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Endocannabinoid control of food intake and energy balance

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Marijuana and its major psychotropic component, Δ^9-tetrahydrocannabinol, stimulate appetite and increase body weight in wasting syndromes, suggesting that the CB_1 cannabinoid receptor and its endogenous ligands, the endocannabinoids, are involved in controlling energy balance. The endocannabinoid system controls food intake via both central and peripheral mechanisms, and it may also stimulate lipogenesis and fat accumulation. Here we discuss the multifaceted regulation of energy homeostasis by endocannabinoids, together with its applications to the treatment of eating disorders and metabolic syndromes.

The natural compound Δ^9-tetrahydrocannabinol (Δ^9-THC), derived from *Cannabis sativa*, is responsible for the psychotropic effects of marijuana and was used in medicine before its mechanism of action was discovered. The anti-emetic and appetite-inducing properties of cannabis have been known for centuries, but only in the last half-century were they assigned to Δ^9-THC[1]. This compound, as well as its synthetic analogue nabilone, have been prescribed to ameliorate vomiting and nausea in cancer patients since the mid-1980s and to prevent weight loss in AIDS patients since 1992.

However, the first receptor for Δ^9-THC was fully characterized[2] only in 1990. This was a G protein–coupled membrane receptor (GPCR)—as could be expected from the fact that Δ^9-THC inhibits adenylyl cyclase and modulates the activity of Ca^{2+} and K^+ channels in neurons in a pertussis toxin–sensitive manner[3]. This first cannabinoid receptor, which is also the most abundant GPCR in the brain, was named CB_1 after the cloning of the second cannabinoid receptor subtype, CB_2, which instead is mostly present in immune cells[3]. The first two endogenous cannabinoid receptor ligands, or endocannabinoids, were discovered in the early 1990s. *N*-arachidonoyl ethanolamine (anandamide)[4] and 2-arachidonoyl glycerol (2-AG)[5,6] are derivatives of arachidonic acid, an ω6-polyunsaturated fatty acid, which is in turn derived from essential fatty acids and is the precursor of several other chemical signals. Phospholipid-dependent pathways for endocannabinoid biosynthesis were discovered, leading to the cloning of the enzymes that catalyze the formation of anandamide and 2-AG from their direct precursors: the *N*-acylphosphatidylethanolamine-selective phospholipase D and the *sn*-1–selective diacylglycerol lipases, respectively[7,8].

The two major endocannabinoids are rapidly hydrolyzed by the fatty acid amide hydrolase and the monoacylglycerol lipase, respectively[9,10], to compounds that are inactive at cannabinoid receptors. The cannabinoid receptors, the endocannabinoids and the enzymes catalyzing their biosynthesis and degradation constitute the endocannabinoid system (**Fig. 1**). Endocannabinoids are not confined to the CNS, but rather act as local mediators in many tissues and are produced 'on demand' to help restore the levels and function of other mediators (including excitatory and inhibitory neurotransmitters) after acute or chronic alterations of the physiological homeostasis of the cell[11].

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Brain endocannabinoids control food intake

Regulation of energy intake by the cannabinoid system was initially assumed to occur centrally. Pharmacological stimulation of CB_1 receptors by systemic administration of plant or endogenous cannabinoids stimulates eating—in the case of Δ^9-THC, even in satiated animals[12–14]. Pharmacological blockade of CB_1 receptors by systemic administration of SR141716A (rimonabant), the first selective CB_1 antagonist[15], attenuates agonists' stimulatory effects on food intake and strongly reduces both the consumption of palatable food (such as sweet foods) by animals fed *ad libitum* and the intake of normal food, but not water, by animals deprived of food[16–19]. Other CB_1 antagonists exert identical effects[20,21]; even a single dose of the antagonist AM251 produces an anorectic effect lasting up to 6 d (ref. 22). Furthermore, CB_1-deficient mice consume much less food in the first hours after food deprivation[23]. These data, together with the established neuromodulatory role of endocannabinoids through CB_1 receptors[11], suggested that the brain endocannabinoid system controls food intake at two levels. First, it tonically reinforces the motivation to find and consume foods with a high incentive value, possibly by interacting with the mesolimbic pathways involved in reward mechanisms. Second, it is activated 'on demand' in the hypothalamus after short-term food deprivation and then transiently regulates the levels and/or action of other orexigenic and anorectic mediators to induce appetite.

The hypothesis of a dual action in mesolimbic and hypothalamic regions was substantiated by the finding that injection of endocannabinoids into these brain areas stimulates food intake in rats[24,25]. Furthermore, endocannabinoid levels vary in both the hypothalamus and the limbic forebrain (but not in the cerebellum, which is not involved in appetite regulation) during the four phases of feeding behavior in rats. These levels are highest during food deprivation and lowest during food consumption, as expected from endogenous

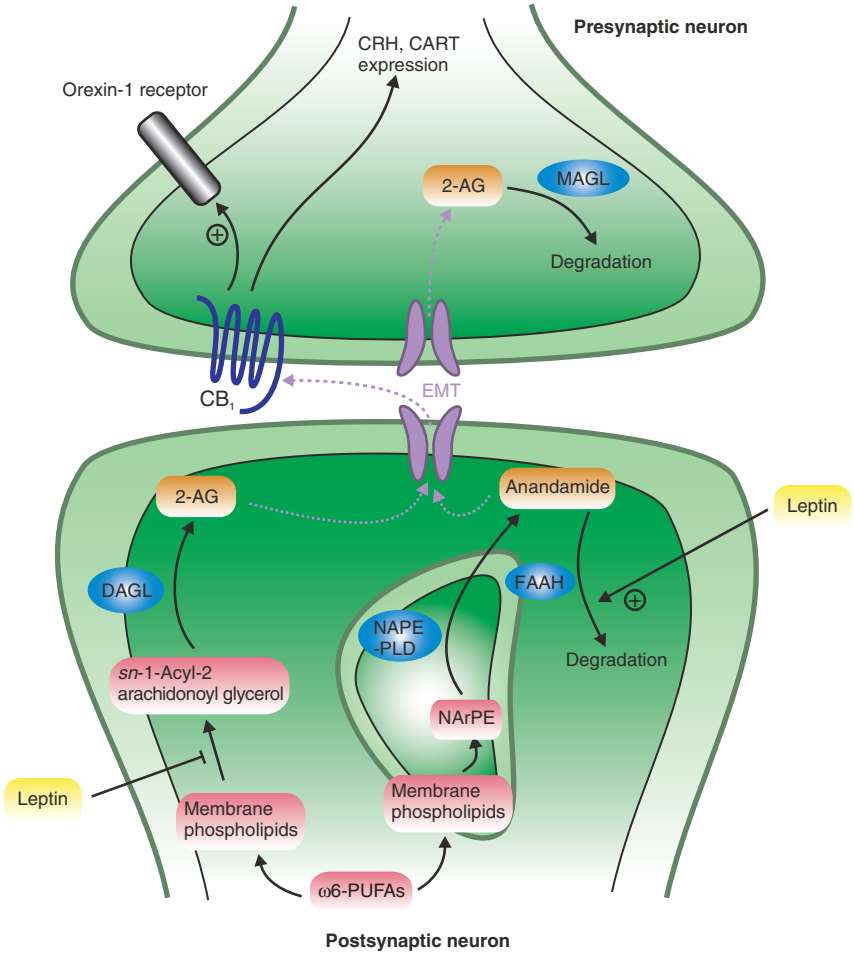

Figure 1 The endocannabinoid system in neurons. Diet-derived ω6-polyunsaturated fatty acids (ω6-PUFAs) are incorporated into membrane phospholipids, which can subsequently be metabolized into the two major endocannabinoids, 2-AG and anandamide, by membrane-associated enzymes. Degradative enzymes for endocannabinoids are localized to internal membranes. Leptin signaling can influence 2-AG biosynthesis in the hypothalamus[23] and anandamide hydrolysis in T-lymphocytes[64]. CB_1 is located mostly presynaptically, allowing for retrograde action[33] of endocannabinoids. CB_1 signaling affects the expression of orexigenic and anoretic mediators in the hypothalamus[32]. DAGL: *sn*-1 selective diacylglycerol lipase; EMT: putative endocannabinoid membrane transporters; FAAH: fatty acid amide hydrolase; MAGL: monoacylglycerol lipase; NArPE: *N*-arachidonoyl-phosphatidyl-ethanolamine; NAPE-PLD: *N*-acyl-phosphatidylethanolamine–selective phospholipase D; CRH: corticotropin-releasing hormone; CART: cocaine-amphetamine–regulated transcript. Blunt-ended line indicates inhibition.

orexigenic mediators[25]. In the hypothalamus, these changes in endocannabinoid levels seemed to be inversely correlated with the changes that are known to occur in blood levels of the neurohormone leptin, which is pivotal in regulating the hypothalamic orexigenic and anoretic signals. Indeed, leptin decreases endocannabinoid levels in the hypothalamus, much as it does for other orexigenic mediators, and obese rodents with defective leptin signaling show significantly higher hypothalamic endocannabinoid concentrations[23]. It has been suggested that an enhanced endocannabinoid tone is also linked to enhanced ghrelin levels in the bloodstream after food deprivation and may underlie some of the orexigenic effects of this peptide when injected into the rat hypothalamus—effects that are in fact blocked by antagonism at CB_1 receptors with rimonabant[26].

After fasting, the endocannabinoid system in the hypothalamus is transiently activated, which increases energy intake. However, recent findings indicate that blockage of CB_1 receptors can also inhibit the intake of normal (not necessarily palatable) food in pre-fed, satiated animals, similar to what was observed with high energy (palatable) food[27,28]. This apparent discrepancy with earlier data[23] may be explained because the separation between hedonically driven and energy deprivation–driven food intake is not so marked. The mesolimbic regions most involved in translating motivation to eat into action (for example, the nucleus accumbens shell) participate in the consumption of normal food as well. Rimonabant blocks food consumption during both the consummatory and the appetitive phases of feeding behavior in pre-fed animals, but does not block the pavlovian response to a palatable stimulus. This finding suggests that endocannabinoids do not reinforce the ability of the stimulus to elicit an approach behavior, but instead maintain stimulus-induced goal-directed behaviors[29]. Accordingly, other authors proposed that stimulation of CB_1 receptors may enhance food palatability[30].

The endocannabinoid system may influence food intake by regulating the expression and/or action of several hypothalamic anoretic and orexigenic mediators. CB_1 receptors colocalize with corticotropin-releasing hormone (CRH) in the paraventricular nucleus (PVN), with melanin-concentrating hormone in the lateral hypothalamus, and with pre-pro-orexin in the ventromedial hypothalamus[31,32]. Genetic deletion of CB_1 increases expression of CRH, pointing to a tonic inhibition of the expression of this anoretic mediator by endocannabinoids[31]. Additionally, in the PVN postsynaptic endocannabinoids retrogradely inhibit glutamatergic release from presynaptic neurons, thus mediating corticosterone-induced fast inhibition of CRH release in this nucleus[33]. Two preliminary reports suggest that retrograde signaling by endocannabinoids released from depolarized postsynaptic neurons[11] also inhibits presynaptic GABA release in the lateral hypothalamus and arcuate nucleus (Y. Jo, S.C. Chua and L.W. Role, *Soc. Neurosci. Abstr.* 47.12, 2004; T. Hentges, M.J. Low and J.T. Williams, *Soc. Neurosci. Abstr.* 76.1, 2004). It remains to be fully determined how this retrograde signaling contributes to energy intake induction by endocannabinoids. Stimulation of CB_1 receptors also causes sensitization of orexin-1 receptors when the two proteins are expressed in the same cell, with possible subsequent enhancement of the appetite-inducing action of orexins[34]. No co-expression of CB_1 receptors and neuropeptide Y (NPY) was found, but it seems that endocannabinoid activation downstream of NPY mediates some of its orexigenic effects, which, accordingly, are attenuated by pharmacological or genetic impairment of CB_1 (ref. 35). In contrast, rimonabant is as effective an anoretic agent in wild-type as in NPY-null mice[23]. This indicates that the induction of food intake by endocannabinoids is not mediated by NPY (in agreement with the lack of coexpression of CB_1 receptors and this neuropeptide) and, additionally, that the surprisingly normal food intake of NPY-deficient mice is not due to compensa-

tion by the endocannabinoid system. Finally, CB_1 receptors seem to inhibit anorectic events downstream of melanocortin-4 receptors[36]. Concerning the mesolimbic system, evidence supports the hypothesis that endocannabinoids increase the drive to eat by enhancing dopamine release in the nucleus accumbens shell[37,38] or by synergizing with opioids through as yet undefined mechanisms[18,19,39–41].

The brain endocannabinoid system seems to be very important for controlling food intake in young rodents[31] and even more in newborn mice, where pharmacological blockade of CB_1 receptors at postnatal day 1 (PND1) leads to suppression of suckling and milk ingestion and eventually to death[42]. Newborn mice lacking CB_1 also ingest less milk, but with less lethal consequences[42]. These observations are particularly notable because 2-AG levels peak in rat brain at PND1 (ref. 43) and high concentrations of 2-AG are found in milk[44].

Another checkpoint at which the endocannabinoid system acts on food intake occurs in the vagus nerve that connects the gastrointestinal tract with medulla and brainstem nuclei involved in control of satiety. In the rat, food deprivation enhances anandamide levels in the duodenum. Here the endocannabinoid may reduce satiety by acting on the vagus, as suggested by the anoretic action of peripherally administered rimonabant and by the reversal of this action following destruction of the vagal capsaicin-sensitive nerves that also mediate cholecystokinin (CCK)-induced satiety[45]. Food deprivation also enhances CB_1 expression in CCK-1 receptor–expressing neurons of the rat nodose ganglion projecting to the duodenum; renewed feeding or treatment with CCK re-establishes low levels of CB_1 receptors in these neurons[46]. These data suggest that reduced endocannabinoid activity may mediate induction of satiety by CCK; they also suggest that fasting overcomes satiety (and possibly emesis) by elevating small intestine endocannabinoid levels and by releasing vagal CB_1 receptors from CCK inhibition, thus disinhibiting the endocannabinoid system in the vagus.

Peripheral control of energy balance by endocannabinoids

The strong evidence supporting the involvement of endocannabinoids in controlling food intake encouraged preclinical studies on the use of CB_1 antagonists against obesity[47]. Rimonabant reduced food intake in genetic models of obesity, the *ob*/*ob* and *db*/*db* mice[23] and Zucker rats[48]. However, the effects of CB_1 blockers on food intake in these models were transient and were significantly outlasted by the effects on body weight[48]. The partial dissociation between the somehow short-lasting anorectic effect of CB_1 antagonists and their longer-lasting effects on body weight was also observed in studies using pair-fed controls and in a model more relevant to human obesity, the diet-induced obese mouse, where obesity is induced by a prolonged high-fat diet. In this model, chronic CB_1 blockade produced a significant reduction of fat mass relative to skeletal muscle mass, and an improvement of metabolic parameters typical of obesity: a reduction in plasma levels of insulin, leptin, non-esterified fatty acids and/or cholesterol, and an increase in the HDL/LDL cholesterol ratio[49–51]. Even more notably, CB_1-null mice, when fed a normal diet from birth, are leaner than their pair-fed wild-type littermates, and have less fat mass[31]. After a high-fat diet, these mice, although they consume as much food as wild-type mice, do not become obese, nor do they develop insensitivity to insulin or leptin[52].

These studies suggest that only part of the reduction of body weight and fat mass effected by CB_1 antagonists is due to their anorectic action and that these drugs also act by counteracting a peripheral tonic action of endocannabinoids on lipogenesis and fat accumulation. Clearly, pharmacological or genetic blockade of CB_1 must be accompanied by increased energy expenditure, and in fact rimonabant was recently shown to increase oxygen consumption and soleus muscle glucose uptake in *ob*/*ob* mice[53]. The effect of the endocannabinoid system on lipogenesis is substantiated by the finding of CB_1 receptors in white adipocytes. In these cells, stimulation of CB_1 leads to activation of lipoprotein lipase[31], whereas its blockade causes up-regulation both *in vitro* and *in vivo*[51,54] of adiponectin, a hormone crucial in reducing the expression of enzymes involved in lipogenesis. Other peripheral organs and tissues, in particular the liver, pancreas and skeletal muscles, might also be involved in the control of energy balance by endocannabinoids. Indeed, stimulation of CB_1 receptors in the liver and hepatocytes increases *de novo* fatty acid biosynthesis by increasing the expression of the lipogenic transcription factor sterol response element–binding protein 1c and two of its targets, acetyl-CoA carboxylase-1 and fatty acid synthase[55]. The finding that a CB_1 agonist enhances the expression of the latter enzyme also in the hypothalamus[55], together with the discovery of adiponectin receptors in the PVN[56], supports the concept that endocannabinoid control of energy balance at the hypothalamic and peripheral levels are likely to be related, cross-talking phenomena.

CB_1 blockers against obesity and metabolic syndromes

The preclinical data outlined above have been confirmed in the context of human obesity by three separate phase III clinical trials carried out with rimonabant. The results of a 2-year study, known as Rimonabant in Obesity (RIO)-North America, with over 3,400 patients subjected to a mild low-calorie diet, were communicated at the 2004 American Heart Association meeting and can be summarized as follows. A 1-year treatment with a 20 mg d^{-1} oral dose of rimonabant causes weight losses of ≥5% and ≥10% in over 62% and 32%, respectively, of subjects completing the study but in only 33% and 16%, respectively, of control subjects receiving placebos. The average weight loss and waist reduction were ~8.8 kg and 8.4 cm, versus 2.9 kg and 4 cm in placebo controls, respectively. After 1 year of treatment, the blood triglyceride levels in subjects completing the study dropped by ~8.5% (versus a ~4.5% increase in placebo controls) and HDL cholesterol levels increased by ~17.5% (versus ~6.3% in placebo controls). Fasting insulin levels decreased by ~2.7 μIU ml^{-1} compared with controls. After randomization into placebo or drug continuation at 1 year, subjects who were kept on rimonabant for another year did not lose further weight but continued to significantly increase their HDL cholesterol levels, whereas the previously treated subjects now taking placebo slowly regained weight to become undistinguishable from the placebo-placebo group only at the end of the trial. Results identical to those in the first year of this study were obtained in two similar 1-year studies: the RIO-Lipids and the RIO-Europe trials. In the RIO-Lipids trials, where a high percentage of patients with metabolic syndrome was selected, and in the RIO-Europe trials, ~50% of the beneficial metabolic effects were dissociated from the observed decrease in body weight, and an increase in adiponectin levels was observed after administration of rimonabant. The pooled data from the RIO studies (5,580 patients) at 1 year also yielded promising results in regard to safety: only 3.6% more rimonabant-treated subjects than placebo-treated subjects experienced any adverse events, and only 5.9% more rimonabant-treated subjects than placebo-treated subjects discontinued treatment as a result of adverse events. These adverse events consisted mostly of nausea (+1.3%), diarrhea (+1.3%), dizziness (+0.6%), depression (+1.4%) and anxiety (+0.7%), and in most cases, subjects showed tolerance to them after the first weeks of treatment, in agreement with results in animal models[48,57].

The 'hyperactive' endocannabinoid system

In summary, animal studies suggest that the endocannabinoid system is important in inducing food intake: it is transiently activated after short-term fasting and/or exposure to palatable foods, thus inducing appetite, reducing satiety and ultimately stimulating lipogenesis and

Table 1 Multisite control of energy balance by the endocannabinoid system.

	The local endocannabinoid system is:	Endocannabinoid activation leads to:
Hypothalamus	Stimulated by fasting	Enhancement of orexin action
	Stimulated by ghrelin[a]	Downregulation of CRH
	Inhibited by leptin	Inhibition of MC4R action
		Increased appetite following food deprivation
Mesolimbic system	Stimulated by palatable (high fat) food	Enhanced dopaminergic signaling in NAc
		Synergism with the opioid system
		Translation of motivation to eat into action
Brainstem	Stimulated by fasting	Effects on nodose ganglion and NTS neurons
	Inhibited by CCK	Inhibition of satiety and emesis
Gastrointestinal tract (duodenum)	Stimulated by fasting	Stimulation of TRPV1/CB_1 neurons in the vagus nerve
		Inhibition of satiety
White adipose tissue	Hyperactivated by fat diet[a]	Downregulation of adiponectin[a]
		Increased lipogenesis

Endocannabinoids and CB_1 receptors are present in all central and peripheral sites involved in the control of energy homeostasis. The external or internal stimuli that regulate endocannabinoid or CB_1 levels are listed for each site, together with the most likely consequences. Evidence is emerging for a role of endocannabinoids in the induction of fatty acid synthesis in the liver as well[55]. CCK, cholecystokinin; CRH, corticotropin releasing hormone; MC4R, melanocortin receptor type 4; NTS, nucleus tractus solitarius; TRPV1, transient receptor potential vanilloid 1 channel for capsaicin; NAc, nucleus accumbens.

[a]Data for which there is only indirect experimental support.

decreasing energy expenditure (**Table 1**). This is consistent with the emerging concept of elevated endocannabinoid levels after stressful stimuli as a strategy to help organisms re-establish homeostasis[11]. However, both preclinical and clinical studies clearly indicate that this system also contributes to pathological conditions such as hyperphagia, exaggerated fat accumulation and dyslipidemia, which are reduced by pharmacologically decreasing the effects of endocannabinoids at CB_1 receptors. A sustained hyperactivity of the endocannabinoid system, limited to tissues controlling energy balance, thus may contribute to the development of obesity and metabolic syndromes. Such hyperactivity might be caused by high-fat diets and the subsequent increased availability of polyunsaturated fatty acid precursors for endocannabinoid biosynthesis and it might be sustained by the resistance to leptin that normally develops with obesity. Indeed, in newborn and adult rodents, dietary ω6-polyunsaturated fatty acids increase brain endocannabinoid levels, whereas prolonged semi-starvation or high dietary levels of ω3-polyunsaturated fatty acids decrease them[58–61]. The hypothesis of a locally hyperactive endocannabinoid system might explain why the appropriate dose of a competitive CB_1 antagonist can be used against abdominal obesity and its consequences, seemingly without causing major side effects.

This hypothesis is also supported by several other findings. First, obese rats are more sensitive than lean rats to rimonabant[48], although the potential for accumulation of this lipophilic compound in the adipose tissue may partly explain these differences as well as its longer-lasting peripheral actions. Second, adipocytes from obese rats and differentiated adipocytes express more CB_1 receptors than adipocytes from lean rats or immature adipocytes[54]. Third, a high-fat diet results in the enhancement of hepatic anandamide and CB_1 levels[55]. Fourth, significantly higher endocannabinoid concentrations are found in the blood or visceral fat of obese humans (ref. 62 and R. Monteleone and V.D.M., unpublished data). However, *in vitro*, CB_1 antagonists act independently of enhanced endocannabinoid levels as inverse agonists, and this property, although not normally observed *in vivo*, may underlie part of their pharmacological actions[63]. Studies with inhibitors of endocannabinoid biosynthesis may help confirm the results obtained with CB_1 antagonists and prove conclusively the hypothesis of a hyperactive endocannabinoid system as a factor contributing to obesity and related disorders. Finally, based on the finding of altered endocannabinoid levels in the blood of women with anorexia nervosa and binge eating disorder, but not bulimia nervosa[62], future investigations should also address the possible role and regulation of the endocannabinoid system in these eating disorders.

Note added in proof: A recent study has shown[65] that overweight and obesity in humans are associated with a potential genetic malfunctioning of one of the endocannabinoid degrading enzymes, further substantiating the hypothesis of a hyperactive endocannabinoid system as a possible cause of obesity.

COMPETING INTERESTS STATEMENT

The authors declare competing financial interests (see the *Nature Neuroscience* website for details).

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