).[17-19] Substantial evidence demonstrates that retrograde signaling underlies a variety of short- and long-term changes in synaptic efficacy.[18]

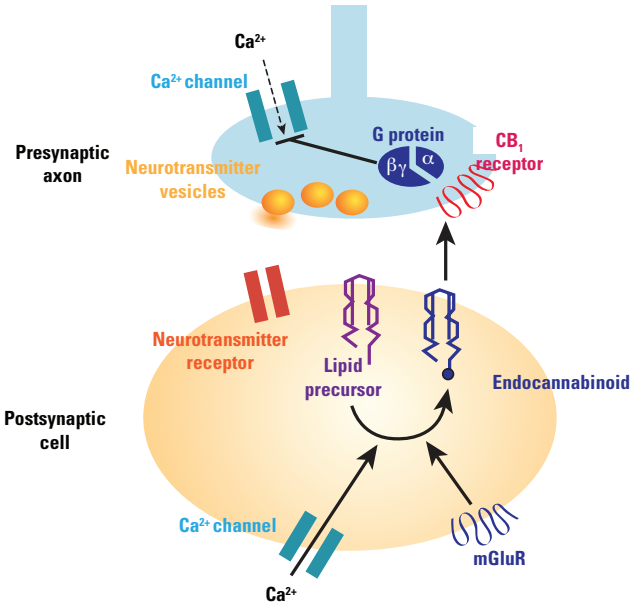

Figure 2. Retrograde signaling by endocannabinoids in the brain. Postsynaptic depolarization opens voltage-dependent Ca^{2+} channels. The influx of Ca^{2+} through these channels activates the enzymes that synthesize endocannabinoids from arachidonic acid-containing membrane phospholipids. Activation of postsynaptic group I metabotropic glutamate receptors (mGluRs) can also generate endocannabinoids. Endocannabinoids then leave the postsynaptic cell and activate CB_1 receptors located in the presynaptic cell membrane. G-protein activation liberates Gbg which inhibits Ca^{2+} influx. This decreases the probability of release of a vesicle of neurotransmitter. Reproduced with permission.[13]

CB_1 receptors are localized mainly on axons and axon terminals where their stimulation is directly coupled to inhibition of certain voltage-activated Ca^{2+} channels.[20,21] Endocannabinoid activation of CB_1 receptors also opens somatic potassium (K^+) channels, and the resulting hyperpolarization inhibits neuronal firing.[22] Thus, inhibition of Ca^{2+} channels and stimulation of K^+ channels both contribute to inhibition of neuronal excitability and suppression of neurotransmitter release.[20] CB_1 receptor activation attenuates GABA and glutamate release from CB_1 receptor–containing nerve terminals.[21] Endocannabinoid-mediated activation of CB_1 receptors on nerve terminals is widespread in the brain. It inhibits neurotransmitter release in many brain regions including the striatum, hippocampus, cerebellum, cortex, hypothalamus, and nucleus accumbens.[23] In addition to inhibiting glutamate and GABA release, CB_1 receptor activation also inhibits serotonin and acetylcholine release at other synapses and inhibits release of neuropeptides.[21,24]

It is likely that some of the CNS effects of anandamide occur through a complex interplay with other systems (see Chapter 2). For example, high concentrations of anandamide activate the transient receptor potential vanilloid (TRPV1) receptor (ion channel found on sensory neurons) causing Ca^{2+} influx and subsequent neurotransmitter release.[25]

Roles of the ECS in the Central Nervous System

Appetite

Ample preclinical data suggest that CB_1 receptor stimulation in the CNS facilitates feeding. For example, short-term food deprivation in rats leads to elevated levels of brain endocannabinoids (see Chapter 3, Figure 1),[26] and administration of 2-AG into the shell subregion of the nucleus accumbens (a limbic forebrain area implicated in eating motivation) induces short-term hyperphagia (abnormally increased consumption of and appetite for food).[26]

CB_1 receptor signaling is intimately involved in several forms of neuronal plasticity; that is, the ability of nerve cells to change their properties for

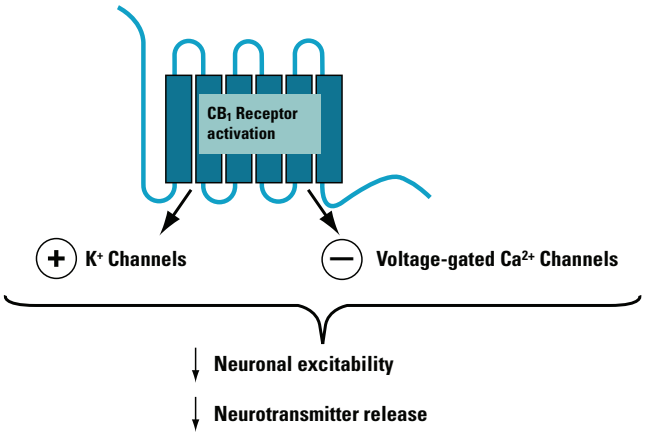

Figure 3. Inhibition of Ca^{2+} channels and stimulation of K^+ channels both contribute to inhibition of neuronal excitability and suppression of neurotransmitter release.[20] CB_1 receptor activation leads to inhibition of voltage-gated Ca^{2+} channels, which may be involved in decreasing Ca^{2+} influx, glutamate release, and subsequent excitotoxic progression. CB_1 receptor activation also inhibits adenylyl cyclase activity and its downstream signaling pathways (see Chapter 2), which may play a role in the therapeutic effects of cannabinoid agonists. K^+ channel-induced hyperpolarization of the cell with the subsequent decrease neurotransmitter release may also be involved in neuroprotection.[22] Adapted from Karanian and Bahr.[80]

example by making new synapses or altering the strength of existing synapses. Energy balance is regulated by a complex interaction of neural signals emanating from the brain, as well as hormones from peripheral organs that act on the brain and other tissues (see Chapter 3). For example, the adipocyte-derived protein, leptin, reduces food intake by activating leptin receptors in the hypothalamus.[27] One influence of leptin on endocannabinoid levels appeared to occur in the hypothalamus, as levels of hypothalamic anandamide and 2-AG decreased in normal rats treated with intravenous leptin (vs rats treated with control solution). Hypothalamic levels of anandamide and 2-AG increased in mice with disrupted leptin signaling (*db/db* mice).[28] In contrast, levels of endocannabinoids in the cerebellum did not differ between *db/db* mice and wild-type mice.[28]

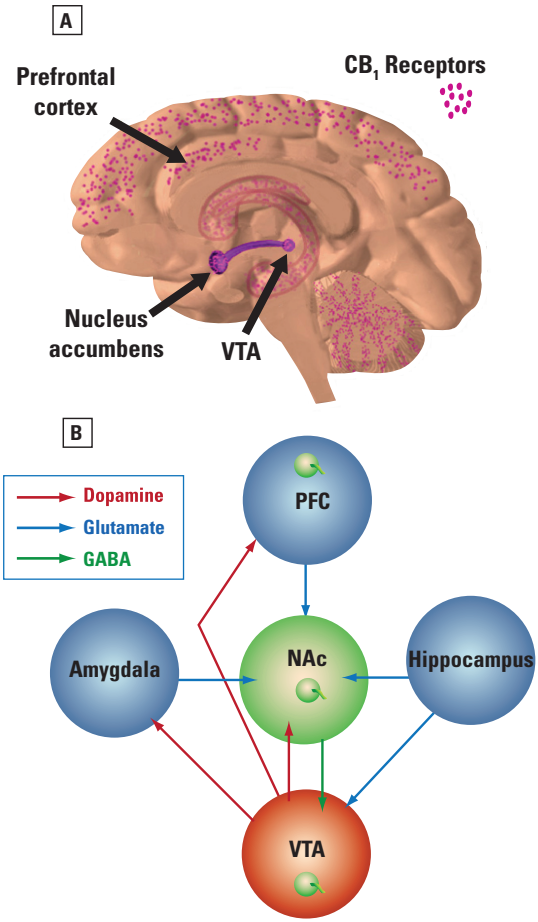

Figure 4. (A) A simplified schematic of the brain reward system: the ventral tegmental area (VTA), the nucleus accumbens (NAc), and the prefrontal cortex (PFC). Dopaminergic axons project from the VTA to the NAc and the PFC. This pathway is activated by the expectation of rewarding stimuli. Expression of CB_1 receptors is represented by pink dots, based on THC-binding studies. (B) Neurotransmitter pathways that CB_1 receptors might modulate in cue- and drug-induced reinstatement of drug-seeking. CB_1 receptor expression is high on neurons impinging on mesolimbic dopamine reward circuits, where perceptions associated with

pleasure/palatability and appetite/incentive stimuli are processed.[119] Functional interactions have been reported between CB_1 receptors and the dopamine, GABA, and glutamate systems. For example, CB_1 receptor activation enhances the release of dopamine from VTA-originating neurons by disinhibition of GABA-containing interneurons in this area. At the level of the NAc, the release of glutamate from neurons originating in several cortical and subcortical areas that are known to be involved in relapse is modulated by CB_1 receptors. Stimulation of NAc CB_1 receptors may suppress glutamatergic activity, with consequent inhibition of GABAergic neurons that normally inhibit VTA dopamine neurons. The basolateral amygdala and hippocampus have an important role in mediating discrete and contextual cue-induced relapse. The anatomical sites where CB_1 receptors exert their modulatory action on drug-seeking behavior are still unknown.

Neurons in the lateral hypothalamus appear to play a role in the motivational aspects of food intake. Jo et al[29] showed that leptin attenuates the CB_1 receptor-mediated suppression of inhibitory postsynaptic currents in perifornical lateral hypothalamic neurons (see Chapter 4, Figure 1). Presumably, this is due to a suppression of endocannabinoid formation by leptin.[29] In addition, hormones from the gastrointestinal tract act in concert with central mechanisms to regulate food intake.[30] Moreover, hormones and metabolites, including insulin and fatty acids, cross the blood–brain barrier in the hindbrain and hypothalamus, thereby modulating hunger and satiety.[31]

Cognition and Memory

Studies in rodents indicate that acute and chronic treatment with CB_1 receptor agonists disrupts working memory (memory of new/recent information).[32-35] However, reference memory (recall of previously learned information) does not appear to be similarly affected.[36] Animal studies also demonstrate that endocannabinoids facilitate selective extinction of aversive memories.[37,38] Electrophysiological data in mice suggest that endocannabinoids can both produce long-term depression and enhance long-term potentiation (both cellular correlates of learning),[39] providing a clue to the cellular mechanisms by which the ECS may influence cognition. Selective CB_1 receptor blockade can enhance mnemonic processes in rats, mice, and birds[40-42] and enhance memory in rodents.[43-45] Moreover, CB_1 receptor knockout mice exhibited enhanced memory.[41] In humans, acute

administration of the cannabinoid THC transiently impairs immediate and delayed free recall of information presented while under the influence of the drug.[46] While the ECS appears to be intimately involved in several forms of cognition, additional studies are needed to fully define this relationship.

Emotionality

There are ample—albeit discordant—data on the ECS and emotionality, likely reflecting the model system used in the various studies. Animal studies suggest that altered ECS activity can affect anxiety- and depression-like behavior.[47] Both high and low levels of ECS activity have been linked with mood disorders.[48-50] Preclinical and human studies are equivocal with regard to the effect of CB_1 receptor blockade and emotional responses to stress.[51-54]

Haller et al[55] observed increased anxiety-like behavior in CB_1 receptor knockout mice compared with wild-type mice when the animals were exposed to a stressful environment. Other data suggest an antidepressant effect of CB_1 receptor blockade. For example, Shearman et al[47] treated wild-type mice with the cannabinoid receptor inverse agonist antagonist AM251 and subjected them to the tail-suspension test (TST) and forced-swim test (FST), in which antidepressant activity is determined by increased mobility. Similar to the antidepressant desipramine, AM251 significantly reduced immobility in both the TST and FST. AM251 induced antidepressant-like effects appeared to be mediated by CB_1 receptors. This is supported by the observations that: 1) co-administration of the CB_1 receptor agonist CP55940, at a dose that did not induce motor impairment or profound hypothermia, reversed effects of AM251 in the TST; and 2) effects of AM251 in the FST were absent in CB_1 receptor knockout mice.

Human studies suggest a relationship between CB_1 receptor activity and affective disorders, and support an antidepressant-like potential for CB_1 receptor blockade (reviewed in Witkin et al).[51] However, the role of the ECS in depressive disorders is complex, and there is scientific debate on the role

of CB_1 receptor antagonism and agonism in major depressive and anxiety disorders.[51-54,56] Clinical studies are needed to determine the potential effects of CB_1 receptor blockade treatment on emotionality in different patient populations. Rimonabant was approved by the European Medicines Agency (EMEA) in June 2006; however, the EMEA recommended that rimonabant be contraindicated in patients with ongoing major depression and in patients being treated with antidepressants following the FDA's advisory committee decision to vote against recommending the approval of rimonabant due in part to lack of safety data in people with depression.[57,58] Although depression and anxiety are manageable disorders, there is a need for prospective analysis of emotionality in clinical trials with CB_1 receptor antagonists.

Nausea and Emesis

Preparations of cannabis were used for the treatment of nausea 3000 years ago. Current studies suggest that the ECS may play an important role in the physiology of nausea and emesis, common side effects of many drugs (eg, cancer chemotherapy) and diseases (eg, irritable bowel syndrome and migraine).

Nausea

Synthetic cannabinoids (Δ^9-THC) are FDA approved for the treatment of nausea and vomiting associated with cancer chemotherapy (ie, nabilone and drobinol).[59,60] Anticipatory nausea and vomiting appear to be best explained by classical conditioning, where a previously neutral stimulus (eg, smells of the chemotherapy environment) elicits a conditioned response (eg, anticipatory nausea and vomiting) after a number of prior pairings.[61] Cancer chemotherapy may be "paired" with a variety of other neutral, environmental stimuli (eg, smells of the setting, oncology nurse, chemotherapy room).[61] These previously neutral stimuli may then elicit anticipatory nausea and vomiting in future chemotherapy cycles. Many patients report anticipatory nausea and vomiting upon re-exposure to the cues previously associated with treatment.[60] In a rat model of anticipatory

nausea, a gaping reaction is induced during exposure to a context previously paired with lithium chloride–induced illness.[62] Pretreatment with THC suppressed the lithium-induced gaping reaction.[62] CB_1 receptor blockade with SR141716 potentiates lithium-induced nausea in rats.[63,64] Interestingly, AM4113 did not induce this conditioned gaping in rats. The CB_1 receptor neutral antagonist AM4113 binds to cannabinoid receptors, but appears not to exhibit inverse agonist effects on cyclic AMP production.[65] These results suggest that AM4113 may decrease appetite by blocking cannabinoid tone, and that this drug may be less associated with nausea than CB_1 receptor inverse agonists.[65] Taken together, these preclinical data may explain how activating CB_1 receptors can be beneficial in treating anticipatory nausea associated with chemotherapy, and suggest that CB_1 receptor antagonists may accentuate the nausea associated with chemotherapy. In clinical trials, the CB_1 receptor antagonist rimonabant has been shown to induce nausea in 11.2%-12.9% of subjects receiving 20 mg of rimonabant compared with 3.2%-6.0% of subjects receiving placebo.[66-69]

In a 12-week weight-loss study, CB_1 receptor blockade with taranabant induced nausea in 10.4%-31.4% of subjects receiving 0.5-6 mg of taranabant compared with 6.7% of subjects receiving placebo.[70]

Emesis

ECS stimulation with THC produces anti-emetic effects.[71] In animal models, cisplatin-induced emesis in shrew was blocked by THC and synthetic cannabinoid agonists.[73] The anti-emetic effects may involve both central and peripheral mechanisms.[71] A study by Van Sickle et al[15] suggests that brainstem CB_2 receptors play a role in the inhibition of emesis. In this study, the anti-emetic effects of 2-AG and the endocannabinoid reuptake inhibitor VDM11 were reversed when ferrets were cotreated with the CB_2 receptor antagonist AM630.[15] Other studies in ferrets showed that the CB_1 receptor mediates the anti-emetic action of cannabinoids in the dorsal vagal complex.[73,74] More recently, data from the ferret model demonstrated the presence of an endogenous tone on CB_1 and TRPV1 receptors that inhibits emesis.[75]

Motor Function

CB_1 receptor agonists typically reduce locomotor activity. This is manifested by both a decrease in spontaneous activity and rigid immobility (at higher doses).[76] Preclinical data suggest that signaling by anandamide acting on TRPV1 receptors may modulate spontaneous and L-3,4-dihydroxyphenylalanine (L-DOPA)–induced locomotion in rats,[77] and that endocannabinoid signaling through the CB_1 receptor may be required for cerebellum-dependent discrete motor learning in mice.[78] At low concentrations (<1 µM) in vitro and low doses (<3 mg/kg) in vivo, SR141716 and AM251 appear to be selective for the CB_1 receptor. However, it is important to note that the effects of a CB_1 receptor antagonist will depend on the endocannabinoid tone of the system (ie, the level of basal ECS activity). When the endocannabinoid tone of that system is low, the physiologic effects of a CB_1 receptor inverse agonist may be observed as the "inverse" of agonist effects in a particular system. Alternatively, studies carried out at high doses may uncover interactions with non-CB_1 receptors, which might explain the hyperactivity observed with SR141716 at <10 mg/kg.[79]

Neuroprotection

The ECS has been implicated in protection against human diseases of the CNS in several animal models (reviewed in Karanian and Bahr).[80] Cannabinoid agonists and cannabinoid antagonists may hold therapeutic potential (both for symptom management and for disease progression) for various CNS disorders, such as multiple sclerosis, Parkinson's disease, and Huntington's disease (reviewed in Pacher, Bátkai, and Kunos, 2006).[81] This therapeutic potential is under investigation.

The ECS, including both CB_1 and CB_2 receptors, may play a role in the pathophysiology of various neuropathologies (reviewed in Howlett et al[82]). Thus, manipulation of ECS activity can be protective or toxic to the CNS.

Examination of human postmortem brains showed that levels of FAAH and CB_2 receptors were increased in Alzheimer's disease.[83] It has been speculated that endocannabinoids might be protective during inflammatory neurodegeneration. For example, enhancing brain 2-AG levels with an inhibitor of endocannabinoid reuptake (VDM11) seems to protect against β-amyloid neurotoxicity in rats.[84] Thus, modulating ECS activity might be a future therapeutic target for the treatment of neurodegenerative diseases.[85]

Neuroexcitability

Excessive activity of excitatory (ie, glutamatergic) systems could lead to the pathological processes of excitotoxicity,[86] and ECS activation may serve to protect neurons against acute excitotoxicity.[84] This effect may involve specific cellular signaling pathways, including decreased Ca^{2+} influx and decreased glutamate release (Figure 3).[87,88]

It is important to consider the relative protective vs excitotoxic CNS effects of ECS activity in the context of the particular animal neurological model, and with regard to acute injury or chronic repair. Indeed, the impact of CB_1 receptor blockade on outcome following various models of brain injury is conflicting. For example, CB_1 receptor blockade with SR141716 produced protective effects in some ischemic models of brain, despite the fact that CB_1 agonists also exhibited the same protective effect in other models.[89-92] The CB_1 receptor antagonists SR141716 and AM251 increased neurogenesis by approximately 50% in the mouse dentate gyrus and subventricular zone.[93] However, this increase in neurogenesis was observed in CB_1 receptor knockout mice, but not in vanilloid TRPV1 receptor knockout mice, suggesting that the effect involved TRPV1 receptors and not CB_1 receptors.[93] Thus, ECS activity is associated with both neuroprotection[94-96] and neurotoxicity[97] in various experimental models. Additional studies are needed to determine the therapeutic potential of interventions via the ECS for various neuropathologies.

Cannabis has been used historically to treat epilepsy.[81] CB_1 receptor activation is typically anticonvulsant and appears to play a role in regulating seizure duration and frequency.[98] CB_1 receptor blockade with SR141716 or AM251 causes status epilepticus-like activity in a hippocampal neuronal culture model of acquired epilepsy.[99] Moreover, CB_1 receptor blockade with SR141716 was shown to induce seizures in pilocarpine-treated rats.[100] These effects may be explained by ECS modulation of excitability in CNS neurons, and the ECS may protect against acute excitotoxicity in CNS neurons.[85] Stimulation of nucleus accumbens CB_1 receptors suppresses glutamatergic activity, with consequent inhibition of GABAergic neurons.[101,102] However, data from animal studies are very dependent on the models used; some of which hold very little relevance to human epilepsy. Thus, additional studies are needed to determine the therapeutic potential for targeting the ECS for the treatment of epilepsy.[81]

Pain Perception

The ECS has a well-established role in modulating pain and represents a validated clinical target. Cannabinoid agonists are effective in animal models of acute and chronic pain.[103-105] Animal studies have revealed an important role of the CB_1 receptor, and likely the CB_2 receptor, in modulating pain perception. Some of these effects are counterintuitive. For example, CB_1 receptor blockade suppressed the antinociceptive effects of paracetamol[106] and THC[107] can produce hyperalgesia.[108]

Genetic deletion of the FAAH gene in mice elevates brain anandamide levels and unmasks the anti-nociceptive effects of this compound. In addition, FAAH knockout mice have attenuated inflammatory responses and exhibit enhanced CB_1 receptor–mediated analgesia.[109] Likewise, pharmacological blockade of FAAH activity reduces nocifensive behavior in animal models of acute and inflammatory pain. In a mouse chronic constriction injury (CCI) model of neuropathic pain, oral administration of the selective FAAH inhibitor URB597 produced a dose-dependent reduction in nocifensive

responses to thermal and mechanical stimuli, which was prevented by the CB_1 receptor blockade with SR141716.[110] The antihyperalgesic effects of URB597 were accompanied by a reduction in plasma extravasation induced by CCI.[110] This effect was prevented by CB_1 receptor blockade and attenuated by the CB_2 receptor antagonist SR144528, further supporting a role for both CB_1 and CB_2 receptors in the nociceptive effects of endocannabinoids.[110] Both the non-selective cannabinoid agonist HU210 and the selective CB_2 receptor agonist JWH-133 attenuated established inflammatory hypersensitivity and swelling in the carrageenan model of inflammatory hyperalgesia in rats.[111] Recently, Agarwal et al[105] studied the analgesic effects of cannabinoids in mice that lacked functional CB_1 receptors specifically in nociceptive neurons in the peripheral nervous system. These mice exhibited reduced antinociceptive effects from local and systemic, but not intrathecal, administration of cannabinoids. These data suggest that CB_1 receptors in peripheral sensory neurons have a prominent role in cannabinoid-mediated analgesia.

Substantial human studies support efficacy of cannabinoid agonists for treating neuropathic pain,[112-114] For example, the analgesic properties of whole plant extracts of *Cannabis sativa* (GW-1000-02) were demonstrated to be effective in a randomized, double-blind, placebo-controlled study in patients with neuropathic pain of peripheral origin.[113] Taken together, these data support a potential role for cannabinoid-based drugs in the treatment of chronic inflammatory pain and neuropathic pain.

Reinforcement

Reinforcement, which occurs when a stimulus is temporally paired with a response that increases the frequency of subsequent responses, plays an important role in the development, maintenance, and recovery from addiction.[115,116] THC self-administration in squirrel monkeys was shown to be CB_1 receptor mediated,[117] and CB_1 receptor blockade with SR141716 reduced cue-induced reinstatement of drug-seeking behavior.[118,119] The ECS likely

plays an important role in brain reward processes through its interactions with the mesolimbic dopaminergic system,[120] primarily by modulating neurotransmitter release (Figure 4).[21,101] Specifically, CB_1 receptors appear to mediate the effects of endocannabinoids in the brain reward process. Endocannabinoids can increase extracellular levels of, dopamine,[120] and CB_1 and dopamine receptors have been shown to interact in rat and monkey striatum.[121] Thus, the ECS may be involved in the reinforcing properties of several drugs of abuse including nicotine, ethanol, and opiates. There is evidence to suggest that stimulation of nucleus accumbens CB_1 receptors suppresses glutamatergic activity, with consequent inhibition of GABAergic neurons that normally inhibit ventral tegmental area dopamine neurons.[101] The therapeutic potential of CB_1 receptor antagonists in the treatment of addiction is being explored with great interest (reviewed in Mackie[122] and Howlett et al[14]).

Summary

The role of the ECS in depressive disorders, epilepsy, motor function, and neuronal function is complex. ECS stimulation is associated with both neuroprotection and neurotoxicity in various experimental models. The effects of acute and chronic CB_1 receptor blockade are often opposite, and this needs to be considered when evaluating the preclinical data. Additional studies are needed to determine potential therapeutic interventions via the ECS for various neuropathologies. Tissue-specific CB_1 receptor knockout mice will facilitate these studies. Both cannabinoid agonists and cannabinoid antagonists may hold therapeutic potential for various disorders of the CNS.

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