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Cannabinoid receptors as therapeutic targets for obesity and metabolic diseases

Luigi Bellocchio, Giacomo Mancini, Valentina Vicennati, Renato Pasquali and Uberto Pagotto

One of the most interesting pharmacological targets proposed in the past ten years for fighting obesity and related metabolic disorders is the endocannabinoid system. The role of the endocannabinoid system is crucial in regulating the rewarding properties of food, in controlling energy balance by acting at the hypothalamic circuitries involved in food intake, and in peripheral metabolism by influencing adipocytes, hepatocytes, myocytes and pancreatic endocrine cells. Obesity seems to be a condition associated with a pathological overactivation of the endocannabinoid system; therefore, restoring a normal endocannabinoid tone by antagonizing the cannabinoid receptor type 1 (CB₁) could help arrest both the development and the maintenance of obesity.

Addresses

Endocrinology Unit and CRBA, Department of Internal Medicine and Gastroenterology, S. Orsola-Malpighi Hospital, Via Massarenti 9, 40138 Bologna, Italy

Corresponding author: Pagotto, Uberto (pagube@med.unibo.it)

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Introduction

If the history of cannabinoids of exogenous and endogenous origin needs to be summarized briefly, it is possible to pinpoint a few distinct crucial moments over the past 40 years. The identification of the chemical structure of the main psychoactive ingredient of marijuana — Δ^9 -tetrahydrocannabinol (THC) — in 1964 represents the milestone of the whole story [1]. However, it took nearly 25 years to clone and characterize the first receptor activated by THC: the so-called cannabinoid receptor type 1 (CB₁) [2]. The discovery that, in the human brain, there are receptors able to recognize the plant-derived substance THC paved the way for the identification of the endogenous lipid ligands for these receptors: arachidonoyl ethanolamine (anandamide; AEA) [3] and 2-arachidonoyl glycerol (2-AG) [4,5]. The number of identified endocannabinoids is now

increasing, but a detailed description of them is beyond the scope of the present review [6]. All together, cannabinoid receptors, endocannabinoids and a set of endocannabinoid-synthesizing and -degrading enzymes constitute the endocannabinoid system (ECS) [7].

This review provides a concise and updated revision of this topic by giving an initial overview of the mechanisms of action by which endocannabinoids are able to affect food intake and energy metabolism. The hypothesis on the association between endocannabinoid overactivity and obesity is also discussed. Finally, the clinical use of rimonabant, the first CB₁ receptor antagonist to be synthesized, in the treatment of obesity and obesity-related metabolic alterations is critically revised in light of the Phase III trials recently published.

Endocannabinoids stimulate the search for palatable food and the motivation to eat

Similar to the orexigenic properties of THC, endocannabinoids are also able to trigger the motivation to eat. The ability of endocannabinoid levels to surge before a meal is consistent with the role of the ECS as a general stress recovery system [8]. If fasting is indeed perceived by living organisms as one of the most stressful conditions, it is logical that endocannabinoids rise up in response to a fasted state. The ECS is proposed to promote food intake centrally by acting at both the mesolimbic and the hypothalamic level [9,10]. Indeed, endocannabinoids are known to play a crucial role in the modulation of reward and reinforcement circuits [11]. This network is implicated in the pleasure produced by natural rewards, and is the neural substrate of drug addiction and addiction-related phenomena. Several studies indicate that the mesolimbic dopaminergic pathway plays a pivotal role in this circuitry, and that this pathway displays close cross-talk with the ECS [11].

The other important neural site of action of endocannabinoids in modulating food intake is at the hypothalamic level [12]. Hormonal and neural signals originating from peripheral organs involved in metabolic control constantly inform the brain about the current state of nutrition; the hypothalamus is also sensitive to metabolic changes associated with energy availability [13]. In turn, this information is used to adjust both caloric intake and energy depositing in peripheral areas.

The production of endocannabinoids in the hypothalamus of rodents on a standard diet is similar to that observed in

the mesolimbic system. Indeed, 2-AG levels have been shown to increase during fasting and to decrease as the animals are re-fed [14]. CB₁ receptors are not as highly expressed in the mammalian hypothalamus as they are in other brain regions [15]; however, studies of functional binding have indicated that there is a high degree of efficiency of G protein coupling, suggesting that in the hypothalamic area the functional activity of the ECS is not correlated with the degree of CB₁ receptor expression [16]. The finding of increased pathological levels of endocannabinoids in rodents with an impairment in leptinergic signalling [17] suggested the existence of cross-talk between leptin and endocannabinoids. A possible site of action for this cross-talk is the lateral hypothalamus [18]. However, the arcuate nucleus and the paraventricular nucleus represent other hypothalamic areas in which a functional interaction between endocannabinoids and neuropeptides involved in food intake might occur. Solid evidence has documented direct cross-talk between CB₁ receptors and corticotrophin-releasing hormone [19], although other findings indicate that the ECS may contribute to the orexigenic action of neuropeptide Y [20] and ghrelin [21,22].

As in the liver and adipose tissue, fatty acid metabolism in hypothalamic neurons acts as a sensor of energy availability. Fatty acid synthase (FAS), the key enzyme of this metabolic pathway, has been shown to play an important role in hypothalamic control of food intake, and its inhibition appears to reduce appetite, probably through modulation of the melanocortin system [23]. Endocannabinoids were reported to increase *FAS* gene expression in the hypothalamus, and increased expression of this gene by fasting/re-feeding could be inhibited by rimonabant, a CB₁ receptor antagonist, at the beginning of the re-feeding period [24]. Although fatty acid synthesis was not measured directly in the hypothalamus, these data suggest that the increase in food intake following food deprivation could involve CB₁-mediated modulation of the FAS pathway, probably through effects on AMP-activated protein kinase activity [25].

The endocannabinoid system and peripheral metabolism

The first indirect indication that cannabinoids could affect energy homeostasis through mechanisms other than food intake came from an observation on marijuana smokers [26]. In this study, the marijuana-induced increase in caloric intake disappeared after a few days, whereas weight gain was seen throughout the rest of the observation period, suggesting the presence of an independent effect on peripheral metabolism [26]. A similar conclusion was reached from rodent studies in which tolerance to the anorectic effect of rimonabant developed after a few days, but the reduction in body weight was maintained throughout the treatment period [27]. Similar observations were reported in several other animal models in which the ECS

was blocked either by pharmacological treatment or by genetic ablation of the CB₁ receptor [19,28–31]. In conclusion, all of these experiments demonstrate that, after blockade of the ECS, the reduction in food intake is transient but a persistent reduction in body weight can still occur, suggesting that factors other than food intake are involved in the final weight reduction [10].

Recently, an impressive series of reports challenged the existence of an exclusive neuronal site of action of endocannabinoids by showing that the ECS can also potently control metabolic functions at many peripheral sites, including adipose tissue [19,32,33,34,35,36], the gastrointestinal tract [37,38], skeletal muscle [39], the endocrine pancreas [36,40,41] and liver [24].

Treatment of obese mice with CB₁ receptor antagonists induces several events in adipose tissue [33], especially at the metabolic level, such as enhanced lipolysis through stimulation of enzymes involved in β -oxidation and the tricarboxylic acid cycle [33]; increased energy expenditure through futile cycle induction [33]; and an improvement in glucose homeostasis through increased expression of glucose transporter-4, a key player in glucose metabolism [33]. Owing to the actions of CB₁ receptor antagonists on factors involved in glucose homeostasis, such as adiponectin [32,34,36] and visfatin [34], it is not surprising that CB₁ receptor antagonists might also be an attractive weapon against insulin resistance and diabetes.

A recent study demonstrated an increase in CB₁ receptor expression in obese mice compared with lean controls [10]. In *ob/ob* mice, treatment with rimonabant is able to stimulate glucose uptake in the soleus muscle [39], and this effect might explain the glycemic improvement observed in diet-induced obese mice after pharmacological CB₁ receptor antagonist treatment [31].

Overactivation of the endocannabinoid system can lead to obesity

The majority of studies on the regulation of food intake control and metabolic processes by the ECS have been performed on lean animals and not in models of obesity. This is one of the reasons why a definitive conclusion failed to be reached on the mode of action through which the ECS contributes to the development of obesity. However, recent reports derived from obese animals and humans seem to identify a close association between the development of obesity and the simultaneous overactivation of the ECS, demonstrated by either a rise in endocannabinoid production or an increase in CB₁ receptor expression [9,10]. To date, five possible sites of pathological ECS overactivation have been identified:

- 1 The hypothalamus, as demonstrated by pathologically increased levels of hypothalamic endocannabinoids in genetically obese (*ob/ob*) mice [17].

- 2 The liver, as shown in rodents by a fourfold increase in AEA content and by the increased density of CB₁ receptors induced by a prolonged period of high-fat diet [24*].
- 3 Skeletal muscle, in which upregulation of the CB₁ receptor has been shown after a high-fat diet [10*].
- 4 Pancreatic cultured β -cells, under conditions mimicking hyperglycemia, in which elevated levels of both AEA and 2-AG [36*] have been found. This suggests dysregulation of endocannabinoid-induced endocrine pancreas secretion in conditions such as prediabetes, type 2 diabetes and obesity.
- 5 White adipose tissue, in which increased levels of endocannabinoids have been found in visceral depots derived from obese patients when compared with those from lean subjects [36*]. The putative CB₁ receptor overexpression in adipocytes derived from obese animals or humans is still under debate [32,42*].

There is, as yet, no clear explanation for the mechanisms responsible for hyperactivation of the endocannabinoid tone in obesity, although considerable evidence seems to point towards a genetic mutation found in a population of obese subjects. This mutation substitutes in threonine for a highly conserved proline residue (P129T) in the sequence of the fatty acid amid hydrolase, the enzyme that rapidly degrades anandamide after its action. Patients with this polymorphism have approximately 50% enzymatic activity; this physiological reduction of function could influence the clearance of endocannabinoids, leading to a sustained and possibly pathological tone of these lipids [43].

Clinical trials with rimonabant to tackle obesity and obesity-related co-morbidities

Clinical trials have shown that rimonabant, the first CB₁ receptor synthesized, has a therapeutic role in the treatment of obesity. A worldwide Phase III trial named RIO (Rimonabant In Obesity) has recently been concluded, and involved more than 6600 obese or overweight patients with or without concomitant co-morbidities. This study consisted of four different clinical trials. The two-year studies — RIO-Europe and RIO-North America — recruited obese or overweight patients with or without co-morbidities. The one-year trials — RIO-Lipids and RIO-Diabetes — were set up to investigate the improvement after rimonabant treatment of specific co-morbidity factors associated with obesity, such as hyperlipidemia and diabetes. The treatment consisted of rimonabant (5 mg or 20 mg) or placebo associated with a hypocaloric diet and counselling to increase the patients' physical activity. However, 5 mg rimonabant did not provide statistically significant changes when compared with placebo; therefore, only data concerning 20 mg rimonabant will be mentioned below (in comparison with placebo treatment). Three of these studies have been published to date: the RIO-Europe *ad interim* analysis of the first

year [44**], RIO-Lipids [45**] and RIO-North America [46**].

Body weight and waist circumference reduction

In the 'intention to treat' analysis with the last observation carried forward of the RIO-Europe study, a weight reduction of 6.6 kg was observed in the 20 mg rimonabant group compared with a loss of 1.8 kg in the placebo-treated group [44**]. Similar data were obtained in the RIO-Lipids (−6.9 kg versus −1.5 kg) [45**] and RIO-North America (−6.3 kg versus −1.6 kg) studies [46**].

As expected, a concomitant reduction in waist circumference of 6.5 cm, 7.1 cm and 6.1 cm was observed in patients treated with 20 mg rimonabant in the RIO-Europe, RIO-Lipids and RIO-North America studies, respectively, whereas the reductions seen in placebo-treated patients were 2.4 cm, 2.4 cm and 2.2 cm, respectively [44**–46**]. The pattern of weight loss showed a sustained profile for 36–40 weeks, followed by a plateau phase in all three trials. In the RIO-North America study, patients who received rimonabant were re-randomized at the end of the first year to either the same dose of rimonabant or placebo, and followed up for an additional year. Patients who were switched from 20 mg rimonabant to the placebo group experienced re-gain of weight, whereas those who continued to receive 20 mg rimonabant maintained their weight loss [46**].

Lipid changes

A significant increase in high-density lipoprotein (HDL) cholesterol and a decrease in triglyceride concentrations in patients treated with 20 mg rimonabant were detected in all three studies. Indeed, HDL cholesterol increased by 22.3%, 19.1% and 12.6% in the RIO-Europe, RIO-Lipids and RIO-North America studies, respectively, in comparison to 13.4%, 11.0% and 5.0% in the placebo groups of the same studies.

The fall in triglycerides in patients treated with 20 mg rimonabant was 6.8%, 12.6% and 5.3% in the RIO-Europe, RIO-Lipids and RIO-North America studies, compared with 8.3%, 0.2% and 7.9% in the placebo cohorts of the same trials [44**–46**].

Changes in glucose metabolism parameters

Plasma glucose and insulin levels were measured before the 75 g oral glucose tolerance test and after 30 min, 60 min and 120 min of the test, both at the beginning and at the end of the one-year treatment. In the RIO-Europe trial, statistically significant reductions in plasma glucose and insulin levels were achieved after 20 mg rimonabant treatment [44**]. Both the RIO-Europe and RIO-Lipids studies showed a significant reduction (from baseline) in 120-min insulin levels in patients receiving 20 mg rimonabant compared with placebo; in addition, the RIO-Lipids study showed that the 60-min and

120-min plasma glucose levels, the 60-min insulin levels, and the glucose and insulin areas under the curve all decreased significantly in the group receiving 20 mg rimonabant [45**].

Blood pressure

In the RIO-Europe and RIO-North-America studies, systolic and diastolic blood pressure was reduced after one year of treatment with 20 mg rimonabant; these changes were not, however, significantly different from placebo [44**,46**]. In contrast, decreases in systolic and diastolic blood pressure in the 20 mg rimonabant group were statistically significant when compared with placebo in the RIO-Lipids study [45**]. Importantly, the decrease was greater among patients with hypertension at baseline [45**].

Other parameters

Among the three studies, only the RIO-Lipids trial examined the changes in circulating leptin and adiponectin levels after rimonabant or placebo treatment. Plasma leptin levels decreased significantly in the group receiving 20 mg rimonabant versus placebo, whereas plasma adiponectin levels significantly increased in the 20 mg rimonabant group when compared with placebo [45**].

Safety data

All three RIO studies showed a slightly higher number of adverse or serious adverse events following rimonabant treatment when compared with placebo. The most common events occurring more frequently were nausea, vomiting, diarrhoea and dizziness. However, they were often mild-to-moderate in intensity and considered to be transient, largely based on their occurrence during the first few months of the studies [44**–46**].

Monitoring for on-treatment anxiety and depression will be necessary in the future to ensure the safe use of rimonabant or any other CB₁ receptor antagonist. As expected, in all three trials, a percentage of patients developed depression or anxiety and this led to discontinuation of the treatment in a moderate but significant number of subjects when compared with placebo.

Rimonabant: current status

Rimonabant (AcompliaTM), the first of a new class of CB₁ receptor antagonist, has been recently approved by the Committee for Medicinal Products for Human Use of the European Medicines Agency (EMA) as a weight loss agent to be used in conjunction with a hypocaloric diet and increased physical activity for the treatment of obese patients (body mass index [BMI] ≥ 30 kg/m²) or overweight patients (BMI > 27 kg/m²) with associated risk factor(s), such as type 2 diabetes or dyslipidaemia (<http://www.emea.eu.int>). The Committee recognized that 50% of the observed improvement in several metabolic parameters in patients who received 20 mg

rimonabant was beyond that expected from weight loss alone. In conclusion, patients most likely to benefit from rimonabant are those with multiple cardio-metabolic risk factors known to be improved by the drug, or those with a BMI > 27 kg/m² who also have abdominal obesity and type 2 diabetes or dyslipidaemia (low HDL cholesterol and/or high triglycerides). Rimonabant is not indicated for patients with a BMI < 27 kg/m² or for those with a BMI between 27 kg/m² and 29.9 kg/m² but who have no associated cardiometabolic risk factor(s). Rimonabant is contraindicated for pregnant or breast-feeding women and is not recommended for children below 18 years of age, patients with uncontrolled serious psychiatric illness such as major depression, or patients receiving antidepressant medication; moreover, it should not be given to patients with severe renal/hepatic impairment.

Rimonabant is currently under review by the Food and Drug Administration for approval in the US.

Conclusions

The ability of the ECS to control food intake and energy balance has recently received great attention, particularly in the light of the different mechanisms of action underlying these functions. The ECS modulates the rewarding properties of food by acting at specific mesolimbic areas in the brain. In the hypothalamus, the CB₁ receptor and endocannabinoids are integrated components of the networks controlling appetite and food intake. Interestingly, the ECS has also been shown to control metabolic functions by acting on peripheral tissues, such as adipose tissue, liver, gastrointestinal tract, skeletal muscle and endocrine pancreas. This system also appears to affect peripheral metabolism through weight-independent mechanisms, and the pathological condition of obesity is strongly associated with overactivation of the ECS.

In the light of this evidence, drugs that interfere with the ECS, especially CB₁ receptor antagonists, should be considered as useful adjuncts to lifestyle and behaviour modifications in the treatment of obesity and obesity-related co-morbidities.

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