Endocannabinoids and Liver Disease.

III. Endocannabinoid involvement in obesity and hepatic steatosis

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Running title: endocannabinoids in obesity and steatosis

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Kunos G, Osei-Hyiaman D. Endocannabinoids are endogenous lipid mediators that interact with the same receptors as plant-derived cannabinoids to produce similar biological effects. The well known appetitive effect of smoking marijuana has prompted inquiries into the possible role of endocannabinoids in the control of food intake and body weight. This brief review surveys recent evidence that endocannabinoids and their receptors are involved at multiple levels in the control of energy homeostasis. Endocannabinoids are orexigenic mediators and are part of the leptin-regulated central neural circuitry that controls energy intake. In addition, they act at multiple peripheral sites, including adipose tissue, liver and skeletal muscle to promote lipogenesis and limit fat elimination. Their complex actions could be viewed as anabolic, increasing energy intake and storage and decreasing energy expenditure, as components of an evolutionarily conserved system that has insured survival under conditions of starvation. In the era of plentiful food and limited physical activity, pharmacological inhibition of endocannabinoid activity offers benefits in the treatment of obesity and its hormonal/metabolic consequences.

Key words: Cannabinoid receptors; metabolic syndrome; appetite; lipogenesis; cardiovascular risk factors;
THE USE OF PLANT-DERIVED SUBSTANCES for medicinal purposes has an age-old history. Beyond their practical importance in the treatment of disease or certain symptoms, these substances have also opened unique windows, looking through which has helped us understand how the body works. As it happened earlier in the case of morphine and opiate receptors, the discovery of specific receptors for cannabis in mammalian cells led to the discovery of endogenous cannabinoids that interact with the same receptors and elicit effects similar to those caused by smoking marijuana. Endocannabinoids identified to date are arachidonic acid metabolites, the most widely studied ones being arachidonoyl ethanolamide or anandamide, and 2-arachidonoylglyceride or 2-AG. These lipid-like substances are enzymatically generated in the plasma membrane from membrane phospholipid precursors and are thought to act as autocrine/paracrine mediators with no hormone-like, distant actions. Once released, they are rapidly metabolized, anandamide being degraded primarily by fatty acid amidohydrolase, whereas 2-AG being metabolized by monoglyceride lipase. The present review will focus on the role of endocannabinoids in the control of energy homeostasis, with its therapeutic implications. References to many of the earlier key findings can be found in recent reviews on this subject (8, 21).

**Endocannabinoids regulate appetite**

Smoking marijuana is known to increase appetite or cause the ‘munchies’, which suggested that blocking the receptors involved in this effect, later determined to be the CB1 cannabinoid receptor subtype, may be useful in the treatment of obesity by reducing food intake. This idea was first put in practice by scientists at the Sanofi company who developed SR141716, subsequently named rimonabant, the first in a growing line of
potent and selective CB₁ receptor antagonists. Early studies with rimonabant have confirmed its ability to reduce both food intake and body weight in various rodent models, which was the prelude for clinical trials to test its effectiveness in the treatment of obesity (21). Although rimonabant (as most other CB₁ antagonists) has inverse agonist activity, which could account for its appetite reducing effect, experiments using mice deficient in CB₁ receptors confirmed that reduction in food intake by rimonabant treatment is due to antagonism of the orexigenic action of endocannabinoids, which are part of the leptin-regulated hypothalamic neural circuitry involved in regulating appetite (6). Recent findings that neutral CB₁ antagonists are also effective as weight reducing agents (4, 23) further supports the notion that CB₁ blockade is the underlying mechanism. Neutral antagonists block receptor binding and activation by an agonist, but do not affect the intrinsic activity of the receptor, therefore do not have an effect on their own. In contrast, inverse agonists reduce the intrinsic activity of the receptor even in the absence of agonist, resulting in an effect opposite to what is produced by agonists.

The hypothalamic appetitive neural circuitry is thought to control the consummatory aspects of food intake, whereas cannabinoids increase appetite for palatable food, suggesting an involvement in the rewarding qualities of food which is controlled by the limbic system. This paradox has been resolved by the recent finding that one of the sites of action of endocannabinoids is lateral hypothalamic neurons that express the orexigenic melanin concentrating hormone and project to the ventral tegmental area, the site of origin of the mesolimbic dopaminergic reward pathway (12). Additional sites in the brainstem and limbic forebrain may also be involved in mediating the orexigenic effect of endocannabinoids (21).
Endocannabinoids and peripheral fat metabolism

One of the first studies that documented the anorectic effect of rimonabant had already indicated that its effect on energy homeostasis is much more complex than can be explained by reduced food intake. Colombo and coworkers reported in 1998 that in rats chronically treated with rimonabant, there was rapid tachyphylaxis to the reduction in food intake, whereas the associated weight loss was maintained throughout the treatment period. This finding, later confirmed by others, was the first clear indication that endocannabinoids must have additional, most likely peripheral, targets in the control of energy metabolism (reviewed in ref. 21). This conclusion was supported by subsequent findings that CB1 receptor knockout mice are resistant to diet-induced obesity despite the fact that their total caloric intake is similar to that of wild-type mice that do become obese on the same diet (21). Furthermore, CB1 knockout mice have a lean phenotype, and the weight difference between knockout and wild-type mice could be eliminated by pair-feeding only in young, but not in adult animals. This indicated that reduced food intake may play a role early on, but in the adult stage other factors, such as increased energy expenditure must contribute to the lean phenotype in the knockouts (5). Nevertheless, an effect on food intake may still contribute to the weight reduction, as suggested by findings that tachyphylaxis to the appetite reducing effect of chronic CB1 antagonist treatment develops more slowly in genetically obese than in lean rats (25), and tachyphylaxis did not develop at all when regular chow was replaced by a ‘palatable’ sucrose diet (9).
Endocannabinoid effects in adipose tissue

Adipose tissue represents one of the peripheral metabolic targets of endocannabinoids. CB₁ receptors have been detected in adipocytes (2, 5), and their stimulation was reported to decrease (18) and their blockade to increase adiponectin gene and protein expression (2). Adiponectin being the primary hormonal stimulus for fatty acid β-oxidation, this may be one of the mechanisms by which CB₁ receptor blockade increases energy expenditure, an effect recently documented by calorimetry in humans treated with a CB₁ antagonist (1). In contrast, increased activity of the endocannabinoid/CB₁ receptor system in adipose tissue may contribute to the development of obesity both in genetically obese Zucker rats (2, 7), which express increased levels of CB₁ receptors in their adipocytes (2), and in obese individuals, in whom the levels of 2-AG are increased in visceral but not subcutaneous fat tissue (3, 18). Although plasma levels of endocannabinoids are too low to produce hormone-like activation of CB₁ receptors, the increased plasma levels of 2-AG detected in individuals with abdominal obesity (3) may reflect spillover from elevated tissue levels and thus could also reflect increased endocannabinoid activity in this group. As to the primary stimulus that leads to such activation, recent evidence suggests the role of proinflammatory changes involving macrophage infiltration and TNF-α production. Visceral adipose tissue from obese versus lean individuals was found to have elevated levels of TNF-α, reduced levels of fatty acid amidohydrolase, the enzyme responsible for the degradation of endogenous anandamide, as well as reduced levels of CB₁ receptors and tissue adiponectin (14). Although tissue levels of anandamide were not quantified in
In this study, macrophages are known to be a rich source of anandamide, the synthesis of which is strongly increased by inflammatory stimuli, such as bacterial endotoxin (16).

**The hepatic endocannabinoid system**

The liver is another potential target of endocannabinoids, given the fact that it plays a major role in *de novo* lipogenesis, and high fat diets paradoxically induce an increase in *de novo* hepatic lipogenesis (15, 24). Although the liver used to be thought of being devoid of cannabinoid receptors, recent studies indicate otherwise. CB₁ receptor mRNA and protein have been detected in mouse, rat and human liver tissue, and both anandamide and 2-AG are present in the liver at levels comparable to those in brain (21). Interestingly, in various animal models of obesity, an upregulation of CB₁ receptors was detected not only in liver (2, 20) but also in adipocytes (2) and skeletal muscle (22), and the hepatic levels of anandamide, but not 2-AG, are also markedly increased in high fat diet-induced obesity (20). This and the reduced expression of the lipogenic transcription factor sterol response element-binding protein 1c (SREBP1c) in the liver of CB₁ receptor knockout mice suggested the involvement of hepatic endocannabinoids in diet-induced obesity. Indeed, treatment of normal mice with a CB₁ agonist increased the expression of SREBP1c and its target enzymes acetyl CoA carboxylase-1 and fatty acid synthase (FAS), as well as *de novo* lipogenesis in the liver, and the increase in hepatic lipogenesis induced by a high fat diet could be attenuated by treatment with a CB₁ antagonist (20).

**Endocannabinoids and hepatic steatosis**
Obesity in humans as well as in experimental animals is often associated with hepatic steatosis or fatty liver, which results from the accumulation of ectopic fat in hepatocytes. Mice deficient in CB1 receptors are resistant not only to high fat diet-induced obesity, but also to the associated steatosis (20), and the steatosis of genetically obese Zucker can be dramatically reduced by chronic CB1 blockade (7). Interestingly, in a recent epidemiological study of subjects with hepatitis C viral infection, daily marijuana use was found to be a predisposing factor for hepatic steatosis (10). Together, these observations indicate a role for the hepatic endocannabinoid system in the development of steatosis. Prompted by these findings, clinical trials are about to begin, testing rimonabant for efficacy in the treatment of non-alcoholic steatohepatitis.

In Western societies, chronic alcohol use and alcoholism is another major source of fatty liver, which can progress to steatohepatitis, cirrhosis and liver cancer. Similar to high fat diet-induced steatosis, alcohol-induced fatty liver is also associated with increased lipogenesis and decreased fat elimination from the liver. These similarities and the ability of chronic alcohol intake to upregulate endocannabinoid levels, at least in the brain, led us to test their involvement in alcohol-induced fatty liver. We have recently reported that the hepatic steatosis induced by chronic ethanol feeding of C57BL6 mice can be attenuated by concurrent treatment with a CB1 antagonist, and that mice with global or hepatocyte-specific deletion of CB1 receptors are resistant to ethanol-induced fatty liver (11). In normal, but not in CB1-deficient animals, ethanol feeding induced the expression of the lipogenic genes SREBP1c and FAS, and decreased the expression and activity of carnitine palmitoyl transferase-1 (CPT-1), the rate limiting enzyme in fatty acid β-oxidation. Furthermore, ethanol feeding increased the expression of CB1 receptors
in hepatocytes and caused a selective upregulation of 2-arachidonoylglycerol (2-AG) levels in hepatic stellate cells. This suggested an unexpected paracrine mechanism whereby stellate cell-derived 2-AG would activate CB₁ receptors on adjacent hepatocytes to induce lipogenic gene expression and lipogenesis and decrease fat elimination. Evidence for this was provided by experiments, in which the presence of stellate cells from ethanol-fed, but not from pair-fed, mice in co-cultures with hepatocytes from pair-fed mice resulted in upregulation of CB₁ receptors as well as SREBP1c and FAS expression in the latter. When hepatocytes were obtained from liver-specific CB₁ knockout mice, the induction of lipogenic gene expression by co-cultured, ethanol-primed stellate cells was blunted, supporting the role of CB₁ receptors in the effect (11). These findings add alcoholic fatty liver as a potential therapeutic target of CB₁ antagonists.

Effects of endocannabinoids on hormonal and metabolic changes associated with obesity

Obesity induced in mice by high fat diets is associated with hormonal and plasma lipid changes similar to those that are used to define the metabolic syndrome in humans. Mice with diet-induced obesity have elevated plasma insulin and glucose levels, indicating insulin resistance, they are leptin resistant with hyperleptinemia, have elevated serum triglyceride and LDL cholesterol and reduced HDL cholesterol levels (21). A role of endocannabinoids and CB₁ receptors in these metabolic changes is indicated by their being blunted or absent in CB₁ receptor deficient mice fed a high fat diet or in wild-type mice with DIO following chronic treatment with a CB₁ antagonist (21). Importantly, in several multicenter, phase III clinical trials, chronic treatment of obese and overweight
individuals with rimonabant caused similar beneficial hormonal and metabolic effects, including improved insulin sensitivity, elevated plasma adiponectin and HDL cholesterol and reduced plasma triglyceride and LDL cholesterol levels, which indicates endocannabinoid involvement in humans as well (reviewed in ref. 8).

Skeletal muscle has an important role in the control of insulin sensitivity, and CB₁ receptors are present on myocytes (22). In obese Zucker rats, chronic treatment with rimonabant resulted in a marked increase in insulin-stimulated glucose uptake and phosphorylation in soleus muscle, suggesting that skeletal muscle is another metabolic target of endocannabinoids (17). Interestingly, we recently found that mice with hepatocyte-specific deletion of CB₁ receptors do become obese on a high fat diet, but develop much less steatosis than wild-type controls. Furthermore, they do not develop insulin and leptin resistance, and their plasma lipid profile remains normal (unpublished observations). These findings strongly suggest a role for hepatic CB₁ receptors not only in diet-induced steatosis, but also in the associated hormonal and metabolic changes.

Endocannabinoids and cannabinoid receptors are also present in the endocrine pancreas, with conflicting reports on insulin-producing β-cells uniquely expressing CB₁ (19) or CB₂ receptors (13), or whether activation of CB₁ receptors stimulates (18) or inhibits (19) glucose-induced insulin release. In any case, the observed suppression of plasma insulin levels by rimonabant treatment of pre-diabetic obese individuals (8) or animals likely reflects improved insulin sensitivity rather than a direct action on insulin secretion. Whether CB₂ receptors may be involved in the in vivo regulation of insulin secretion, or other components of the obesity/metabolic syndrome, remains to be tested.
Future directions

An important problem that has emerged during clinical trials with rimonabant is its centrally mediated side effects, including anxiety and depression. Although the incidence of such side effects was relatively modest compared to placebo, they may have contributed to the nearly 50% attrition of enrolled subjects during the course of these studies, which will need to be further explored. Because these side effects are most likely due to antagonism of CB₁ receptors in the CNS, it will be difficult to separate them from the therapeutically desirable consequences of CB₁ blockade. However, this may not be impossible in view of the findings in hepatocyte-specific CB₁ knockout mice, which provide strong evidence for the role of hepatic, i.e. peripheral, CB₁ receptors in steatosis. As a corollary, peripherally restricted CB₁ antagonists may be effective in reversing steatosis with fewer or no centrally mediated side effects. Alternatively, using a neutral, as opposed to an inverse, antagonist may also offer some advantage, as such antagonists have been reported effective in reducing weight in obese rodents, but had fewer centrally mediated side effects (4, 23).

Another therapeutic approach may envision combination treatments including CB₁ antagonists, where complementary and/or synergistic effects may allow reducing the dose of each drug, resulting in fewer side effects. For example, the insulin sensitizing action of CB₁ antagonists may warrant their testing in diabetes as a combination with insulin or thiazolidinediones, where their weight reducing effect would counteract the weight increasing effect of the latter two. Clinical trials with rimonabant in subjects with obesity/metabolic syndrome also documented its ability to cause a substantial increase in HDL cholesterol (8). This could warrant its testing in combination with statins, whose
effect is mainly on reducing LDL cholesterol, in the treatment of dyslipidemias. We can look forward to many of these ideas being tested in the near future.
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