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OPINION

Antagonizing the cannabinoid receptor Type 1: A dual way to fight obesity

D. Cota[1], S. Genghini[2], R. Pasquali[2] and U. Pagotto[2]

[1]Neuroendocrinology Group, Max-Planck-Institute of Psychiatry, Munich, Germany; [2]Endocrinology Unit, Department of Internal Medicine and Gastroenterology, Center for Applied Biomedical Research (C.R.B.A.), S.Orsola-Malpighi Hospital, Bologna, Italy

The energy balance equation requires energy intake to be equal to energy output in order to maintain a steady body weight. Obesity is defined as a disease caused by a constantly high caloric intake exceeding energy expenditure needs and resulting in an excess of body fat storage by the organism. In terms of epidemiology, obesity has been recognized as one of the top 10 health problems worldwide, in fact more than 300 million adults are obese and one billion are overweight (1).

Over the centuries, the human body has developed powerful biological drives to defend itself actively against famine. Conversely, control mechanisms to limit caloric intake are less powerful. Nowadays, greater food availability and changes in life styles work together against maintenance of a healthy body weight (2). Given the heterogeneity of the etiology of the disorder and the not yet complete understanding of the complexity of the systems regulating energy balance, the development of more effective weight management therapies is absolutely necessary to limit the harmful consequences of obesity, such as an increased risk for Type 2 diabetes, cardiovascular diseases and cancer (3).

THE ROLE OF THE ENDOGENOUS CANNABINOID SYSTEM ON CENTRAL CIRCUITS REGULATING FOOD INTAKE

Marijuana (*Cannabis sativa*) and its psychoactive derivatives are the most widely consumed illegal drugs in western countries. Despite the ethical and social concerns raised by the abuse of these substances, their therapeutic potentials, highlighted by increased scientific research in the last 30 yr, represent an extremely attractive frontier. The ability of marijuana to increase hunger and stimulate appetite, particularly for sweet and palatable food, was described many years ago. In the '90s, the discovery of the cannabinoid receptors (at least two subtypes known, CB1 and CB2) and of their endogenous ligands, lipid compounds derived from arachidonic acid and collectively named endocannabinoids, provided evidence of an endogenous cannabinoid system. This clue provoked accelerating research to discover the physiological functions of this system, whose high degree of evolutionary conservation across species further underlines its fundamental role in mammalians (4).

Several studies on the behavioral pharmacology of cannabinoids have shown that the administration of the main psychoactive compound of marijuana, Δ^9-tetrahydrocannabinol (Δ^9-THC), determines increased food intake in rodents and humans. Synthetic cannabinoid agonists are able to cause hyperphagia and an increase in body weight in experimental animals. Moreover, it is noteworthy that dronabinol (a synthetic compound derived from Δ^9-THC) was approved more than 15 yr ago for the treatment of anorexia and nausea in patients with cancer and for the treatment of AIDS-induced wasting syndrome (5, 6).

Even more intriguing was the finding that central or peripheral administration of the endocannabinoid anandamide determines overfeeding in rodents, confirming a direct role for endocannabinoids in the stimulation of food intake. Therefore, it has been suggested that an endocannabinoid tone could play a key role in the neuro-chemical regulation of appetite. These observations on the orexigenic effect of the endogenous, synthetic or plant-derived cannabinoids have been reinforced by the

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Correspondence: U. Pagotto, MD, Unità di Endocrinologia, Dipartimento di Medicina Interna e Gastroenterologia, Ospedale S.Orsola-Malpighi, Via Massarenti 9, 40138 Bologna, Italy.

E-mail: pagube@med.unibo.it

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remark that in short-term studies selective CB1 antagonists, such as SR141716A and AM281, can block and even reverse this effect, determining a decrease in food intake (6, 7).
The effects of endocannabinoids on appetite are clearly related to the production of the ligands and the expression of their membrane receptor (CB1) at the level of the hypothalamus and of other feeding-associated brain regions such as the limbic forebrain, mainly involved in the reward processes mediating the incentive value of food (6).
In 2001, Di Marzo et al. described that leptin is able to down-regulate hypothalamic endocannabinoid levels and that genetically obese, hyperphagic rodents with defective leptin signaling are characterized by elevated hypothalamic levels of endocannabinoids. Moreover, mice with defective leptin signaling treated with SR141716A showed a decreased food intake (8). All these data allowed the scientific community to include the endocannabinoids as neuromodulators belonging to the increasing family of the appetite-stimulating neuromediators, such as NPY, orexins/hypocretins and melanin concentrating hormone (MCH) (8). Moreover, reliable data seem to indicate that the levels of endocannabinoids, especially in the brain areas involved in eating motivation, may be deeply modified by fasting and feeding states (9). As a counterpart, when animals are fed with palatable food, CB1 mRNA expression is down-regulated in areas of the limbic forebrain consistently with increased activation of the receptors by endocannabinoids (10).
Acting in brain regions like the *nucleus accumbens* and hippocampus, endocannabinoids may stimulate the appetite for tasty food and it may therefore be hypothesized that they play a role in obesity induced by eating-disorders. Some reports have suggested that cannabinoids and endocannabinoids may induce overconsumption by increasing the motivation to search for palatable food. Therefore, cannabinoids and endocannabinoids act on appetitive processes, inducing eating even in satiated animals. The evidence that CB1 antagonists are able to reduce the motivation for sucrose, alcohol and beer intake further indicates that a positive incentive could be under a CB1-mediated control (5, 6). The interactions of the endogenous cannabinoid system with other pathways, like the opioidergic one, could possibly explain part of the cannabinoid effects on reward processes (6).
Moreover, exciting results are emerging from the application of reverse genetics. The study of the CB1 knockout ($CB1^{-/-}$) phenotype has thrown further light on the role of the endogenous cannabinoid system in feeding and body weight regulation. It has been reported that mice with genetically impaired CB1 receptors ($CB1^{-/-}$) show a reduced hyperphagic response to fasting as compared to their wild-type (WT) littermates (8). Very recently, we have conducted a more comprehensive study of the $CB1^{-/-}$ phenotype showing that these mice are characterized by hypophagia, decreased body weight and reduced fat mass compared to their WT littermates (11). It is noteworthy that the high number of appetite-stimulating factors is unable to compensate for the lack of the endocannabinoid action in maintaining energy homeostasis in $CB1^{-/-}$ mice, whose phenotype is principally related to the decreased caloric intake (11). Because CB1 mRNA has been co-localized with several hypothalamic neuropeptides, such as corticotroph releasing hormone (CRH), cocaine- and amphetamine-related transcripts (CART), orexins/hypocretins and MCH, it is reasonable to expect that endocannabinoids directly alter signaling flow within central appetite regulating circuits (11). In support of this hypothesis, $CB1^{-/-}$ mice show altered levels of hypothalamic CRH and CART transcripts that could partially account for their modified feeding behavior (11).
However, the hyperphagic action of cannabinoids seems not exclusively mediated by CB1 located in brain circuits controlling feeding. Recent evidence suggests that they may influence energy homeostasis by acting at peripheral sites as well.

THE ROLE OF THE ENDOGENOUS CANNABINOID SYSTEM ON PERIPHERAL ORGANS IN THE REGULATION OF ENERGY BALANCE

A recent paper has described that the content of the endocannabinoids produced in the intestinal tract is modulated by feeding (12). In the small intestine starvation determines a 7-fold increase in the orexigenic anandamide and a decrease of the anorexic non-cannabinoid anandamide-analog oleylethanolamide; interestingly this effect is reversed on re-feeding (12). Therefore, the activation of CB1 on nerve terminals innervating the gastrointestinal tract is involved in mediating satiety signals originating in the gut. Moreover, evidence shows that cannabinoids are able to modulate gastric emptying and intestinal peristalsis and anandamide produced *in situ* could reasonably act as an integrative signal regulating both gastrointestinal motility and food intake (12).
As mentioned above, the most commonly used CB1 antagonist SR141716A has been shown to have anorectic efficacy in a number of short-term studies performed in experimental animals, where the decrease in food intake due to the compound

was particularly evident for palatable and tasty foods (5, 6). In two very recent reports, researchers investigated the long-term efficacy of different CB1 antagonist compounds, SR141716A and AM251 respectively, in a diet-induced obesity (DIO) model that more realistically reflects the most common cause of human obesity (13, 14). The repeated administration of the compounds in DIO mice induced a transient central anorectic effect which was overcome by a more significant and sustained reduction in body weight, due to the decrease in body fat mass. These findings strongly suggest that peripheral, feeding-independent mechanisms may contribute to the action of the drugs (13, 14).

Interestingly, we have found that CB1 is expressed in white adipocytes and is involved in lipogenesis (11). Experiments performed *in vitro* have demonstrated that CB1 agonists stimulate adipocyte differentiation, increasing the activity of the lipoprotein lipase enzyme, an effect completely blocked by the SR141716A treatment. As a consequence, these findings have suggested that the leanness and the reduced body fat in $CB1^{-/-}$ mice could be explained by both hypothalamic and adipocyte dysfunctions (11).

It should be mentioned that obesity is considered a pro-inflammatory state and that adipose tissue is an important source of cytokines (adipokines) known to be involved in the onset of endothelial dysfunction and altered insulin sensitivity that lead to atherosclerosis and diabetes mellitus Type 2, diseases typically associated with increased adiposity (15). In this context, recent intriguing studies have further supported a metabolic role exerted by the endogenous cannabinoid system describing a direct action of cannabinoid compounds on adipose tissue. Liu et al. have shown that a synthetic analog of Δ^9-THC (named ajulemic acid or AJA) is able to bind and to activate the peroxisome proliferator-activated receptor γ (PPARγ). It is well known that the activation of this nuclear receptor (e.g. through the action of PPARγ agonists, such as thiazolidinediones) highly expressed in fat is directly linked to anti-inflammatory processes, amelioration of insulin sensitivity and adipocyte differentiation (16). Moreover, it has been described that CB1 expression is up-regulated in differentiated adipocytes and that the administration of SR141716A in obese fa/fa rats determines a decrease in body weight and an increase in adiponectin (Acrp 30) expression, therefore inducing an advantageous metabolic change that potentiates and sustains weight loss (17). In fact, Acrp30 is a secreted protein exclusively produced by adipose tissue that, among the other known adipokines, appears to improve insulin sensitivity, induces fatty acid oxidation, reduces body weight and inhibits vascular inflammation (17).

However, the downstream molecular pathways involved in this novel metabolic function of the endogenous cannabinoid system still have to be elucidated. In fact, these recent findings seem to suggest that both CB1 agonists and antagonists and/or cannabinoid compounds that directly bind nuclear receptors, without involving CB1, are able to modulate adipose tissue processes, altogether determining an amelioration of metabolic parameters usually altered by the development of obesity.

Therefore, even taking into account that further research is mandatory to clarify the different mechanisms underlying the effects of the endocannabinoids both at central and peripheral level, a consideration should be formulated. Endocannabinoids are centrally and peripherally produced, they stimulate appetite and are strongly involved in energy-regulating processes. The possibility of interfering with a system that contemporarily acts on central nervous pathways and on peripheral signals of food intake and of fuel storage is therefore an extremely attractive proposal, paving the way for the clinical use of one of the most promising drug targets for the treatment of eating disorders and obesity. In this perspective, CB1 antagonists remarkably seem to represent one of the most promising therapeutical candidates in the fight against obesity. Clinical phase III trials are ongoing to evaluate the effects of SR141716A (pharmaceutically named Rimonabant) in obese patients with and without comorbidities, with dyslipidemia and Type 2 diabetes (http://www.clinicaltrials.gov). Data from these trials are eagerly awaited, since they will definitively provide the key information as to the real opportunities of application for this class of molecules to treat obesity.

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