The Endocannabinoid System

A New Target for the Regulation of Energy Balance and Metabolism

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Abstract: Recent studies have provided evidence that the endocannabinoid (EC) system has very significant effects on energy balance and metabolism through the central control of appetite and by affecting peripheral metabolism. Endocannabinoids are endogenous phospholipid derivatives which bind and activate cannabinoid receptors type 1 and type 2 (CB_1 and CB_2 receptors). The CB_1 receptor, a G-protein coupled receptor, is believed to be responsible for the majority of the central effects of endocannaboids on appetite. Chronic positive energy balance and obesity have been associated with an overactivation of the endocannaboid system which has been suggested to contribute to the development of abdominal obesity and to associated metabolic abnormalities which increase the risk of cardiovascular disease and type 2 diabetes. Animal studies had shown that stimulation of the cannabinoid CB_1 receptor with endocannaboids such as anandamide could induce first an increase in food intake leading to body weight gain. Furthermore, an exciting development in this field has been the discovery of CB_1 receptors in many peripheral tissues, including key organs involved in carbohydrate and lipid metabolism such as the adipose tissue and liver. Thus, blocking CB_1 receptors located in the liver and adipose tissue could have an additional impact on the metabolic risk profile beyond what could be explained by the reduction in food intake and the related body weight loss. Preclinical studies have shown that rimonabant, the first CB_1-receptor blocker to be available in clinical practice, could not only induce a reduction in food intake, but could also produce body weight loss beyond what could be explained by its effect on food intake. Thus, the evidence from preclinical studies have suggested that CB_1 blockade could represent a relevant approach to reduce food intake, to induce body weight loss, and, most importantly, to "fix" the dysmetabolic state of viscerally obese patients at increased cardiometabolic risk.

Key Words: cardiometabolic risk, CB_1 receptor, endocannabinoid system, rimonabant, abdominal obesity

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There is now solid evidence to support the notion that the endocannabinoid (EC) system has very significant effects on energy balance and metabolism.[1] Moreover, it appears that the EC system cannot only control appetite centrally but it can also modulate carbohydrate and lipid metabolism through its effect on peripheral tissues. ECs are endogenous phospholipid derivatives (the 2 most studied being anandamide and 2-arachidonoylglycerol, [2-AG]) which bind and activate cannabinoid receptors type 1 and type 2 (CB_1 and CB_2 receptors). The CB_1 receptor, a G-protein coupled receptor, has been shown to be responsible for the central effects of endocannaboids on appetite. In normal conditions, the EC system is activated on demand to reduce pain and anxiety, modulate temperature, regulate blood pressure, inhibit motor behavior and sedate, as well as to promote food intake. In the presence of chronic conditions such as long-term overeating, obesity, and smoking, the EC system becomes overactivated and this state has consequences on numerous physiologic and metabolic processes.

Recent pharmacological advances have led to the synthesis of cannabinoid receptor agonists and antagonists. Such discoveries have enabled the study of the physiological roles played by the EC system and have led to the development of new approaches that may have a significant role in the management of high-risk abdominal obesity and potentially for the prevention of cardiovascular disease and type 2 diabetes.

A brief history of how knowledge of the EC system has evolved is provided and recent advances that have specifically linked the physiologic properties of the EC system to metabolism are reviewed. Finally, the emergence of cannabinoid receptor antagonism as a promising therapeutic alternative for treating abdominal obesity and cardiometabolic risk is also discussed.

The Discovery of the EC System: Some Historical Perspectives

In 1964, Δ-9-tetrahydrocannabinol was isolated and identified by Rafael Mechoulam's team as the active psychotropic constituent of *Cannabis sativa*.[2] Considered a recreational drug in most parts of the world, cannabis exerts a wide range of effects including analgesia, anti-inflammation, immunosuppression, anticonvulsion, alleviation of intraocular pressure in glaucoma, attenuation of vomiting, and appetite stimulation. However, the clinical application of cannabinoids has been limited by their psychoactive effects, thus

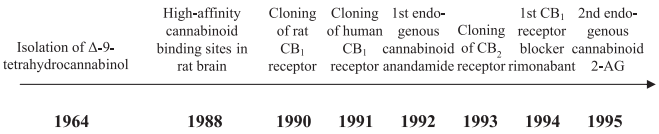

FIGURE 1. Some key historical discoveries related to the EC system.

leading to interest in the biochemical bases of their action. Indeed, the EC system has been extensively studied by academicians and industry scientists, resulting in contributions that have provided a greater understanding of how this system works (Fig. 1).

Devane and coworkers[3] discovered the presence of high-affinity cannabinoid-binding sites in the rat brain, which led to the determination and characterization of a cannabinoid receptor. This discovery was followed by the cloning of a guanine-nucleotide–binding protein (G-protein)-coupled receptor (coined the CB_1 receptor), first from rats[4] and then in man.[5] In 1992—28 years after Δ-9-tetrahydrocannabinol was isolated—Devane and colleagues[6] discovered anandamide, the first endogenous cannabinoid. The CB_2 receptor, which was found not to be involved in the regulation of energy balance but rather associated with immune function, was cloned in 1993.[7] In 1994, there was the characterization of the first selective CB_1 receptor blocker rimonabant.[8] In 1995, another endogenous cannabinoid, 2-AG, was also isolated from the gut and in the brain.[9]

On the basis of these findings, it was hypothesized that the nonpsychoactive effects of cannabinoids were either mediated centrally or through direct interaction with other nonreceptor proteins. This hypothesis led to the pursuit of therapeutic targets and to the development of the first selective CB_1 blocker, rimonabant.[8] The newly discovered compound displayed affinity for the central cannabinoid receptor (CB_1 receptor) but was found not to be active on the peripheral CB_2 receptor.[9a]

Thus, it is now understood that the EC system comprises a family of locally produced, short-lived, endogenous phospholipid-derived ligands known as ECs. The 2 most abundant ECs discovered to date, anandamide and 2-AG, are produced on demand postsynaptically by neuronal cells. These ECs, along with the G-protein coupled CB_1 receptor, localize in several areas of the brain, including key areas in the hypothalamus involved in the regulation of hunger, appetite, and satiety. An exciting development has been the discovery that the CB_1 receptor was also expressed in other organs and tissues, such as the GI tract, the liver, and adipose tissue, whereas CB_2 receptors are quite abundant in immune cells.

Animal studies have shown that the stimulation of the CB_1 receptor subtypes by ECs leads to the inhibition of neurotransmitter release in central and peripheral neurons, inducing numerous physiological responses such as food intake and fat accumulation. Thus, by blocking the CB_1 receptors, the effect of the elevated EC concentrations observed in animal models of obesity can be substantially reduced. In view of the potential impact of EC system modulation, the CB_1 receptor was therefore considered as a possible therapeutic target for human pharmaceutical intervention.

Modulating the EC System: Impact on Energy Balance

CB_1 Agonism

The psychoactive constituent of cannabis, Δ-9-tetrahydrocannabinol, also known as dronabinol, has been used to promote appetite in some clinical situations requiring stimulation of food intake. For example, dronabinol can be used in anorectic cancer patients or in patients with AIDS in situations where severe body weight loss threatens the survival of afflicted patients.

For instance, Beal and colleagues[10] have published an interesting study evaluating the effects of CB_1 agonism (using dronabinol) on appetite and weight in a sample of 139 patients with AIDS-related anorexia who had lost ≥2.3 kg of body weight. Patients were randomized to receive 2.5 mg dronabinol twice daily or placebo and were then asked to rate their appetite, mood, and nausea on a 100-mm visual analog scale 3 days weekly. After 42 days of treatment, the results indicated that dronabinol therapy increased appetite when compared with placebo. In fact, stimulating the CB_1 receptor with dronabinol led to an average increase of body weight of 1 kg, which from an energy balance standpoint indicates that these patients were in positive energy balance and gaining some body fat.

In a phase 2 clinical trial, 19 patients with various malignancies who had cancer-associated anorexia were started on a regimen of dronabinol 2.5 mg 1 hour after meals for 4 weeks.[11] Evaluations for side effects, efficacy, acceptability, and satisfaction were conducted at 2 and 4 weeks. Of the 18 evaluable patients, 13 reported an improved appetite, suggesting again that CB_1 agonism with dronabinol was an effective approach to stimulate appetite and food intake in patients with advanced cancer.

These studies provide supporting evidence for CB_1 receptor stimulation leading to an increase in food intake. One reason for this effect is attributed to the location of the CB_1 receptors in key areas of the hypothalamus involved in the regulation of hunger and satiety.

However, a chronic stimulation of the CB_1 receptor in the brain could not only promote food intake and a positive energy balance, but it may also lead to obesity and to related metabolic abnormalities. In addition, the discovery of CB_1 receptors in other peripheral tissues such as the liver and the adipose tissue has allowed the study of the specific effects of CB_1 agonists on these tissues. For instance, the powerful CB_1 agonist HU-210 was found to promote lipogenesis in the liver whereas such an effect was prevented by pretreatment with the CB_1 blocker rimonabant.[12] The CB_1 receptors are also located in fat cells, and their modulation has been shown to also cause metabolic changes in adipose tissue. For instance, incubation of adipose tissue with a CB_1 agonist has been shown to increase lipoprotein lipase activity.[17] These findings from animal studies are fully

compatible with the expanded visceral adipose tissue mass and with the low plasma adiponectin levels found in high-risk, abdominally obese patients.[14,21] On that basis, one could put forward the hypothesis that CB_1 blockade with rimonabant could induce a reduction in food intake and weight loss through a central effect, but that this class of drug could also reduce lipogenesis in the liver and stimulate adiponectin production by fat cells.[24]

CB Antagonism

Animal studies have shown that blocking the CB_1 receptor or absence of the CB_1 receptor (CB_1 knockout mice) was associated with reduced food intake in animals. Williams and colleagues[15] examined whether the EC anandamide could induce overeating via a specific action at central CB_1 receptors. Eighteen presatiated male rats received subcutaneous injections of anandamide (0.5, 1.0, 5.0, and 10.0 mg/kg) before 3-hour nocturnal food intake tests. In a second series of intake tests, 8 rats received an anandamide injection (1.0 mg/kg) preceded by injection of the CB_1 receptor antagonist rimonabant (0.1, 0.5, and 1.0 mg/kg SC). All doses of anandamide significantly increased food intake, but in the presence of increasing doses of rimonabant, the hyperphagia previously observed with anandamide was completely blocked.

Another study conducted in presatiated rats administered anandamide directly into the ventromedial hypothalamus.[16] The rats received an intrahypothalamic injection of anandamide followed by measurement of food intake at 3 hours postinjection. Similar to the study conducted by Williams and coworkers,[15] this study demonstrated that anandamide induced food intake by stimulation of CB_1 receptors; however, this effect was blocked in the presence of the CB_1 receptor antagonist rimonabant.

Cota and associates[17] reported in a seminal paper that the lack of CB_1 receptor in mice (CB_1 knockout mice) was associated with a reduced food intake as well as with leanness. The growth curves for the wild-type littermates and the CB_1 knockout mice are depicted in Figure 2a. When compared with wild-type mice, the CB_1 knockout mice exhibited reduced spontaneous caloric intake and subsequent decreased body weight. The authors also reported the presence of CB_1 receptors in epididymal adipocytes. One of their key findings was the following: CB_1-specific activation enhanced lipogenesis in primary adipocyte cultures. The absence of a CB_1 receptor resulted in leaner mice than in wild-type animals and analysis of body composition using nuclear magnetic resonance imaging confirmed a markedly decreased body fat mass and a slightly increased lean body mass in CB_1 knockout animals compared with wild-type mice (Fig. 2c).

The leaner body composition phenotype of CB_1 knockout mice compared with wild-type mice could not be explained by differences in food intake between the 2 groups of animals. To reach that conclusion, Cota and colleagues[17] performed pair-feeding studies to test the possibility that peripheral metabolic processes could also contribute to the lean phenotype of CB_1 knockout mice. Under pair-feeding

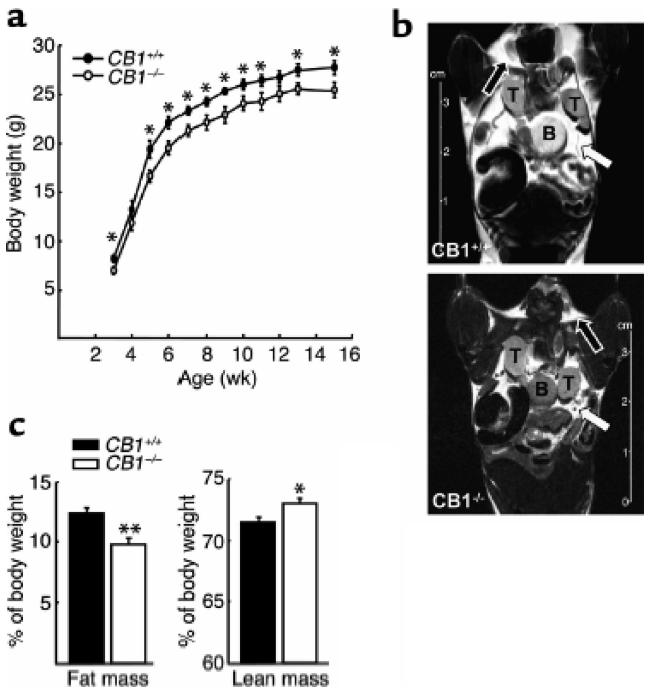

FIGURE 2. Body weight and body composition of CB_1 knockout mice. (**a**) Body weight curves in CB_1 wild-type male mice versus CB_1 knockout mice starting at 3 weeks of age. Each data point represents mean ± SEM of 15 mice. $^*P < 0.05$ versus CB_1 wild-type littermates. (**b**) NMR imaging performed in 16-week-old mice. White arrow indicates visceral fat; black arrow, subcutaneous fat; T, testis; B, bladder. (**c**) Analysis of body composition by quantitative NMR. Fat mass and lean mass expressed as percentage of the body weight. The columns represent the mean ± SEM of 15 CB_1 wild-type and knockout mice, respectively. $^*P < 0.05$ and $^{**}P < 0.005$ versus CB_1 wild-type controls. Reproduced with permission from *J Clin Invest.*[17]

conditions, adult CB_1 wild-type remained fattier than CB_1 knockout littermates. Thus, the lean phenotype observed in the CB_1 knockout mice must be attributed to peripheral metabolic effects that are independent from the variation in food intake.

In search of the mechanism responsible for the peripheral effects of ECs on energy balance and metabolism, Ravinet Trillou and colleagues[18] investigated the short-term effects of rimonabant in mice subjected to a high-fat diet. Over the 5-week treatment period, rimonabant induced a transient reduction of food intake (−48% on week 1) and a marked but sustained reduction of body weight (−20%) and adiposity (−50%) in mice exposed to the high-fat diet. However, the food intake of animals under treatment with the CB_1 blocker rimonabant eventually returned to the value found in placebo treated animals. Thus, the lower body weight of treated animals at the end of the study could not be attributed to an effect on food intake, suggesting a direct metabolic effect of CB_1 blockade. Rimonabant had no effect in CB_1 receptor knockout mice, which confirmed the implication of CB_1 receptors in the activity of the compound.

The EC System and Adiponectin Secretion by Fat Cells

As mentioned earlier, a remarkable development in the understanding of the EC system was the discovery of CB_1 receptor expression in fat cells.[17] Also aforementioned, adipose tissue, in addition to being an organ specializing in the storage and mobilization of fat, is now recognized as a remarkable endocrine organ producing numerous cytokines (adipokines) among which we include adiponectin and TNFα.

Adiponectin is a specific adipose tissue-derived cytokine that has recently received considerable significant attention in metabolic and epidemiological studies. In obese animals, adiponectin elicits the following properties: reduction of body weight with no effect on food intake, induction of fatty acid oxidation in muscles, decrease in hyperglycemia and hyperinsulinemia, and improved insulin resistance.[19] In human subjects, a negative correlation between plasma adiponectin levels and BMI, fasting insulin, triglycerides, and insulin resistance has been noted.[20] More specifically, when indices of total adiposity and of body fat distribution are measured, it has been reported that viscerally obese patients are the subgroup showing the lowest plasma adiponectin levels.[21] Indeed, when we have compared plasma adiponectin levels as well as metabolic risk variables in 2 groups of equally obese patients with either low or high accumulation of intra-abdominal adipose tissue, the obese patients with little intra-abdominal or visceral fat were notably not characterized by reduced concentrations of adiponectin.[21] Only obese patients with excess visceral adiposity were characterized by markedly reduced adiponectin levels. Although all adiposity and adipose tissue distribution indices were negatively correlated with plasma adiponectin levels, multiple regression analyses revealed that visceral adipose tissue accumulation was the only independent predictor of adiponectin levels. These results support the notion that adiponectin concentration is influenced to a greater extent by visceral than subcutaneous obesity. Considering the inhibiting effects of ECs on the production of adiponectin by fat cells, the low plasma adiponectin concentrations of viscerally obese patients provide indirect evidence of a chronically activated EC system in visceral obesity.

Both TNFα and adiponectin have been shown to have important metabolic properties. Although it is still not clear whether TNFα induces systemic insulin resistance, it certainly does so in adipose tissue and inhibits the synthesis and secretion of adiponectin by fat cells.[22] Conversely, increased concentrations of adiponectin could possibly play a protective role against the aggression of adipose tissue by infiltrating macrophages. In vitro data suggest that the properties of these adipose tissue-derived cytokines are compatible with a ying-yang role of these 2 cytokines in the regulation of the cardiometabolic risk profile.[23]

Bensaid and colleagues[24] have used reverse transcription polymerase chain reaction (PCR) analysis of CB_1 receptor mRNA expression in obese Zucker (fa/fa) rats. Total RNA isolated from adipose tissues of these obese rats or their lean littermates and from differentiated or undifferentiated adipocyte cells were reverse transcribed and submitted to PCR amplification. The results showed that differentiated adipocytes and adipose tissue expressed CB_1 receptor mRNA, confirming the findings previously reported by Cota and colleagues.[17]

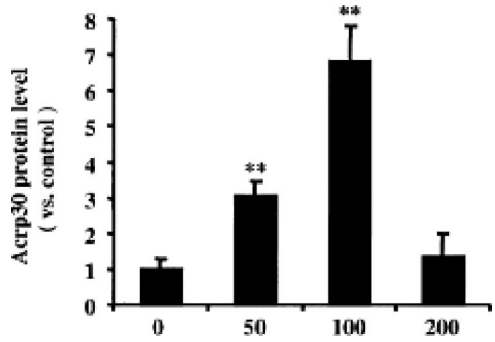

FIGURE 3. CB_1 blockade by rimonabant stimulates adiponectin production in adipocytes. Subconfluent mouse adipocytes were exposed or not (control) to various concentrations of rimonabant (50–200 nM) every day for 4 days. The chart shows the concentration-dependent effect of rimonabant on adiponectin protein level. *$P \leq 0.05$; **$P \leq 0.01$, compared with control. Reproduced with permission from *Mol Pharmacol*.[24]

Relative quantification analysis also revealed an upregulation of the CB_1 receptor expression in adipose tissue of obese rats, which was much higher than that observed in lean rats. In differentiated adipose cells, the expression of CB_1 receptor mRNA was at least 4-fold higher than that obtained in undifferentiated adipocytes.[24]

Moreover, the addition of rimonabant (100 nM) to subconfluent cultures of adipocytes induced a rapid and strong increase in adiponectin expression. The stimulation rate of adiponectin mRNA expression, compared with that of control cultures, was 1.4- and 1.8-fold after 30 and 60 minutes of rimonabant incubation, respectively. After 4 days of treatment, rimonabant increased the adiponectin levels in adipocytes in a concentration-dependent manner (from 50 to 200 nM), with a maximal effect at 100 nM (Fig. 3)[24]. In summary, the Bensaid study[24] showed that rimonabant not only blocked the CB_1 receptor to limit fat accumulation in fat cells, but it also stimulated adiponectin mRNA expression in adipose tissue in obese rats. From the above observations, CB_1 blockade with rimonabant could be especially helpful not only to induce a reduction of food intake and body weight loss, but even more importantly to alter liver and adipose tissue metabolism while boosting adiponectin production by fat cells among high-risk abdominally obese patients who have an excess mass of visceral adipose tissue, low adiponectin levels, and the whole cluster of atherogenic and diabetogenic abnormalities that we often refer to as the metabolic syndrome.

CONCLUSION

The EC system is an endogenous and physiological system which plays a key role in the regulation of food intake and fat accumulation as well as in the control of lipid and glucose metabolism. An overactivated EC system in the

context of chronic overeating promotes fat accumulation, alters adipose tissue adiponectin production, and may promote liver steatosis through increased hepatic lipogenesis. Rimonabant selectively blocks CB_1 receptors centrally and peripherally, including in adipose tissue, to help normalize an overactivated EC system and its consequences on food intake as well as on liver and adipose tissue metabolism, thereby contributing to improve insulin sensitivity and other features of the so-called metabolic syndrome. On that basis, targeting the EC system may represent a novel approach to the management of abdominal obesity and associated cardiometabolic risk.

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