Role of the Endocannabinoid System in Regulating Cardiovascular and Metabolic Risk Factors

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ABSTRACT

Increased endocannabinoid (EC) system activity promotes excessive food intake and obesity in animals and humans. The EC system regulates food intake and hedonic reward through central mechanisms located within the hypothalamus and limbic forebrain. In rodent models, cannabinoid1 (CB1) receptor blockade reduces appetite and weight and prevents obesity and insulin resistance. The EC system also regulates food intake and metabolic factors through peripheral CB1 receptors located at multiple sites throughout the body, including adipose tissue, skeletal muscle, liver, and the gastrointestinal (GI) tract. In rodent models, CB1 receptor antagonists act in the liver to decrease lipogenesis, act in the GI tract to increase satiety, and function in adipose tissue to normalize adiponectin levels and reduce fat storage. The CB1 receptor antagonist rimonabant has been shown to reduce food intake and improve metabolic parameters, such as insulin resistance and fatty liver, in animal models of obesity. In preliminary human studies, upregulation of the EC system has been linked to obesity through mechanisms that include high-fat diet, insulin resistance, and genetic malfunction of an EC inactivation enzyme. Evidence suggests that CB1 receptor blockade is a novel therapeutic strategy that addresses the underlying mechanisms of both obesity and cardiometabolic risk. © 2007 Elsevier Inc. All rights reserved.

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The endocannabinoid (EC) system is an intercellular signaling system that plays an important role in regulating cardiovascular risk factors associated with excess body weight and obesity.1 Increased EC system activity promotes excessive food intake and fat accumulation in animal models and humans.1,2 Little was known about the pharmacology or neurobiology of the EC system until the discovery of endogenous cannabinoids (ECs) and their receptors over the past 2 decades.3

THE ENDOCANNABINOID SYSTEM

The endogenous cannabinoids, anandamide and 2-arachidonoylglycerol (2-AG), are phospholipid-derived precursor ligands of the 2 cannabinoid receptors, cannabinoid1 (CB1) and cannabinoid2 (CB2).5 (Figure 1). Both are 7-transmembrane, G-protein–coupled receptors, similar to receptors for many hormones and neurotransmitters; however, each of these receptors has its own unique structure.3,5

The ubiquitous CB1 receptor is found in numerous organs involved in the regulation of energy homeostasis, including the brain, adipose tissue, muscle, liver, and gastrointestinal (GI) tract.3 CB2 receptors are located predominantly in the immune system3 and are not discussed further in this review.

Unlike traditional hormones and neurotransmitters, which are preformed and stored in secretory vesicles until needed, the ECs are synthesized as needed from phospholipids upon activation of their synthetic enzymes. The phospholipid agonists, anandamide and 2-AG, activate cannabinoid receptors locally, in close proximity to the parent cell, and once activated, they are immediately metabolized and rapidly degraded.3 Within the brain, ECs act as retrograde neurotransmitters (or messengers) that inhibit synaptic ac-
activity. Although ECs influence numerous behaviors, in general the net effect of ECs at diverse sites in the brain and throughout the body is anabolic, facilitating increased energy intake, decreased energy expenditure, and increased accumulation of body fat.2

NEUROMODULATING EFFECTS OF ENDOCANNABINOIDS

Neurotransmitters, such as norepinephrine, dopamine, \(\gamma\)-aminobutyric acid (GABA), and serotonin, transmit signals from neuron to neuron across synapses. The synapse itself consists of a presynaptic terminal containing presynthesized and packaged neurotransmitters, a postsynaptic terminal containing receptor sites for neurotransmitters, and a synaptic cleft between the presynaptic and postsynaptic membranes.

Traditional or canonical neurotransmitters activate receptors on postsynaptic neurons. Upon receiving a nerve impulse, the presynaptic neuron is activated, causing an increase in intracellular calcium which, in turn, results in the release of preformed neurotransmitters, such as serotonin. When serotonin interacts with receptors on the postsynaptic membrane, numerous events occur such as excitation or inhibition of the postsynaptic cell.6

The CB1 receptor differs in that it is located primarily on presynaptic cell membranes, for example on presynaptic neurons that release the inhibitory neurotransmitter GABA. When these neurons release GABA into the synaptic cleft, the GABA inhibits activity in postsynaptic cells. However, when intracellular calcium levels become elevated in the postsynaptic neuron, enzymes that synthesize ECs are activated, causing the formation and release of endocannabinoids. These molecules then cross back in a retrograde fashion across the synaptic cleft, stimulating CB1 receptors on the presynaptic membrane and thereby preventing increased calcium levels in the presynaptic cell. This has the effect of reducing the amount of neurotransmitter (i.e., GABA) release,6 a process known as retrograde suppression of neurotransmitter release.7 Stated more simply, the postsynaptic cell inhibits input from the presynaptic cell by activating ECs. In this example, GABA would normally act on a postsynaptic cell to halt an ongoing meal, i.e., to make an individual feel full. EC activation in the postsynaptic cell might occur in response to the sight or taste of a pleasing dessert, and the result would be attenuation of presynaptic GABA release and a tendency to keep eating. ECs therefore act in part by stimulating appetite and prolonging food intake during meals, resulting in fat accumulation over time.

CENTRAL MECHANISMS OF ENDOCANNABINOID ACTIVITY

The basic biologic activity of the EC system on food intake, body weight, and metabolic syndrome components has been described chiefly in animal models.1,8,9 The EC system modulates both the homeostatic (i.e., necessary for survival) and pleasurable aspects of eating through central mechanisms located within the hypothalamus and limbic forebrain, respectively.1

Because the hypothalamus regulates the quantity of food consumption, the injection of ECs directly into the
hypothalamus should promote increased food intake. This hypothesis was confirmed in a pioneering study, which showed that anandamide injection into the ventromedial hypothalamus of presatiated rats induced significant appetite stimulation through stimulation of CB1 receptors. Conversely, when a CB1 receptor antagonist was injected 30 minutes before anandamide administration, it inhibited anandamide-induced food intake. The researchers concluded that cannabinoids modulate appetite by activating CB1 receptors located in the hypothalamus.

Kirkham and colleagues measured 2-AG levels in the hypothalamus and limbic forebrain of rats in relation to fasting, feeding, and satiation. They found that feeding decreased and food increased raised 2-AG levels. No changes were observed in satiated control animals (Figure 2). 2-AG robustly stimulated eating and appeared to regulate appetite and body weight through its activity on brain systems that mediate incentive and reward. This hypothesis was confirmed in another experiment in which the same investigators injected an EC into a different part of the brain, the nucleus accumbens of the limbic forebrain, which controls the hedonic or reward aspect of stimulants such as food. Injection of 2-AG significantly increased the amount of food eaten during the first hour after administration, whereas the injection of a CB1 receptor antagonist inhibited food intake.

The ability of CB1 receptor antagonism or deletion to blunt food intake after food deprivation was demonstrated in a study comparing CB1 receptor-deficient mice, in whom the CB1 receptor was genetically deleted or “knocked-out,” and wild-type control mice. The results showed that, following fasting, CB1 receptor-deficient mice ate less compared with their wild-type littermates. Further proof of concept was shown in the capability of the CB1 antagonist rimonabant to reduce food intake in wild-type mice but not knockout mice lacking the CB1 receptor. Additionally, the investigators found that increased hypothalamic levels of ECs contribute to the hyperphagia that promotes certain kinds of genetic obesity. Daily treatment of these obese mice with rimonabant resulted in a reduction in body weight, a finding indicative of the centrally mediated role played by ECs in the development of obesity.

In summary, studies have established that cannabinoids mediate food intake both in the hypothalamus, which controls energy homeostasis, and in the limbic system, which controls the pleasurable aspects of eating.

CANNABINOID1 RECEPTOR BLOCKADE IN ANIMAL MODELS OF OBESITY
Cannabinoids control food intake through centrally mediated mechanisms that involve interactions between feeding behavior and hedonic reward. The demonstration in animals that CB1 receptors control food intake and body weight, whereas CB1 receptor blockade reduces appetite and weight, has profound implications for the treatment of obesity in humans.

In an important study by Ravinet Trillou and colleagues, a population of CB1 receptor-deficient mice was compared with wild-type control mice to assess their response to standard and high-fat feeding regimens. Over the course of 11 weeks on a high-fat diet, the control mice became obese. By contrast, the mice lacking CB1 receptors remained as lean as they were on a standard diet. At the age of 20 weeks, the mean body weight and adiposity of the knockout mice were, respectively, 24% and 60% lower than those of the controls. In addition, CB1 receptor deletion reduced plasma insulin and leptin levels. Although the knockout mice preferred the high-fat diet, they did not become obese, and they maintained the same weight as normal control animals. Throughout the study, the knockout mice were lean and resistant to diet-induced obesity. In this animal model, the inability to activate the CB1 receptors prevented the development of obesity and insulin resistance.

In another study by Ravinet Trillou and colleagues, the effect of CB1 receptor blockade, as opposed to genetic deletion, was evaluated in a mouse model of diet-induced obesity. During a 5-week treatment period, rimonabant (10 mg/kg per day orally) induced a transient reduction in food intake of 48% in the first week and a significant and sustained reduction of body weight (~20%) and adiposity (~50%). Rimonabant also exerted favorable effects on metabolic parameters, correcting insulin resistance and lowering plasma leptin, insulin, and free fatty acid levels. After an initial sharp, but transient, reduction in food intake and weight, although the rats ate normally for the remainder of the study, they sustained their weight loss compared with controls, despite consuming a high-fat diet.

In summary, in animal models of obesity, CB1 receptor blockade with rimonabant transiently decreases high-fat dietary intake and persistently lowers body weight and insulin resistance.

PERIPHERAL MECHANISMS OF ENDOCANNABINOID ACTIVITY IN OBESITY
The EC system regulates food intake through peripheral as well as central mechanisms. CB1 receptors are located at multiple peripheral sites, including adipose tissue, muscle, the liver, and the GI tract (Figure 3). Ghrelin, a gut peptide, is a potent appetite stimulator. In addition to acting on the brain, it has important peripheral actions, including beneficial effects on the ischemic heart and increasing adipose tissue deposition; it also has direct effects on carbohydrate metabolism.

In the GI tract, cannabinoid and ghrelin levels increase in response to fasting and decrease on administration of a CB1 receptor antagonist. In a rat model, food deprivation increased anandamide content 7-fold in the small intestine, an effect that was reversed upon refeeding. The administration of rimonabant reduced food intake in both 24-hour food-deprived rats and partially satiated rats. In another study, intraperitoneal administration of rimonabant signifi-
sarily decreased ghrelin levels and reduced food intake in rats whose ghrelin levels were elevated in response to a 24-hour fast. Because both ghrelin and anandamide increase food intake by reducing satiety signals from the GI tract, the implication is that endocannabinoids act in the GI tract, as they do in the brain, to favor increased energy intake.

In the liver and in adipose tissue, CB₁ receptors are expressed and CB₁ agonists facilitate the formation and storage of triglycerides, thus acting synergistically with actions in the brain (i.e., enhanced food intake) to favor weight gain. The fundamental role of hepatic EC system activation in diet-induced obesity in mice has been characterized by Osei-Hyiaman and colleagues. Specifically, activation of hepatic CB₁ increases the activity of several lipogenic enzymes, causing the liver to synthesize more fat and thereby promoting both obesity and fatty liver disease. In wild-type control mice, CB₁ receptors are located primarily in hepatocytes around the centrilobular veins and, as expected, were absent in CB₁ knockout mice. Administration of a CB₁ receptor agonist to wild-type mice increased hepatic gene expression of the lipogenic transcription factor SREBP-1c (sterol regulatory element–binding protein-1c) and its target enzymes, acetyl-CoA carboxylase-1 and fatty acid synthase. The net result of this activity was to increase fatty acid synthesis. In this animal model, anandamide, 2-AG, and CB₁ receptor levels were all elevated in diet-induced obesity, probably owing to a

Figure 2  Endocannabinoid levels in the hypothalamus and cerebellum of rats in relation to fasting, feeding, and satiation. Feeding lowers and food deprivation raises 2-arachidonoylglycerol (2-AG) levels in the hypothalamus but has no effect in the cerebellum, an area not directly involved in the control of food intake. *P < 0.05. (Adapted with permission from Br J Pharmacol.)

Figure 3  Multisite location of cannabinoid₁ (CB₁) receptors, and effects of a CB₁ antagonist on metabolism. GI = gastrointestinal; ↑ = increased; ↓ = decreased.
marked reduction in fatty acid amide hydrolase (FAAH).\textsuperscript{15}

Importantly, this study implicated CB\textsubscript{1}-mediated stimulation of fatty acid synthesis to fatty liver disease. Wild-type mice fed a high-fat diet for 3 weeks, though not yet obese, had a significant increase in the basal rate of hepatic fatty acid synthesis and the development of hepatic steatosis.\textsuperscript{15} This deleteriously increased rate of hepatic fatty acid synthesis was inhibited by rimonabant in wild-type mice, but not in CB\textsubscript{1} receptor knockout mice, implicating increased levels of anandamide and CB\textsubscript{1} as the causative mechanisms. These findings strongly suggest that an early EC-mediated increase in de novo lipogenesis in the liver is a critical component in diet-induced obesity, and raise the possibility that CB\textsubscript{1} antagonists may be effective not only as antiobesity agents but also in preventing or reversing the development of fatty liver.\textsuperscript{15}

Adipose tissue also expresses CB\textsubscript{1} receptors, and the level is increased in obese animals.\textsuperscript{9} In obese Zucker rats, cannabinoid administration increased the level of adipose tissue lipoprotein lipase, the enzyme that enables adipocytes to store fat, while rimonabant blocked this activity. Rimonabant exerted its activity in part by increasing expression of Acrp30 (adiponectin) messenger RNA (mRNA), a plasma protein that decreases hyperinsulinemia and weight.\textsuperscript{9} Conversely, in adipose tissue of CB\textsubscript{1} receptor knockout mice, rimonabant had no effect on Acrp30 mRNA expression, demonstrating a CB\textsubscript{1} receptor-mediated effect. Thus, adipocyte CB\textsubscript{1} receptor mRNA is upregulated in obesity and downregulated by CB\textsubscript{1} receptor blockade, resulting in reduced hyperinsulinemia and suggesting that rimonabant regulates metabolism peripherally, as well as centrally, through mechanisms independent of weight loss alone.\textsuperscript{9}

In another study, CB\textsubscript{1} receptor agonism induced adipocyte lipoprotein lipase activity in primary cultures of mouse adipocytes.\textsuperscript{16} These findings suggest that the EC system modulates energy homeostasis through dual peripheral lipogenic and central orexigenic mechanisms and, therefore, that CB\textsubscript{1} antagonists offer a novel approach to the treatment of both obesity and cardiometabolic risk.\textsuperscript{16} Finally, rimonabant administration, but not pair feeding (where control animals receive the same amount of food voluntarily consumed by the rimonabant-treated animals), normalized plasma adiponectin in mice that were fed a high-fat diet. The effect of CB\textsubscript{1} blockade with rimonabant normalized adiponectin, not only by reducing food intake but also by increasing levels of adiponectin, which increases insulin sensitivity.

In summary, the EC system regulates food intake through peripheral and central mechanisms located at multiple peripheral sites. CB\textsubscript{1} receptor antagonists act in the liver to decrease lipogenesis, act in the GI tract to increase satiety, and function in adipose tissue to normalize adiponectin levels and reduce insulin sensitivity. In animal models, the CB\textsubscript{1} receptor antagonist rimonabant reduces food intake and improves metabolic parameters, such as insulin resistance and fatty liver.

**Cannabinoid\textsubscript{1} Blockade in Human Obesity**

Preliminary data in humans indicate that higher levels of cannabinoids are present in obese individuals. In a recent study, Engeli and associates\textsuperscript{2} found that circulating levels of anandamide and 2-AG were significantly increased (35% and 52%, respectively, in obese compared with lean women) (Figure 4).\textsuperscript{2} In addition, some forms of human obesity are associated with a mutation in FAAH, the enzyme that degrades ECs. In the Engeli group’s study, obese women had a reduction in adipose tissue FAAH gene expression of 59% compared with lean women (see Figure 4).\textsuperscript{2}

Related to this, Sipe and colleagues\textsuperscript{17} observed that an FAAH gene mutation was associated with overweight and obesity in both white and black subjects ($P = 0.05$). In whites, genetically defective FAAH expression was correlated with increasing body mass index (BMI) and obesity, and a similar trend was observed in black subjects.\textsuperscript{2} These data suggest that a genetic defect in FAAH, the enzyme that inactivates ECs, renders some individuals more susceptible to obesity and supports the use of CB\textsubscript{1} blockade as a therapeutic strategy.

Important questions remain regarding the use of CB\textsubscript{1} receptor antagonists to treat cardiometabolic risks. For example, since none of the clinical data published to date have included patients being treated for depression, it is unknown how rimonabant may interact with antipsychotic therapeutics such as some selective serotonin reuptake inhibitors that tend to cause weight gain. Likewise, there are no data yet on possible interactions of rimonabant with specific dietary nutrients, such as low-fat diets, or the consumption of omega-3 fats; and studies combining rimonabant with exercise have not yet been reported.

In summary, the hyperactivity of the EC system in obesity involves several factors, including a high-fat diet, insulin resistance, and genetic malfunctioning of EC inactivation mechanisms. CB\textsubscript{1} receptor blockade appears to represent a viable therapeutic strategy that addresses the underlying mechanisms of obesity.

**Summary**

Obese animals and humans have elevated levels of endogenous cannabinoids. The EC system exerts its effect through a synergistic interaction between central and peripheral mechanisms at multiple sites, thereby promoting dysregulation in metabolic and energy homeostasis, which culminates in a net anabolic effect. Administration of CB\textsubscript{1} agonists, such as anandamide and 2-AG, increases food intake and body weight, whereas administration of CB\textsubscript{1} antagonists reduces food intake and body weight and improves blood glucose and lipid profiles.

Evidence suggests that CB\textsubscript{1} receptor antagonists act centrally in the brain to reduce food intake and peripherally in the liver to decrease lipogenesis, act in the GI tract to increase satiety, and function in adipose tissue to normalize adiponectin levels and reduce insulin sensitivity. In animal models, the CB\textsubscript{1} receptor antagonist rimonabant reduces food intake and improves metabolic parameters, such as insulin resistance and fatty liver.
adipose tissue to increase adiponectin and in the liver to decrease lipogenesis. Preliminary evidence indicates that CB1 blockade increases glucose uptake and oxygen consumption in muscle, decreases lipogenesis in the liver, and increases satiety in the GI tract. The subject of EC action is a timely one because CB1 antagonists have shown promise in clinical trials as novel therapeutic tools for the treatment of obesity and its associated cardiometabolic risk factors.

References

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**Figure 4**  Endocannabinoid system dysregulation in human obesity. *Top* Plasma anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are elevated in obesity. *Bottom* Genetically defective fatty acid amide hydrolase (FAAH) is associated with body mass index (BMI) (left). Obese patients have decreased adipose tissue FAAH gene expression (right). AU = arbitrary units; GAPDH = glyceraldehyde 3-phosphate dehydrogenase. (Reprinted with permission from *Diabetes* and *Int J Obes (Lond)*.)


