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REVIEW

The role of the endocannabinoid system in the control of energy homeostasis

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The endocannabinoid system has recently emerged as an important regulator of energy homeostasis, involved in the control of both appetite and peripheral fat metabolism. We briefly review current understanding of the possible sites of action and cellular mechanisms involved in the central appetitive and peripheral metabolic effects of endocannabinoids. Studies in our laboratory, using leptin-deficient obese rodents and CB1 cannabinoid receptor (CB1)-deficient mice, have indicated that endocannabinoids acting via CB1 are involved in the hunger-induced increase in food intake and are negatively regulated by leptin in brain areas involved in appetite control, including the hypothalamus, limbic forebrain and amygdala. $CB1^{-/-}$ mice are lean and are resistant to diet-induced obesity (DIO) despite similar energy intake to wild-type mice with DIO, suggesting that CB1 regulation of body weight involves additional peripheral targets. Such targets appear to include both adipose tissue and the liver. CB1 expressed in adipocytes has been implicated in the control of adiponectin secretion and lipoprotein lipase activity. Recent findings indicate that both endocannabinoids and CB1 are present in the liver and are upregulated in DIO. CB1 stimulation increases *de novo* hepatic lipogenesis through activation of the fatty acid biosynthetic pathway. Components of this pathway are also expressed in the hypothalamus where they have been implicated in the regulation of appetite. The fatty acid biosynthetic pathway may thus represent a common molecular target for the central appetitive and peripheral metabolic effects of endocannabinoids.
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Endocannabinoid regulation of appetitive behavior

Cannabis has been used for recreational purposes for thousands of years, and its effects on appetite have been well recognized.[1] In colloquial terms, getting the 'munchies' means craving for food, particularly sweets. Endocannabinoids are endogenous lipids that bind to and activate the same receptor which mediates the effects of plant-derived cannabinoids. The two most widely studied endocannabinoids are arachidonoyl ethanolamide, called anandamide[2] and 2-arachidonoylglycerol (2-AG).[3,4] The discovery of these substances raised the obvious question: are endocannabinoids orexigenic and are they involved in the physiologic regulation of appetite and energy homeostasis? The present paper provides a brief overview of the central appetitive and peripheral metabolic effects of endocannabinoids. For more detail, the reader is referred to recent excellent reviews on this topic.[5–7]

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An important step toward understanding the biological role of endocannabinoids was the introduction by the sanofi-aventis company of selective cannabinoid receptor antagonists for both major types of cannabinoid receptor:[8,9] the CB1 receptor expressed at very high levels in the brain[10] but also present in various peripheral tissues,[11] and CB2 receptors expressed predominantly in immune and hematopoietic cells.[12] In the late 1990s, several studies have demonstrated that treatment with the CB1 selective antagonist, now called rimonabant, reduces food intake in rodents,[13–15] and both anandamide[16,17] and 2-AG[18] were found to stimulate food intake. However, it was not possible to conclude that the effect of rimonabant was due to blocking of the tonic orexigenic effect of an endocannabinoid because of the well-known inverse agonist properties of rimonabant.[19,20] This problem can be circumvented through the use of mice deficient in CB1 receptors. The mouse strain preferred for studies of obesity is the C57Bl6, a hedonic strain with high preference for both palatable food and a propensity to develop obesity when maintained on a high-fat diet. CB1 receptor knockout mice developed by Zimmer *et al*.[21] had been backcrossed to a C57Bl6 background, and heterozygote breeding pairs yielded CB1 knockout mice and

wild-type littermates used as controls in a study to determine the role of endocannabinoids and CB1 receptors in the control of food intake.[22]

When food intake was measured in such mice under free-feeding conditions, there was no difference between basal food intake in knockout and wild-type mice.[23] However, when appetite was increased by fasting for 24 h prior to testing, the hunger-induced increase in food intake was much greater in wild-type than in knockout mice.[22] Furthermore, when these fasted mice were pretreated with 3 mg/kg rimonabant 10 min prior to the test period, food intake in the wild-type animals was reduced to the same level as seen in the knockouts, in which rimonabant was ineffective.[23] The observed parallel displacement of the cumulative food intake curve in wild-type mice treated with rimonabant means that all of the reduction occurred in the first hour of testing. This suggests that rimonabant acts on the appetitive rather than the consummatory phase of food intake. These findings clearly indicate that a part of the hunger-induced increase in food intake is mediated by endocannabinoids acting via CB1 receptors.[22]

Hyperphagia, which can be induced by food restriction in normal animals, is present in the early stages of genetically determined obesity, where the appetite suppressing effect of leptin is missing because of the absence of leptin, such as in the *ob/ob* mice, or a defect in leptin signaling, such as in *db/db* mice or Zucker (*fa/fa*) rats. In young *ob/ob* and *db/db* mice, rimonabant treatment reduced food intake even when the animals had free access to food prior to testing.[22] This suggests that in these animals, the activity of the endocannabinoid system is increased and contributes to their hyperphagia. Indeed, the hypothalamic levels of endocannabinoids, measured using gas chromatography/mass spectrometry, were found elevated in *db/db* mice and in Zucker rats compared to their respective lean controls.[22] Furthermore, the hypothalamic levels of anandamide were markedly reduced when *ob/ob* mice were treated with leptin.[22] These findings suggest a hypothalamic site of action for the orexigenic effects of endocannabinoids.

Hypothalamic site(s) of action are also suggested by findings that microinjection of anandamide into the dorsomedial hypothalamic nucleus increases food intake in rats,[24] and endocannabinoid levels in the hypothalamus decrease during feeding[18] and increase during short-term fasting.[18,25] In rats made tolerant to Δ^9-tetrahydrocannabinol (THC), rimonabant suppressed food-maintained operant responses and hypothalamic metabolism, measured by 2-deoxyglucose uptake.[26] CB1 receptors are expressed in areas of the hypothalamus involved in appetite control, such as the paraventricular nucleus (PVN), where they are expressed in CRH- and CART-containing neurons, and in the lateral hypothalamus, where they are present in orexin- and melanin concentrating hormone (MCH)-containing neurons.[27] This latter site is of particular interest, as orexin- and MCH neurons also express functional leptin receptors,[28,29] and they have dense projections to dopaminergic neurons in the ventral tegmental area,[30] where they modulate the mesolimbic dopaminergic pathway involved in food reward. Thus, they are a likely site for the proposed leptin/endocannabinoid interaction in the control of food intake,[22] and could also represent a site of integration of hypothalamic and extrahypothalamic structures involved in the orexigenic effect of endocannabinoids. However, activation of hypothalamic centers, such as the PVN, by endocannabinoids may also occur indirectly via CB1 receptors on peripheral afferent nerve terminals,[31] most likely located in the GI tract. Such an 'indirect' pathway is compatible with recent findings that CB1 mRNA is present in cholecystokinin (CCK)-containing neurons in the nodose ganglion, where CB1 mRNA expression is upregulated by fasting and downregulated by refeeding.[32] This latter effect could be mimicked by the satiety hormone CCK, which may thus suppress feeding indirectly via reducing orexigenic signaling via CB1.[32]

Endocannabinoids and CB1 receptors are integral components of the mesolimbic reward pathway,[33] and cannabinoids are known to increase the taste for palatable food.[14,34] Not surprisingly then, sites in the limbic forebrain have also been implicated in their orexigenic effects. Alterations in endocannabinoid levels in response to feeding status were more pronounced in the limbic forebrain than in the hypothalamus,[18] and rimonabant suppressed 2-deoxyglucose uptake in the limbic forebrain of rats even in the absence of tolerance to THC, when no changes were observed in the hypothalamus.[26] In rats, a high-fat diet-induced downregulation of CB1 receptors, interpreted to indicate increased endocannabinoid activity, was detected in several regions of the limbic forebrain including the nucleus accumbens, but not in the hypothalamus.[35] Additionally, cannabinoids can increase the intake of palatable foods - by acting at sites in the brainstem,[36] which are known to have reciprocal neural connections with forebrain limbic structures.[37]

The adipocyte-derived hormone leptin suppresses appetite indirectly by increasing the expression of anorexigenic mediators, such as alpha-MSH[38] and decreasing the expression of orexigenic factors, such as neuropeptide Y.[39] The increased tissue levels of endocannabinoids in animals with defective leptin signaling therefore suggest that leptin may negatively regulate the orexigenic endocannabinoids. Indeed, in preliminary experiments, we found that in mice with DIO, elevated plasma leptin levels were associated with marked reductions in tissue levels of anandamide in the hypothalamus, the amygdala, and the limbic forebrain, but not in the cerebellum.

Endocannabinoids regulate peripheral energy metabolism

It is well recognized that energy intake and utilization are regulated in a coordinated fashion, and factors involved in

the central regulation of appetite may also affect peripheral energy metabolism.[40] For example, the appetite-reducing hormone leptin also regulates the activity of stearoyl coenzyme-A desaturase in the liver, the enzyme involved in fatty acid desaturation.[41] Studies with rimonabant indicated that chronic treatment of mice with diet-induced obesity reduced appetite transiently but caused a sustained reduction in body weight.[42] This suggests that factors other than appetite must be involved in the weight-reducing effect of rimonabant. Further evidence for this came from observations that mice lacking CB1 receptors are resistant to diet-induced obesity even though their total energy intake is similar to that of wild-type littermates, which do become obese on the same diet.[43,44] When C57Bl6 mice are placed on a high-fat/high-carbohydrate diet, they become obese in 3–4 months: they gain weight and their adiposity index is increased, indicating increased adipose mass. These changes are associated with hyperleptinemia and elevated plasma insulin levels, reduced plasma adiponectin, and elevated plasma triglycerides,[44] changes compatible with the presence of the metabolic syndrome. In contrast, CB1 knockouts on the same high-fat diet do not gain weight or increase their fat deposits, and their plasma leptin levels also remain largely unchanged compared to CB1 knockouts on regular chow, even though their total energy intake during the 4 month period is not different from the intake of the obese wild-type mice on the same high-fat diet.[44] This clearly indicates that a CB1-mediated mechanism other than increase in appetite must be involved in the development of DIO. Indeed, adipocytes have been found to express CB1 receptors,[27,45] stimulation of which may affect lipid metabolism through regulating the level of adiponectin production[45] or by increasing lipoprotein lipase activity.[27] The latter study also demonstrated that CB1 knockout mice are lean and display hypophagia early in their lives. As they get older, their food intake becomes normal but their energy expenditure is increased as compared to their wild-type littermates.[27]

However, the role of adipose tissue in lipogenesis is minor compared to that of the liver.[46] In a recent study, the tissue mRNA level of the lipogenic transcription factor sterol response element-binding protein-1c (SREBP-1c) was consistently lower in both liver and adipose tissue from CB1$^{-/-}$ compared to wild-type mice, suggesting that SREBP1c expression may be tonically increased by endocannabinoids acting at CB1 receptors.[44] SREBP1c regulates the expression of genes involved in fatty acid synthesis, both acetyl coenzyme carboxylase-1 (ACC1) and fatty acid synthase (FAS),[47] and treatment of normal mice with the potent CB1 agonist HU-210 (20 ng/g) was found to increase the hepatic mRNA levels for SREBP1c, ACC1, and FAS, which could be prevented by pretreatment with 3 μg/g rimonabant.[44]

As a functional correlate of increased FAS gene expression, *de novo* hepatic fatty acid synthesis, as analyzed by measuring the incorporation of tritium into fatty acids in the liver following intrahepatic injection of ^3H_2O, was significantly increased by HU-210 treatment. Again, this effect was prevented by pretreatment with 3 μg/g rimonabant, and it was absent in CB1$^{-/-}$ mice. The CB1-mediated increase in fatty acid synthesis could be reproduced in isolated hepatocytes from wild-type mice, which strongly suggested that the receptors mediating this effect were located in the liver.[44] This was then verified by multiple approaches, including RT–PCR, *in situ* hybridization, immunohistochemistry, and Western blotting.[44] The latter technique required the use of purified liver plasma membranes because of the very low level of hepatic CB1 receptors, which may have been the reason why earlier studies failed to detect CB1 message or protein in liver tissue.[48] Interestingly, CB1 receptors have been recently detected in rat liver stellate cells[49] as well as rat hepatocytes maintained in organoid culture.[50]

The resistance of CB1-deficient mice to diet-induced obesity also manifested in the absence of fatty liver in these animals, whereas wild-type mice on the same high-fat diet developed fatty liver.[44] The striking difference between wild-type and CB1$^{-/-}$ mice in their response to high-fat diet suggests a role for CB1 in the hepatic lipogenic response to high-fat diet. Indeed, when wild-type mice were tested 3 weeks following the initiation of the high-fat diet, before a significant effect on body weight could be detected, the basal level of *de novo* fatty acid synthesis was markedly increased compared to that in lean controls, and pretreatment of the mice on the high-fat diet with 3 μg/g rimonabant significantly reduced the rate of fatty acid synthesis.[44] In mice killed at the same time, the hepatic levels of anandamide were elevated threefold compared to the lean controls, with no difference in the levels of 2-AG. The hepatic level of CB1 receptors, quantified in Western blots of purified liver plasma membranes, was also markedly increased in mice on the high-fat diet.[44] These findings indicate that intake of a high-fat diet activates the hepatic endocannabinoid system, which contributes to the increased lipogenesis.

Since total energy intake was not different in wild-type and CB1$^{-/-}$ mice on the high-fat diet,[43,44] the absence of CB1 must be associated with increased energy expenditure. Exposing C57Bl6 mice to a high-fat diet decreases energy expenditure, as documented by indirect calorimetry.[51] This accounts for the increase in feed efficiency in wild-type mice on a high-fat compared to normal diet, whereas in CB1$^{-/-}$ mice feed efficiency was not significantly altered by high-fat diet (Figure 1). One of the factors that could contribute to this difference is adiponectin, the adipocyte-derived hormone that promotes fatty acid β-oxidation.[52] Plasma adiponectin levels declined significantly in wild-type mice with diet-induced obesity, whereas the same high-fat diet failed to affect plasma adiponectin levels in CB1$^{-/-}$ mice,[44] suggesting that energy expenditure was not reduced in the latter group.

Fatty acid metabolism in hypothalamic neurons acts as a sensor of nutrient availability,[53] and its pharmacological modulation influences appetitive behavior.[54] Interestingly, activation of CB1 was found to increase hypothalamic SREBP1c and FAS mRNA levels, and this effect was abrogated

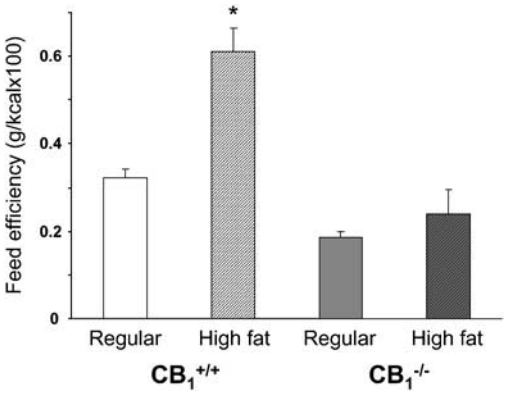

Figure 1 Increased feed efficiency induced by a high-fat diet in wild-type, but not in CB1 knockout mice. Male and female CB1$^{-/-}$ mice and their wild-type littermates (CB1$^{+/+}$) were fed regular mouse chow or a high-fat diet from age 4 to 14 weeks, as described.[44] Feed efficiency was calculated as the grams of body weight gained divided by the kcal consumed during this period, times 100. *Indicates significant difference ($P<0.005$) from the corresponding group on normal diet. $N=6$–10 per group.

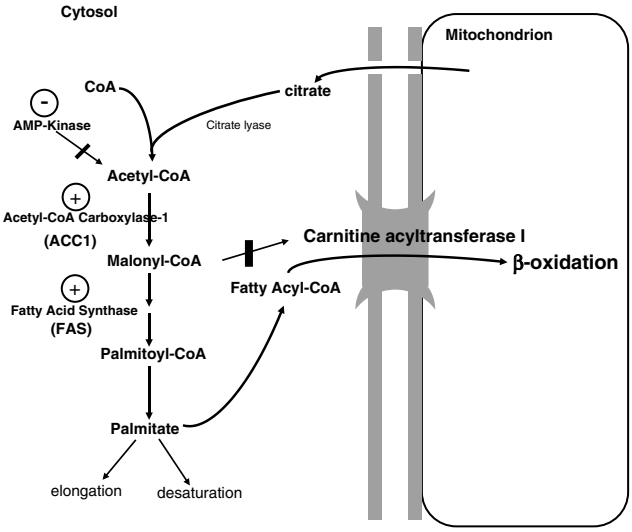

Figure 2 Schematic representation of cannabinoid modulation of fatty acid synthesis and β-oxidation. In the liver, cannabinoids inhibit AMPK,[57] which results in reduced phosphorylation, thus activation of ACC1. Cannabinoids also increase the expression of ACC1 and FAS.[44] All of these effects are likely involved in the observed increase in fatty acid synthesis.[44] Fatty acid β-oxidation is likely reduced because of an increase in malonyl-CoA, although this has not yet been directly tested.

in animals pretreated with 3 μg/g rimonabant, although rimonabant alone did not reduce SREBP1c and FAS mRNA levels.[44] Fasting followed by refeeding with a high-carbohydrate diet is a potent inducer of SREBP1c and FAS gene expression.[55] In mice subjected to a cycle of fasting/refeeding, the elevated basal levels of SREBP1c and FAS mRNA were significantly reduced by treatment with 3μg/g rimonabant at the beginning of the refeeding period, which also reduced food intake. Although fatty acid synthesis was not measured directly in the hypothalamus, these findings suggest that the increase in food intake following fasting may involve a CB1-mediated modulation of the fatty acid synthetic pathway.

Recent work has highlighted the key role of the AMP-activated protein kinase (AMPK) as a sensor of cellular energy status. AMP-activated protein kinase is activated by an increase in AMP:ATP ratio triggered by a decline in cellular ATP, and results in increased food intake.[56] AMP-activated protein kinase activation also inhibits lipogenesis and increases fatty acid oxidation, by phosphorylating and thus inhibiting the activity of ACC1, the rate-limiting enzyme in fatty acid synthesis. The resulting decrease in malonyl CoA represents a metabolic switch that results in reduced fatty acid synthesis and increased β-oxidation. Interestingly, a recent study provided evidence for the regulation of AMPK by cannabinoids, which were found to stimulate AMPK activity in the hypothalamus and myocardium, and to inhibit it in the liver and adipose tissue, via activation of CB1.[57] These effects are compatible with the proposed CB1-mediated increase in hepatic lipogenesis and the increase in food intake via activation of hypothalamic CB1. It would be interesting to see whether a similar mechanism is present in extrahypothalamic brain regions implicated in the orexigenic effects of cannabinoids. The reason why activation of CB1 results in inhibition of AMPK activity in some tissues but activation in others,[57] also remains to be determined. Figure 2 is a schematic representation of the proposed targets of cannabinoids in the fatty acid synthesis/β-oxidation pathways in the liver.[44,57]

The studies described in the second section indicate that endocannabinoids acting at CB1 receptors in adipose tissue and liver regulate peripheral energy metabolism. The findings that the AMPK/ACC1/FAS pathway, which controls fatty acid synthesis and β-oxidation, is targeted by cannabinoids in both the liver and the hypothalamus suggest that a common molecular pathway is involved in the central regulation of appetite and the control of peripheral energy metabolism by the endocannabinoid system.

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