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As the focus of this issue of the CMReJournal, the endocannabinoid system (ECS) has provided unique pharmacological tools that, in combination with moderate dietary restriction, can ameliorate several cardiometabolic risk factors simultaneously in obese and overweight individuals, and do this partly independently of the associated weight loss. These targets include visceral (intra-abdominal) obesity, high plasma triglyceride and low HDL cholesterol levels and several measures of insulin resistance, as well as glycated hemoglobin in type 2 diabetic patients [1-3]. The twelve short articles in this special issue summarize the contents of the corresponding lectures given during the 6th International Chair on Cardiometabolic Risk (ICCR) bi-annual meeting held on June 20-22, 2008, under the theme “The endocannabinoid system (ECS): the evidence relating to its sage targeting in the treatment of cardiometabolic risk”. At that time, the future of CB1 receptor antagonists/inverse agonists as unique therapeutics against obesity and related metabolic disorders still seemed very promising, despite existing information about their psychiatric side-effects. At this meeting, renowned experts in the fields of the ECS and cardiometabolic risk research summarized current knowledge of the physiological role of the ECS in the brain and peripheral tissues, and of its malfunctioning in obesity and the accompanying hormonal and metabolic abnormalities. The aim of the meeting was to further our understanding of the mechanisms underlying the efficacy and adverse event profiles of several CB1 receptor antagonists/inverse agonists that were under clinical development at that time, and to outline some principles for optimizing their risk/benefit ratio.

The short articles are organized as mini-reviews, each with key points and/or suggestions for future studies, and reflect the various subjects discussed at the meeting, i.e., 1- the role of the ECS and cannabinoid CB1 receptors in the central nervous system (CNS) and its disorders, including stress and maladaptation to new environmental conditions (articles by B. Lutz and by C. Hillard), and in the control of food intake (T. Kirkham); 2- the involvement of the ECS in peripheral functions not necessarily related to the regulation of energy balance, such as the immune response and bone formation (A. Zimmer), and gastrointestinal function (K. Sharkey); 3- the crucial role of endocannabi-
noids and CB1 receptors in the control of adipocyte function (U. Pagotto), which can be explored also by examining the effects of CB1 antagonists in a dog model of the “metabolic syndrome” (R. Bergman); and 4- the role of CB1 and/or CB2 receptors in liver diseases (S. Lotersztajn) and cardiovascular dysfunctions (P. Pacher), including atherosclerosis (F. Mach). Finally, G. Le Fur, who at the time of the conference was CEO of Sanofi-Aventis, describes the history of the development of the first CB1 receptor antagonist/inverse agonist, rimonabant, as an anti-obesity drug, whereas ICCR Chairman J.-P. Després and ICCR International Academic Board member L. Van Gaal, summarize, based on the available data from clinical trials with rimonabant, who should be the ideal patient for this drug [see also ref. 3]

On October 23, the European Medicines Agency (EMEA) announced the suspension of the marketing authorization of rimonabant (Acomplia) in the European Union, based on the conclusion of the EMEA’s Committee for Medicinal Products for Human Use (CHMP) that the benefits of this first-in-its-class compound, indicated as an adjunct to diet and exercise for the treatment of obesity in patients with body mass index (BMI)>30 kg/m² and in patients with BMI>27 kg/m² and dyslipidemia or type 2 diabetes, “no longer outweigh its risks”. A few weeks earlier, Merck had announced that they were also discontinuing the clinical development of their CB1 receptor antagonist/inverse agonist, taranabant, based on safety issues. On November 5, Sanofi-Aventis, the developer of rimonabant, announced the interruption of the clinical trial program with rimonabant, apparently based on the decision of several national health authorities to withdraw patients from the ongoing clinical trials with this compound. On the same day, Pfizer also announced a similar decision regarding yet another compound with a similar mechanism of action, otenabant (CP-945,598), stating that “although Pfizer believes that the CP-945,598 compound has the potential to be a safe and effective treatment for weight management…. the Company has decided to discontinue the development program based on changing regulatory perspectives on the risk/benefit profile of the CB1 class and likely new regulatory requirements for approval.” This snowballing of events [4] will likely compromise the future prospect of using CB1 receptor antagonists/inverse agonists not only for treating obesity (the indication they were all originally designed for), but also for the treatment of other cardiometabolic risk factors that, as discussed in this issue, these compounds may target directly and independently of their effects on body weight [3].

Indeed, since the publication of the first phase III studies with rimonabant, additional evidence has emerged pointing to altered CB1 receptor signalling as a key mechanism that contributes to the development of visceral obesity and the associated adipose tissue lipotoxicity, insulin resistance, ectopic fat accumulation (including liver fat) and atherogenic inflammation [5-7]. In obese rodents and humans, the amount of lipogenic endocannabinoids was found to be elevated in visceral adipose depots and reduced in subcutaneous adipose tissue [1, 8, 9], thus possibly contributing to the selective deposition of visceral fat at the expense of subcutaneous fat, which acts as a protective metabolic buffer to cope with excess dietary energy [6]. Activation of CB1 receptors in mature adipocytes decreases adiponectin expression [8], thus potentially contributing to the typical hypoadiponectinemic state of visceral obesity, whereas hepatocyte CB1 receptors are sufficient per se to cause fatty liver, dyslipidemias, as well as insulin and leptin resistance in mice subjected to a high fat diet [10]. High plasma levels of the endocannabinoid 2-arachidonoylglycerol (2-AG) in obese patients are strongly
associated with high visceral obesity independently from BMI, and also with high triglycerides, low HDL cholesterol and insulin resistance [11, 12]. A comprehensive lifestyle modification program leading to substantial waist circumference and visceral fat reduction was recently shown to be accompanied by a strong reduction in 2-AG levels [13]. The causative role of the elevated endocannabinoid tone in visceral adipose tissue in the development of abdominal obesity and, hence, type 2 diabetes and atherosclerosis, is suggested by the finding in animal models that CB1 receptor antagonists/ inverse agonists significantly ameliorate the altered metabolic and lipoprotein profiles, and the hepatic, pancreatic and renal damage that accompany obesity [14-16]. Conversely, overactivation of CB1 receptors, induced indirectly by inhibiting the degradation of endocannabinoids, was found to cause hypertriglyceridermia in lean mice [17]. Importantly, blockade of CB1 receptors reduces visceral fat more than total fat or hepatic fat in rats fed a candy diet [18], and inhibits the formation of atherosclerotic plaques in a mouse model of atherosclerosis, in a way independent from its effects on food intake and total cholesterol [19]. These findings in animals were paralleled by data obtained in the ADAGIO-Lipids and STRADIVARIUS clinical trials, which indicated that although rimonabant increases the incidence of psychiatric side-effects, including depression, these are usually mild to moderate in severity, and suggested that patients with high-risk abdominal obesity and no history of depression may represent the ideal therapeutic target for CB1 antagonists in terms of an optimal benefit to risk ratio [20, 21]. It may be pointed out that obesity as well as body weight reduction per se have been linked to an increased incidence of depression [22, 23], and therefore the recent statement of EMEA’s CHMP recommending the suspension of the marketing of Acomplia may have overemphasized the risk and, consequently, minimized the benefit of CB1 receptor blockade achieved through the use of rimonabant and other CB1 receptor antagonists with a similar pharmacological profile.

We believe that the twelve articles of this special issue, apart from providing a snapshot of the general role of the ECS in various disorders, including the metabolic syndrome, might also help suggest future strategies for the optimal use of the next generation of these compounds, including non brain-penetrant CB1 antagonists [2], for the safe and efficacious pharmacological treatment of residual cardiometabolic risk.

References


The increase in the prevalence of obesity is well recognized and has been seen not only in North America but in Europe and Asia as well. Associated with increased adiposity is increased risk for a variety of diseases, including diabetes and associated comorbidities, cardiovascular disease, and cancer. Thus, it is clear that if the rate of increase of adiposity could be reduced, there could be important consequences regarding public health.

The pathogenesis of the so-called “metabolic syndrome” or the closely associated cardiometabolic risk remains less than clear. It is well documented that visceral (intra-abdominal) adiposity in particular is associated with increased risk as well as with insulin resistance [1]. Thus, the relationship between visceral adiposity, insulin resistance, and risk for diseases remains to be explored. Much of the data relating visceral fat to increased risk has been epidemiological, from cross-sectional studies [2, 3]. In our laboratory we have taken an experimental approach, examining the pathogenesis of visceral fat and insulin resistance as well as associated risk factors in the canine model [4]. The latter model allows us to make longitudinal measurements of pathophysiological events and to intervene with appropriate drugs. One such agent is rimonabant, which is an antagonist of the cannabinoid system and limits binding to CB1 receptors.

Similar to human subjects, dogs exhibit a wide range of adiposity in visceral and subcutaneous depots. After fat feeding, we observe a modest weight gain associated with doubling of fat in visceral and subcutaneous depots [5]. The patterns of fat deposition are different between visceral and subcutaneous depots. In the visceral depot we see evidence of progenitor cells that can develop into large adipocytes, and the visceral fat cells are particularly sensitive to lipolysis stimulated by adrenergic agonists. The overall pattern of insulin resistance in this model is related to increases in expression of enzymes that favour lipolysis and enzymes that favour gluconeogenesis. This data supports a
model in which excess lipolysis from the visceral fat depot raises free fatty acids (FFA) in the portal circulation, leading to hepatic and peripheral insulin resistance.

The sympathetic nervous system (SNS) also plays a special role: we have found evidence for cyclic stimulation of lipolysis that can be blocked by adrenergic antagonists [6]. These results implicate the SNS in stimulating lipolysis from the visceral fat depot, causing increased portal FFA.

Studies in which we have extirpated all or part of the visceral fat depot in rodents and in the dog model have shown remarkable enhancement of insulin sensitivity. The extirpation studies also support a very important role of visceral fat in pathogenesis of the metabolic syndrome.

But, if FFA are implicated in causing insulin resistance, why have many laboratories failed to show an increase in levels of FFA under fasting conditions? In our laboratory we took samples over a 24-hour window, before and after feeding of fat. We discovered that there is a powerful increase in FFA levels in the middle of the night (3 a.m. to 6 a.m.) due to increased visceral lipolysis during that period [5]. We hypothesize that it is the outpouring of FFA from the visceral depot in the middle of the night that is responsible for the insulin resistance in the metabolic syndrome (Figure).

We have examined the efficacy of the CB1 inhibitor rimonabant on the development of the metabolic syndrome in the dog model. Feeding of fat to dogs causes continual weight gain; rimonabant stops the gain in weight even in the face of a palatable high fat diet. Rimonabant reverses many of the deleterious effects of fat feeding, including fat deposition, stemming the increase in body weight and increasing energy expenditure. Rimonabant virtually reverses fat deposition in liver, even with increased food fat content. Finally, rimonabant increases energy expenditure, explaining its effects on body weight distribution.

Figure: Night-time pathogenesis of the metabolic syndrome.
References


Introduction

All the constituents of the endocannabinoid system (ECS) are widely expressed throughout the adult nervous system, but they are also highly present during gestation in the developing central nervous system. A particularly interesting observation is that the cannabinoid receptor type 1 (CB1 receptor) is expressed in two very different neuronal subpopulations: in inhibitory GABAergic neurons and in excitatory glutamatergic neurons. This implies that CB1 receptors have divergent roles in physiological processes throughout the entire organism. In addition, pathological dysregulations of the ECS may occur in either or both of these neuronal populations. Consequently, it is very critical to determine dysregulations at the exact neuronal level in order to be able to draw valid conclusions about the pathological processes. Such conditions include pathological states such as obesity, epilepsy, depression and anxiety disorders.

State of the Art

It has been known for several years that CB1 receptors are expressed in numerous brain regions but also in different neuronal subpopulations, including GABAergic and glutamatergic neurons [1]. Recently, it has been recognized that CB1 receptors are also present in serotonergic, noradrenergic and cholinergic neurons [2-4], but even dopaminergic neurons may contain CB1 receptors [4]. At the synaptic level, endocannabinoids are involved in retrograde signalling processes, where endocannabinoids are released from the postsynapse and, after crossing the synaptic cleft, they bind to presynaptic CB1 receptors, leading to the suppression of neurotransmitter release. As CB1 receptors are present on the synaptic terminals of different neurotransmitters, the physiological and pathophysiological consequences of retrograde endocannabinoid-mediated suppression of neurotransmission may be rather divergent, depending on the exact neurotransmitter that is modulated.

Key Points

- The ECS is activated on demand and thereby executes distinct functions in different behaviours.
- Dysregulations of the ECS may occur differentially, in a neuronal subtype specific manner. Thus, to draw valid conclusions, it is crucial to make an exact determination of the dysregulated neuronal subpopulation. The ECS can be over or underexpressed.

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This particular feature of the ECS is illustrated in the endocannabinoid-mediated control of seizure threshold. In a rat model of febrile seizures, CB1 receptors are up-regulated specifically in hippocampal GABAergic interneurons upon prolonged hyperthermia in early postnatal stages, leading to a life-long decrease in GABA transmission. This dysregulation lowers the seizure threshold, making these animals prone to epilepsy [5]. A follow-up study showed that pharmacological blockade of CB1 receptors during the time of hyperthermia inhibited the emergence of febrile seizures in adulthood [6]. On the other hand, it was shown that lack of CB1 receptors specifically in cortical glutamatergic neurons also leads to an increased susceptibility to seizure-inducing agents, such as kainic acid, while the loss on GABAergic neurons had no effect on seizure susceptibility [7]. Postmortem analysis of human tissues from subjects with temporal epilepsy showed a specific decrease in hippocampal CB1 receptors in glutamatergic neurons, while no change in hippocampal GABAergic interneurons was observed [8]. Thus, these are intriguing results, indicating that depending on the site of dysregulation of the ECS, either blocking or enhancing endocannabinoid signalling may be of therapeutic benefit [9].

A dichotomy of endocannabinoid function is emerging for other processes. 1-During neural development, CB1 receptors are present on cortical glutamatergic neurons. Here, the functions include processes such as proliferation of neural progenitor cells, neuronal migration and axon fasciculation [10]. In GABAergic interneurons, CB1 receptors do not serve an apparent function in proliferation and migration, but rather in the growth cone and during synaptogenesis [11]. 2-In endocannabinoid-controlled fear extinction, CB1 receptors on cortical glutamatergic neurons play a crucial role [12]. However, data on CB1 receptor function on GABAergic interneurons is still pending for further evaluation, but preliminary results suggest that CB1 receptor function on this neuronal population might have the opposite effect. Further studies in other behavioural paradigms, such as anxiety and stress coping, will help validate whether CB1 receptor function is dichotomized in GABAergic and glutamatergic neurons.
But it will certainly be a challenging task to evaluate the specific function of CB1 receptors in a particular neuronal subpopulation within a distinct brain region. An illustration of this is endocannabinoid-controlled fear extinction, where the complete loss and the glutamatergic-specific loss of CB1 receptor leads to impaired fear extinction. However, considering the complex expression of CB1 receptors in neuronal networks involved in fear extinction (Figure), sophisticated genetic experiments will be required to pinpoint in detail CB1 receptor function to a particular behaviour.

Priorities for Future Studies

In any investigation addressing possible dysregulations of the ECS in the central nervous system, it will be very important to detail the sites of dysregulation. It will be rather straightforward to localize CB1 receptor expression and quantify over or underexpression in a particular neuronal subpopulation, but it will be very difficult to draw strong conclusions on dysregulated endocannabinoid levels, as endocannabinoids diffuse within 50-80 micrometers and influence a broad area where CB1 receptors are present. Further investigations on dysregulated ECS may focus on anxiety, stress coping, obesity and depression-like behaviours in animal models.

Due to the fact that CB1 receptors are present at a myriad of different nerve terminals in the brain, the question arises as to how this system is able to regulate specific behaviours, as it apparently does. One hypothesis is that the ECS is active only in neuronal networks that fire and are activated. Thus, this neuromodulatory system is active on demand at particular circuits and thereby executes specific functions. It will be important to decipher such endocannabinoid-regulated neural circuits.

References


PERIPHERAL FUNCTIONS OF THE ENDOCANNABINOID SYSTEM: PATHOLOGY AND PHYSIOLOGY

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Introduction

Although the endocannabinoid system (ECS) is best known for its important neuromodulatory role, it has become apparent in recent years that the physiology of almost every organ is affected by the ECS. This is especially true under pathological conditions, in which an up-regulation of the expression of cannabinoid receptors and increased endocannabinoid production can often be observed. The ECS in peripheral tissues thus seems to constitute an important stress-response system that becomes activated in situations that challenge homeostasis.

State of the Art

Transgenic and knockout mouse models, in combination with high affinity small molecules, are very powerful tools in the analysis of protein functions. We have generated mutant mouse strains deficient in CB1 and CB2 receptors [1, 2], as well as CB1/CB2 double knockout mice [3, 4]. The lack of each of these receptors has severe deleterious consequences on animal physiology. While most of the initial studies on CB1 receptor knockout animals have focused on central nervous system phenotypes, it has recently been demonstrated that CB1 signalling affects numerous peripheral organs and tissues including bone, liver, skin, the immune system, endothelial cells, adipose tissue, embryo development and implantation, etc. [4-8]. Likewise, CB2 receptor signalling modulates immune cell functions and thus affects many organs and different pathologies, such as atherosclerosis, liver fibrosis, osteoporosis, and neuropathic pain [9, 10].

Neuropathic pain conditions resulting from nerve injury are difficult to treat even with potent analgesic compounds, but recent pharmacological studies indicate that CB2 selective agonists show analgesic efficacy in neuropathic pain conditions. Studies in CB2 knockout mice exposed to sciatic
nerve injury have demonstrated that CB2 receptors contribute to the local containment of neuropathic pain by modulating glial activation in response to nerve injury [11]. In the absence of CB2 signalling, neuropathic pain spreads beyond the site of nerve injury. The underlying molecular mechanisms were identified by expression profiling studies that showed an enhanced interferon-γ (IFN-γ) response in the absence of CB2 signalling [12]. IFN-γ is produced by neurons and astrocytes in the spinal cord segment ipsilateral to the nerve injury in wild-type animals and also in the contralateral region in CB2 deficient mice, thus matching the pattern of nociceptive hypersensitivity in these animals. The most direct support for a functional involvement of IFN-γ as a mediator of CB2 signalling was obtained with a double knockout mouse strain deficient in CB2 receptors and IFN-γ, which no longer showed the contralateral hyperalgesia observed in CB2 knockouts.

The effect of CB2 signalling in neuropathic pain exemplifies the immunomodulatory role of this system under pathological conditions. However, there is still relatively little information about the significance of CB2-mediated effects in normal physiology. In fact, the only obvious defects in CB2 deficient animals, in the absence of pathogenic challenges, have been observed in the skeletal system [13]. During their first 2-3 months of life, CB2-deficient mice accrue a normal peak trabecular bone mass but subsequently show markedly enhanced age-related bone loss. At one year of age, their trabecular bone volume density is approximately 50% lower than that of wild type control animals. Young mice and other vertebrates alike undergo a rapid skeletal growth phase accompanied by accrual of peak bone mass, which remains constant in adult animals until the beginning of age-related bone loss [9]. These changes are referred to as bone remodelling and involve a continuous process of resorption by osteoclasts and formation of new mineralized matrix by osteoblasts. Imbalanced bone remodelling leads to bone mass accrual or bone loss. CB2-deficient mice show an increase in bone resorption and formation, with a net negative balance [13, 14]. This phenotype is reminiscent of human postmenopausal osteoporosis and suggests that CB2 is associated with maintaining bone remodelling at balance. Importantly, CB2-selective agonists were shown to prevent bone loss after ovariectomy [13]. Thus, CB2 is an attractive new target for the pharmacotherapy of osteoporosis.

To determine if CB2 receptors also contribute to the regulation of bone mass in humans, we therefore studied polymorphisms in the human CNR2 locus, encoding the CB2 receptor, in a case-control sample of osteoporotic patients [15]. The CNR2 locus is located on chromosome 1p36. Although this region and its mouse ortholog on chromosome 4 have been previously linked to bone mineral density and osteoporosis in several independent studies [16], none considered CNR2 as a potential candidate gene. We analyzed 26 SNPs spanning approximately 300 kb around the CNR2 locus and found a significant association with the disease phenotype with several SNPs. The most significant P-values were observed with SNPs located within the CB2 coding region. We therefore sequenced the CB2 coding exon in all 388 patients and controls and identified two missense variants, Gln63Arg and His316Tyr, with the Arg63 variant being more common in the osteoporotic patients than in the healthy controls. Our findings were recently confirmed in an independent study using a large cohort of Japanese women and men [17]. Together, these studies strongly suggest that a common variant of the CB2 receptor contributes to the etiology of osteoporosis in humans.
Priority for Future Studies

One important focus in the analysis of human CB2 signalling will be to investigate potential differences in the biochemical properties of the two CB2 receptor variants. These studies have to consider intracellular signalling cascades, receptor sorting and desensitization, receptor heterodimerization, etc. Many of these studies can probably be done in heterologous expression system or in cells isolated from human probands. However, it may ultimately be necessary to generate mice carrying the human receptor isoforms in order to fully elucidate the functional relevance of the variants in the context of pathology. Considering the widespread effects of the ECS on different pathologies, it will also be important to study the association of human receptor polymorphisms with other relevant diseases, such as liver fibrosis.

References


Introduction

One of the environmental factors that precipitates and exacerbates mental illnesses, including depression, anxiety disorders, and substance abuse, is repeated life stress. For example, homotypic stressors that occur on a daily basis, such as poverty or medical problems, are associated with increased depressive symptoms [1]. Not everyone exposed to stress has pathological consequences. The factors that protect against the “allostatic” load of repeated stress include: an ability to habituate to the stress, maintenance of hedonic tone and reward in the face of stress, and extinguishing of fearful memories [2]. It is clear from preclinical studies that CB1 receptor signalling increases sensitivity to reward [3] and is critical for the extinction of aversive memories [4]. Similarly, data is accumulating to support a role for the endocannabinoid system (ECS) in reducing stress responsivity, including a decrease in endocrine and behavioural responses to the initial presentation of the stress and in the development of habituation.

Key Points

- Stressful life events or situations contribute to many human diseases, including depression, anxiety, and cardiovascular disease.
- Data from animal studies suggests that the endogenous cannabinoid signalling system is a mechanism by which stress is buffered or dampened.
- In mice exposed to an acute stress, loss of endocannabinoid signalling, either through pharmacological blockade or genetic deletion, results in an exaggerated activation of the HPA axis, as well as exaggerated behavioural responses evoked by the stress or threat.
- Repeated exposure to the same stress results in habituation. Pharmacological blockade of endocannabinoid signalling reinstates the behavioural response to stress in habituated mice. In other words, we hypothesize that habituation is accompanied by an activation of endocannabinoid signalling in response to the stress, which serves to reduce the response.
- Repeated exposure to variable stressors and administration of the glucocorticoid corticosterone results in decreased CB1 receptor expression in the hippocampus and an increase in the prefrontal cortex. This data is consistent with the behavioural effects of chronic stress on inducing symptoms of depression.
State of the Art

A simple model that reflects our current understanding about the relationships between stress and the ECS is presented in the Figure.

The interactions between the ECS and stress are bidirectional; stress alters the ECS and the ECS alters stress responses. As a result of this interrelationship, the ECS is in an excellent position to provide feedback to the stress circuit. This speculation has been confirmed by many types of studies.

In acute stress, a primary role of the ECS is to dampen hypothalamic-pituitary-adrenal (HPA) axis activation. Inhibition of the ECS increases and augmentation of the ECS decreases HPA axis activation [5, 6]. Acute stress also produces behavioural effects, including reduced exploration, defensive postures, and anhedonia. These behavioural manifestations of stress are also dampened by the ECS [7-9]. On the other hand, data demonstrating that the ECS plays a role in dampening immobility responses in the forced swim assay (interpreted as a measure of despair in response to a stressful situation) is not consistent. Some investigators find that CB1 activation reduces immobility [10, 11] while others report that CB1 receptor inhibition reduces immobility [12]. These discrepancies likely lie in the lack of reliability of immobility as a measure of stress in rodents. In summary, the available data indicates that the ECS dampens stress responses.

Tissue contents of anandamide (AEA) are reduced in the hippocampus and amygdala and 2-arachidonoylglycerol (2-AG) is reduced in the hypothalamus following acute stress exposure [13]. On the one hand, this data is puzzling because the hormonal and behavioural data described above suggest that ECS signalling becomes activated during stress. However, an alternative explanation is that ECS signalling is high under non-stressed conditions and that the effects of stress are related to the degree to which endocannabinoid presence at the receptor is decreased [6].

Chronic exposure to the same stressor produces habituation, revealed as a reduction in the hormonal and behavioural consequences of stress exposure. Data is accumulating that habituation requires the
ECS. For example, habituation to neuronal activation in the prefrontal cortex is blocked by CB1 receptor antagonist treatment [14]. Furthermore, preliminary data from our laboratory demonstrates that habituation to HPA axis activation by restraint is lost in CB1 receptor null mice. Repeated restraint stress alters tissue contents of the endocannabinoids in a region- and ligand-dependent manner [14, 15]. Interestingly, the changes are consistent with the hypothesis that repeated exposure to the same stressor increases activation of the ECS, due to enhanced ligand release, which down-regulates stress responses.

Chronic exposure of rodents to variable stressors at random times (CUS) results in behavioural changes that mimic those seen in human depression. CUS, which results in a chronic elevation of serum corticosterone, has significant effects on the ECS. CB1 receptor density is decreased in the hippocampus [16] and increased in the prefrontal cortex [17] in rats exposed to CUS. We have also identified similar changes in mice at the mRNA level. The effect of CUS on hippocampal CB1 receptor density is mimicked by chronic treatment with corticosterone [18]. CUS also causes perseverative behaviour in the Morris water maze test, which is reduced when a CB1 agonist is present. This data suggests that down-regulation of CB1 signalling, perhaps in the hippocampus, contributes to some of the behavioural manifestations of chronic stress. Repeated restraint stress under conditions in which there was no habituation results in decreased sucrose consumption in mice. This effect is reversed by CB1 receptor activation [9], providing another example of a role for dysregulation of the ECS in the consequences of stress.

Priority for Future Studies

Our understanding of the interactions between stress and the ECS are at the beginning of their maturity. Better tools are needed to apply to this issue. For example, selective and efficacious pharmacological inhibitors of the synthesis and degradation of the endocannabinoid ligands are a high priority for many in vivo studies. In addition, more selective genetic deletions will be helpful in sorting out the neurocircuitry of the interactions of the ECS with stress. Most importantly, we need to understand whether the role of the ECS as a stress buffer contributes to human disease. For example, does early life stress alter the ECS and does this contribute to an increased propensity for depression and addiction? Can augmentation of the ECS be used as a therapeutic approach for the treatment of stress-related diseases, including depression and anxiety?

References
ENDOCANNABINOIDS AND NON-HOMEOSTATIC CONTROLS OF APPETITE

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Introduction
The predominant models that guide current development of drugs designed to address obesity and its associated diseases concentrate on homeostatic regulation of energy balance and body weight. However, it is arguable that neither body weight nor food intake are regulated variables in the generally accepted physiological sense. The increasing prevalence of obesity—arising from the overconsumption of palatable, energy-dense foods and from sedentary lifestyles—indicates the failure of any efficient mechanisms to curtail energy intake with increasing adiposity and of any effective break on the accumulation of body weight once the much vaunted ‘set points’ for weight or adiposity are attained. Rather, the mechanisms that underlie hunger, and particularly food-craving and hedonic responses to food, are considerably greater influences on the frequency, quantity, and variety of consumption. Consequently, should we wish to develop pharmaceutical interventions to restrict food intake, these positive motivational factors represent crucial targets for investigation and are likely to provide more effective therapies than agents that aim to reinforce putative inhibitory ‘satiety signals’. The endocannabinoids appear to be critical to the normal biopsychological mechanisms that create appetite and stimulate eating, specifically contributing to incentive processes and the hedonic evaluation of food stimuli.

Key Points
- CB1 agonists stimulate eating.
- CB1 antagonists suppress food intake.
- Endocannabinoids mediate specific motivational aspects of appetite.
- Endocannabinoid activity is linked to:
  - Increased salience and incentive value of food and food-related stimuli through activation of mesolimbic dopamine incentive (‘wanting’) circuits.
  - Enhanced palatability/reward of food via modulation of nucleus accumbens shell circuitry and interactions with endogenous opioids.
- CB1 agonists promote hunger, food craving, anticipation of food pleasure, and heightened enjoyment of food.
- CB1 antagonists can reduce food craving and hunger.
- The endocannabinoid system is key to overconsumption and weight gain as a component of the biological systems that have evolved to ensure positive energy balance.
- Pharmacological modulation of brain endocannabinoid activity may permit effective modification of ‘greedy’ behaviours and increased restraint overeating in the obese.
State of the Art

The well-documented appetite-stimulating actions of *Cannabis sativa* result from agonist actions of phytocannabinoids, such as delta-9-tetrahydrocannabinol (THC), at CB1 receptors. That these actions reflect a physiological role of endocannabinoids in appetite control was confirmed by the demonstration in animal models that 1-CB1 blockade suppresses food intake and 2-the endogenous CB1 ligands anandamide, 2-arachidonoylglycerol (2-AG), and noladin ether all promote eating [1-4]. More detailed behavioural analysis indicates that endocannabinoids specifically modulate food wanting and liking. Thus, CB1 agonists and antagonists respectively increase or reduce the amount of effort an animal will expend to obtain food [5-7]. Additionally, CB1 knockout mice exhibit lower levels of responding for sweet food than wild-type mice [8]. THC, 2-AG, or anandamide advance the onset of meals, inducing eating even in fully-satiated animals [9-11]. The orexigenic actions of cannabinoids resemble the changes that occur with food deprivation, and regional brain levels of anandamide and 2-AG increase after fasting [12].

In humans, a principal effect of THC is the amplification of preprandial hunger [13]. Conversely, rimonabant selectively lowers hunger and desire to eat at the start of a meal, while having no effect on post-meal ratings of hunger or fullness. Importantly, with repeated administration, rimonabant reduces the frequency and strength of food cravings [14]. These data are compatible with the known effects of CB1 agonists and antagonists on mesolimbic dopaminergic neurons that subserve incentive motivation (Figure). For example, the accumbens dopamine release that is provoked by presentation of a novel, palatable food is blocked by rimonabant [15]. Overall, the data imply that endocannabinoids may be essential to the orientation to motivationally significant stimuli, the attribution of incentive salience and reward anticipation, and the elicitation of food seeking and eating initiation.

**Figure:** Endocannabinoids modulate activity in mesolimbic dopaminergic (DA) incentive pathways and opioidergic reward circuits, and these actions underlie the orexigenic potency of cannabinoids. Cannabinoid-induced eating is prevented by DA and opioid receptor antagonists as well as by CB1 blockers. Stimulation of CB1 receptors facilitates activity in incentive pathways, promoting orientation to food stimuli and stimulating the motivation to eat. CB1 agonists also act in the accumbens to facilitate opioid mediation of the sensory pleasure of food as it is ingested. Incentive and reward circuits are likely to interact through cannabinoid-mediated mechanisms: cannabinoid activity may thus contribute to the anticipation of orosensory pleasure that is experienced when we are hungry or food is craved.
Endocannabinoids also appear to have a secondary role in mediating the liking of food. Positive hedonic reactions to sweet fluids are respectively enhanced or diminished by CB1 agonists and antagonists [16-18]. Moreover, the nucleus accumbens shell that mediates palatability responses is highly sensitive to the stimulatory actions of endocannabinoids. Anandamide and 2-AG are effective orexigenic in this region, as are agents that increase endocannabinoid levels by blocking their enzymatic breakdown or reuptake [19]. Intra-accumbens administration of anandamide specifically enhances the hedonic impact of sweet taste [20], while accumbens CB1 receptors are down-regulated in rats that overconsume palatable food supplements [21], which is consistent with increased endocannabinoid activity.

Opioid receptor agonists and antagonists respectively increase or reduce food intake by altering the hedonic evaluation of foods [22-26]. There is now convincing evidence for interactions between endocannabinoids and endogenous opioids in relation to feeding. Thus, THC hyperphagia is attenuated by sub-anorectic doses of naloxone [27], and THC stimulates beta-endorphin release in the accumbens [28]. Importantly, the facilitatory effects of both CB1 and opioid receptor agonists on responding for palatable ingesta are reversed by either rimonabant and naloxone [5, 6]. Moreover, low doses of rimonabant and opioid antagonists that are behaviourally inactive when administered singly, combine synergistically to produce a profound anorectic action when co-administered [29, 30]. As with anandamide, administration of morphine into the accumbens shell increases the liking of sweet solutions, with a very close correspondence between opioid- and cannabinoid-sensitive sites [20, 31].

Independent manipulations of endocannabinoid or opioid processes produce distinct behavioural/motivational consequences, indicating that cannabinoids primarily affect appetitive processes while opioids mainly influence consummatory processes. We suggest that endocannabinoids principally mediate the motivational processes that drive us to eat, but—through interactions with opioid peptide systems—may also contribute significantly to the hedonic evaluation of foods during eating. Arguably, endocannabinoid-opioid activity underlies food craving, the anticipation of delight from eating, and the actual experience of pleasure derived from the sensory properties of food [9].

**Priorities for Future Studies**

As this paper indicates, central endocannabinoid systems are implicated in the principal psychological processes that govern eating motivation and may represent critical components of the mechanisms that lead us to overconsume, a major contributor to weight gain. As such, the endocannabinoids are potentially important therapeutic targets for pharmacological treatments designed to modify eating behaviours and attitudes/responsiveness to foods. Modification of endocannabinoid activity or blockade of CB1 receptors may allow us to limit our susceptibility to the temptations of food and to learn to restrain our excessive appetites. As these factors contribute more than any others to the development of obesity, there is an urgent need to define the psychological consequences of CB1 receptor manipulations in human studies. Insights obtained from the exploration of the subjective ef-
ffects of CB1 ligands would shed important light on the true physiological role of endocannabinoids in appetite control.

References


THE ENDOCANNABINOID SYSTEM AND THE CONTROL OF GASTROINTESTINAL FUNCTION

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Introduction

Cannabinoids (CBs) have long been used to attempt to improve gastrointestinal (GI) function in a variety of conditions associated with disordered intestinal motility, inflammation, and pain. After the discovery of delta-9-tetrahydrocannabinol, a substantial effort was made to discover the mechanism of action of cannabis in the GI tract. These studies were largely focused on GI motility and revealed that cannabinoids reduced the release of acetylcholine from nerve terminals in the enteric nervous system [1]. This effect leads to a slowing of motility and is seen throughout the length of the gut. With the discovery and cloning of the CB1 and CB2 receptors and the isolation of endogenous ligands for these receptors, notably 2-arachidonoylglycerol (2-AG) isolated from the canine GI tract [2], it has become apparent that the gut is a major site of action of the endocannabinoid system (ECS). The ECS is active under physiological and pathophysiological conditions and serves to regulate a variety of GI functions. In this paper, some of the recent findings on the localization and action of the ECS in the GI tract will be highlighted. The main findings are illustrated schematically in the Figure.

Key Points

- The ECS is a novel regulatory system involved in the control of gut function in health and disease.
- The ECS in the GI tract is involved in the regulation of gastrointestinal motility, secretion, sensation, and inflammation.
- CB1 and CB2 receptors are expressed on selective populations of neurons of the enteric nervous system. They modulate synaptic and junctional transmission in the GI tract under physiological (CB1 receptors) and pathophysiological conditions (CB1 and CB2 receptors). They are also expressed on the extrinsic innervation of the gut and regulate visceral sensitivity under pathophysiological conditions.

State of the Art

CB1 receptors were the first components of the ECS to be localized in the GI tract [3]. Consistent with the actions of CBs in the gut, they were found on cholinergic neurons, which are the excitatory motor neurons, major classes of interneurons, and primary afferent neurons of the enteric nervous system. Double-labelling studies also revealed that CB1 receptors were absent from the intrinsic neurons that regulate relaxation of the gut and express nitric oxide synthase. CB1 receptors are also
present on the epithelium of the human gut. Recently, CB2 receptors were localized on neurons of the enteric nervous system and on immune cells in the lamina propria of the mucosa [4]. Like CB1 receptors, these were also largely found on excitatory neurons and were mostly absent from inhibitory motor neurons. Under physiological conditions, these receptors do not appear to be functional in the control of motility. In intestinal inflammation, CB2 receptor expression is upregulated on epithelial cells and the neuronal receptors are able to regulate enhanced motor function [4]. CB1 and CB2 receptors are also found on extrinsic nerves innervating the gut. Vagal afferent neurons express CB1 receptors and spinal afferents express both CB1 and CB2 receptors, being involved in nociceptive transmission and pain sensation from the gut [5].

As noted above, both major endocannabinoids have been isolated from the gut. Anandamide levels are higher in the colon than the ileum [6] and are elevated in states of intestinal inflammation [7, 8]. 2-AG is found at higher levels than anandamide in the GI tract, is more abundant in the small intestine than in the colon, and unlike anandamide is not altered in intestinal inflammation [6, 7]. Degradation of anandamide is largely accomplished by fatty acid amide hydrolase (FAAH), which is distributed throughout the wall of the gut [9]. Monoacylglycerol lipase, which degrades 2-AG, is found in the intestinal epithelium and also in the enteric nervous system [10]. To date, the localization of the biosynthetic enzymes of the ECS has not been determined.

The functions of the ECS in the GI tract have yet to be fully elucidated, but there is good evidence that there is endocannabinoid “tone” in the GI tract [1, 3]. This is defined as a baseline activity of the ECS in the gut that is reduced by blocking CB receptors. Consistent with this concept, when CB1 antagonists are given to animals and humans, there is enhanced gut motility [11, 12], possibly due to some degree of enhanced secretion in the gut, and intestinal inflammation is exacerbated [11, 13]. In states of inflammation, visceral hyperalgesia is observed after treatment with a CB1 receptor antagonist, suggesting
that the ECS is able to attenuate visceral sensitivity in inflammation [14]. Under baseline conditions, the ECS is not apparently active in regulating sensitivity of the gut, since neither CB1 nor CB2 antagonists alter baseline visceral sensitivity to graded colorectal distension [14]. Some of the observations noted above with regard to CB1 receptors have been confirmed by the use of genetically modified mice lacking these receptors.

In order to raise local levels of endocannabinoids, animals have been treated with compounds that inhibit FAAH or block the activity of the putative endocannabinoid membrane transporter (EMT). Under these conditions, GI motility is reduced, consistent with an action of the ECS in limiting the extent of propulsion in the gut [6]. These effects are completely reversed by a CB1 receptor antagonist, being presumably mediated by anandamide. It is likely that they occur at the level of the enteric nervous system, but this has yet to be shown conclusively. The role of 2-AG in motor function is not yet as well established. FAAH inhibitors are capable of attenuating the degree of colitis induced by chemical agents administered intraluminally [7, 8]. Recently, it was shown that this effect was mediated by CB1 and CB2 receptors, both of which completely abolish the protective effects of specific FAAH inhibitors and EMT blockers in colitis [8]. In a similar vein, the EMT inhibitor VDM11 was shown to block the secretory effects of cholera toxin in the mouse ileum, an action effect shown to be mediated by CB1, but not CB2 receptors [15]. Of note also is a role for CB2 receptors in regulation of intestinal hypersensitivity in states of colonic irritation. Here a CB2 receptor antagonist was found to reverse the degree of analgesia induced by a strain of probiotic bacteria given to rats that had been treated with a butyrate enema as a model of irritable bowel syndrome (IBS) [16]. Further evidence for the involvement of ECS in the pathophysiology of IBS was provided when it was shown in a clinical study that single nucleotide polymorphisms of the FAAH gene were more strongly associated with some forms of IBS [17].

**Priorities for Future Studies**

There is considerably more work required to fully establish the roles of the specific elements of the ECS in the various organs of the gut, as well as the many functions that have been only studied to a limited extent. The role of the ECS in the regulation of secretion is not well established and whether the ECS regulates intestinal blood flow has yet to be determined. The biosynthetic enzymes of the ECS are not as well characterized as the other components of this system and are not yet described in the gut. Similarly, there are novel endocannabinoid ligands that have been discovered and which may be expressed in the gut, as may some new putative receptors of the ECS, such as GPR55. In pathophysiological states, the ECS has already been shown to play important roles as noted above, however, there are many conditions where this has yet to be examined and where it is important to do so in the near future.

**References**


THE ENDOCANNABINOID SYSTEM IN ADIPOSE TISSUE

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Introduction
Historically, the endocannabinoid system has been thought to affect energy metabolism by its motivational and orexigenic neuronal effect. However, the weight loss induced by the antagonization of CB1 receptor has only recently been linked to a reduction in caloric intake, which remains a mechanism limited in time. In addition, this weight loss has been shown to be mainly controlled by sustained food intake-independent mechanisms that are more related to energy dispersion. The finding of a weight loss beyond the reduction in food intake led to the examination of the putative role of the endocannabinoid system (ECS) in the various organs implicated in energy dispersion. Very soon, it appeared evident that adipose tissue represents one of the most important targets to examine. This paper will describe the recent advancements in the interaction between adipose tissue and the ECS.

State of the Art
The relevant role of endocannabinoids in adipocyte physiology is highlighted by the evidence of the presence of a functional ECS in the adipocytes. In fact, these cells not only express the CB1 receptor but are also endowed with the full biochemical machinery to synthesize and degrade endocannabinoids [1-9]. Although recent documentation of CB2 expression in adipocytes is noteworthy, its functional role is not well understood at present [6, 7, 9].

The expression of the CB1 receptor is more prominent in mature adipocytes than in pre-adipocytes [3, 6, 7]. Through these dynamic changes in CB1 profile expression, endocannabinoids promote adipocyte growth and differentiation, a function resulting from a cross-talk with the peroxisome prolif-
operator-activated receptor-γ (PPAR-γ) [10]. Even more importantly, the CB1 receptor has been shown to be differently expressed in different compartments of adipose tissue, such as adipocytes derived from the omental and the subcutaneous fat pad, respectively. This finding makes it possible to speculate that the ECS, similar to other hormones and peptides, may act differently in these two districts [7].

The role of the endocannabinoids is not limited to increasing mature adipocyte proliferation. As in the brain, CB1 activation in adipose tissue acts to increase energy storage. Endocannabinoids influence a number of intracellular mechanisms to stimulate lipogenesis by inducing triglyceride accumulation through the inhibition of adenylate cyclase [4, 11] and the consequent reduction of lipolysis and via activation of lipoprotein lipase to provide exogenous fatty acids for the adipocytes [2]. In addition, endocannabinoids stimulate de novo lipogenesis in adipocytes by increasing the expression and activity of enzymes involved in fatty acid and triglyceride biosynthesis [12]. Finally, CB1 activation in adipocytes modulates insulin signalling and glucose uptake in order to increase energy storage [7, 13]. Recent data seems to show that endocannabinoids determine the sensitivity of the insulin response in adipocytes through an involvement of Akt [13].

In the past few years, it has been established that adipose tissue may also act as an endocrine organ. In fact, adipocytes express and secrete a number of adipokines that may deeply influence local adipocyte biology as well as systemic metabolism at sites as diverse as the brain, liver, muscle, pancreatic β-cells, gonads, lymphoid organs, and systemic vasculature [14]. Endocannabinoids have been recognized as important players in the regulation of adipokine secretion in down-regulating adiponectin [1, 3, 6, 11] and stimulating visfatin secretion [11, 15], contributing to the impairment of insulin sensitivity and a decrease in glucose uptake in skeletal muscle.

Recently, it has been found that the administration of CB1 antagonists first in diet-induced obese rodents and after in obese subjects induced an increase in energy expenditure [16-18]. These findings, together with an analysis of the intra-adipocyte gene modulations induced by CB1 blockade chronic

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Figure: Beneficial effects of CB1 blockade on adipocytes.
treatment, indicated that the drug-induced reduction of adipose mass is attributable to increased energy expenditure, mainly through futile cycling (calcium and substrate) [11]. Rimonabant treatment also altered gene expression, which favoured energy dissipation through mitochondrial heat production in brown adipose tissue [11]. Several reports have documented a direct role of the ECS in the modulation of proteins involved in thermogenesis [1, 11]. Treatment of differentiated brown adipocytes with a CB1 agonist decreased the expression of uncoupling protein 1 [11, 15]. However, unlike with rodents, the role of brown adipose tissue in humans is not yet well established. Physiological and pharmacological stimuli may be capable of trans-differentiating white adipocytes into brown adipocytes in humans. Therefore, CB1 receptor antagonist treatment may lead to an eventual increase in energy expenditure by increasing brown adipocytes. Genetic and pharmacological blockade of the cannabinoid CB1 receptor increases mitochondrial biogenesis in white and brown adipocytes [19]. Treatment of adipocytes with rimonabant, a selective antagonist of CB1 receptors, increases mitochondrial biogenesis genes, including PGC-1α and Tfam. This possibly implies that SR141716 is antagonizing an endocannabinoid tone present in cultured fat cells. Moreover, the genetic CB1 receptor blockade in vivo increases eNOS expression and mitochondrial biogenesis both in whole white adipose tissue and isolated mature white adipocytes, and this is accompanied by prevention of high fat diet-induced fat accumulation. This might increase oxidative metabolism in white adipocytes by counteracting the inhibitory effects of endocannabinoids, whose levels are increased in fat tissues of obese rodents and humans [19].

A possible mediator of these functions may be the AMPK system, which acts as a fuel sensor to regulate energy balance both at the cellular level and within the whole body, by inhibiting anabolic pathways and stimulating catabolic processes in order to increase the ATP/AMP ratio. Endocannabinoids act to decrease AMPK activity in the adipose tissue, contributing to an increase in adiposity and lipogenesis and resulting in decrease in energy expenditure [19]. Accordingly, induction of AMPK activity has been found to increase mitochondrial content in adipocytes. Thus, the enhanced β-oxidation of free fatty acids elicited by AMPK activation and by eNOS-dependent mitochondrial biogenesis might functionally link rimonabant treatment to its anti-obesity effects.

As mentioned before, a close association between the development of obesity and a simultaneous overactivation of the ECS in the adipose tissue, expressed as a rise in endocannabinoid production or an increase in CB1 receptor expression, has been shown [6, 7]. Significantly higher levels of 2-arachidonoylglycerol (2-AG), but not anandamide, have been detected in the visceral (intra-abdominal), but not subcutaneous, fat of obese patients [7]. A similar pathological increase in endocannabinoids has been detected in mice. However, in rodents, anandamide and not 2-AG seem to be elevated [12]. Importantly, this increased intra-adipocitic tone of endocannabinoids has been demonstrated to be under the control of hypothalamic leptin signalling [12].

Increased levels of plasma 2-AG were present in patients with visceral obesity compared to patients with subcutaneous obesity and compared to lean controls [20-22]. The increase in 2-AG was also shown to positively correlate with some important cardiometabolic risk factors, such as body mass index, waist circumference, fasting plasma triglyceride and insulin levels, low HDL cholesterol, and adiponectin levels [21].
Altogether, this data enables us to include the CB1 receptor and the endocannabinoids in the group of agents that play a determinant role in the physiology and the pathophysiology of these cells (figure).

References


THE ENDOCANNABINOID SYSTEM AS A THERAPEUTIC TARGET FOR LIVER DISEASES

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Introduction

Chronic liver disease is responsible for about 800,000 deaths a year due to cirrhosis and its complications. The most common causes of liver disease worldwide are viral hepatitis, chronic alcohol consumption, and non-alcoholic fatty liver disease (NAFLD) associated with the metabolic syndrome. All these conditions generate liver injury and inflammation, thereby activating liver fibrogenesis. Progression of fibrosis leads to cirrhosis and the life-threatening complications of liver failure and portal hypertension, as well as to incident hepatocellular carcinoma [1].

Accumulating evidence indicates that the endocannabinoid system (ECS) plays a crucial role in the pathophysiology of liver diseases, both as a key player in hepatic injury and as a mediator of cirrhosis complications. Indeed, CB1 and CB2 receptors have emerged as mediators of non-alcoholic and alcoholic fatty liver disease and regulate hepatic inflammation, liver fibrosis, and complications of infection.

Key Points

- Chronic liver disease is responsible for about 800,000 deaths a year due to cirrhosis and its complications.
- Viral hepatitis, chronic alcohol consumption, and non-alcoholic fatty liver disease are the most common causes of liver disease worldwide. All these conditions generate liver injury and inflammation, thereby activating liver fibrogenesis, which can progress to cirrhosis and the life-threatening complications of liver failure and portal hypertension, as well as to incident hepatocellular carcinoma.
- The ECS is highly up-regulated during liver injury.
- CB1 receptors promote fatty liver via direct activation of CB1 receptors expressed in hepatocytes.
- CB1 receptors are profibrogenic.
- CB1 receptors contribute to the hemodynamic complications of cirrhosis.
- Beneficial effects of CB1 antagonists are expected in patients with non-alcoholic or alcoholic fatty liver disease, at multiple steps of liver disease progression.
- The development of peripherally restricted CB1 antagonists will constitute a major challenge within the next few years.
- CB2 receptors also participate in the pathogenesis of non-alcoholic fatty liver disease via a pathway distinct from that activated by CB1 receptors: CB2 receptors indirectly enhance metabolic steatosis, following increased obesity-associated fat inflammation and insulin resistance.
- CB2 receptors are antifibrogenic.
- Potential therapeutic indications of CB2-specific molecules are expected but will require additional preclinical studies in order to precisely define the conditions associated with CB2-dependent pro or anti-inflammatory effects.
cirrhosis, including cirrhotic portal hypertension and cirrhotic cardiomyopathy [2, 3].

**State of the Art**

The normal liver produces endocannabinoids [2, 4], originating from both hepatocytes and non-parenchymal cells [5], and expresses low levels of cannabinoid receptors. In contrast, the ECS is up-regulated during liver injury and affects several physiopathological processes associated with acute or chronic liver disease.

The ECS and fatty liver disease

NAFLD is linked to the metabolic syndrome and is a rising cause of liver injury in Western countries. NAFLD shares common pathologic features and pathophysiological mechanisms with alcoholic fatty liver disease. Both diseases can present as steatosis but may evolve towards alcoholic or non-alcoholic steatohepatitis, when associated with liver inflammation and hepatocyte injury, that promotes liver fibrogenesis, with a 20% risk of cirrhosis after 10 to 20 years [6].

Recent studies have demonstrated the major role of CB1 receptors in fatty liver disease. Hence, hepatic endocannabinoids are overproduced and hepatocyte CB1 receptors are up-regulated in response to high-fat diet or chronic alcohol feeding [7-9]. Moreover, CB1 receptor-deficient mice do not develop steatosis, and high fat diet or ethanol-induced fatty liver is prevented by rimonabant treatment [7-9]. Interestingly, mice with hepatocyte-specific deletion of CB1 receptors are resistant to fatty liver, thereby supporting a direct role of hepatic CB1 receptors in this process [8]. This data is reinforced by clinical evidence showing that daily cannabis use is an independent predictor of steatosis severity in patients with chronic hepatitis C [10].

CB2 receptors also contribute to the pathogenesis of NAFLD. Indeed, mice deficient in CB2 receptors are more resistant to high fat diet-induced obesity than wild type animals. Moreover, CB2 antagonism improves insulin sensitivity and blunts hepatic steatosis following inhibition of obesity-associated inflammation in the adi-
pose tissue [4]. These results demonstrate that both CB1 and CB2 play a role in the pathogenesis of NAFLD via distinct pathways. Hepatic inflammation is prominently involved in the process.

The ECS and liver fibrogenesis

The frequent inability to eradicate the cause of chronic liver disease warrants the development of liver-specific antifibrotic strategies that generally aim at inhibiting the accumulation of liver fibrogenic cells and/or reducing extracellular matrix accumulation. In addition, inhibition of parenchymal injury or reduction of liver inflammation has also been shown to have some beneficial antifibrogenic effects [1]. However, despite encouraging experimental results, proof of efficacy of potential antifibrogenic molecules in a clinical setting is currently lacking.

Recent studies have shown that the ECS may be a crucial regulator of liver fibrogenesis. CB1 and CB2 receptors are up-regulated in the cirrhotic human liver, predominantly in liver fibrogenic cells. Moreover, endogenous activation of CB2 receptors limits progression of experimental liver fibrosis by reducing accumulation of liver fibrogenic cells, thereby demonstrating the antifibrogenic properties of CB2 receptors [11]. Interestingly, CB2 receptors also display anti-inflammatory properties during liver ischemia-reperfusion injury [5]. However, whether CB2 agonists may, in addition to directly limiting hepatic fibrogenic cell accumulation, also indirectly regulate fibrogenesis by inhibiting the inflammatory response to chronic liver injury remains to be determined.

In contrast to CB2 receptors, CB1 is profibrogenic in the liver. Administration of rimonabant or genetic inactivation of CB1 receptors inhibits fibrosis progression in three models of chronic liver injury by a mechanism involving reduced proliferation and increased apoptosis of liver fibrogenic cells [12]. Moreover, daily cannabis use is an independent predictor of fibrosis severity in patients with chronic hepatitis C, suggesting that CB1 signalling predominates over CB2 in these patients [13].

These findings unravel CB1 and CB2 receptors as potential novel targets for antifibrogenic therapy during chronic liver diseases and suggest that combined therapy with selective CB1 antagonists and/or CB2 agonists might open novel perspectives for the treatment of liver fibrosis.

Endocannabinoids as mediators of vascular and cardiac abnormalities in cirrhosis

The ECS contributes to the hemodynamic alterations associated with cirrhosis. Indeed, endocannabinoids trigger vasorelaxing effects, and CB1 receptors contribute to the pathogenesis of portal hypertension via enhanced mesenteric vasodilation [14, 15]. Moreover, the cardiac expression of the ECS is increased in experimental models of cirrhosis and is associated with a CB1-dependent impairment of cardiac contractility, demonstrating the role of the CB1 receptor in the development of cirrhotic cardiomyopathy [16, 17].
Priorities for Future Studies

The ECS is increasingly incriminated in several pathophysiological aspects associated with chronic liver disease progression (Figure). Steatogenic and profibrogenic properties of CB1 receptors and their harmful impact on hemodynamic complications of cirrhosis suggest that CB1 receptors trigger several deleterious effects that may enhance progression of chronic liver disease to cirrhosis and its complications. Beneficial effects of CB1 antagonists are therefore expected in patients with non-alcoholic or alcoholic fatty liver disease, at multiple steps of disease progression. However, the increased incidence of anxiety and depression in obese patients treated with rimonabant has led to its recent withdrawal by the European Medicines Agency (EMEA). The development of peripherally restricted CB1 antagonists will therefore constitute a major challenge within the next few years. CB2 receptors play a key role in the regulation of the liver inflammatory response [18]. These findings may open novel therapeutic perspectives on clinical development of CB2 specific molecules. However, potential therapeutic indications will require additional preclinical studies in order to precisely define the conditions associated with CB2-dependent pro- or anti-inflammatory effects.

References


THE ENDOCANNABINOID SYSTEM AND CARDIOVASCULAR DISEASE

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Introduction
Cannabinoids and their endogenous and synthetic analogs exert complex cardiovascular effects both in vitro and in vivo mediated by cannabinoid receptor-dependent and -independent mechanisms. The cannabinoid CB1 and CB2 receptors and the endocannabinoid degrading enzymes are expressed in the myocardium [1-3], human coronary artery endothelial and smooth muscle cells [4, 5], and infiltrating inflammatory cells, among many other tissues/cells [6]. In experimental animals and in humans (depending on the route of administration, duration, and the dose) these cardiovascular effects may include CB1-mediated bradycardia/tachycardia, hypotension, and depressed cardiac contractility involving modulation of autonomic outflow through sites of action at presynaptic autonomic nerve terminals and in the central nervous system, as well as direct effects on myocardium and the vasculature [7]. In spite of the above mentioned cardiovascular effects of endocannabinoids, the endocannabinoid system (ECS) appears to play a limited role in cardiovascular regulation under normal physiological conditions. However, in various disease conditions, the ECS may become overactivated and play important protective and/or detrimental roles.

Key Points
- The cannabinoid CB1 and CB2 receptors and endocannabinoid degrading enzymes are present in cardiovascular tissues.
- Activation of cardiovascular CB1 receptors leads to hypotension and decreased cardiac contractility. However, the role of myocardial CB2 receptors is still elusive.
- The ECS plays a limited role in cardiovascular regulation under normal physiological conditions.
- In various forms of shock and heart failure, the ECS may become overactivated and contribute to depressed cardiovascular function, which can be prevented or attenuated by CB1 antagonists.
- The ECS may also be activated as a compensatory mechanism in various forms of hypertension to limit pathologically increased blood pressure and myocardial contractility.
- CB1 antagonists exert various cytoprotective and anti-inflammatory effects in multiple unrelated preclinical disease models and also in patients with obesity and/or metabolic syndrome.
- Activation of CB2 receptors in inflammatory cells and endothelium attenuates TNF-α-induced endothelial inflammatory response, chemotaxis, and adhesion of inflammatory cells to the activated endothelium, and consequent release of various proinflammatory mediators, which may underlie the beneficial effects of CB2 agonists in vascular inflammation, atherosclerosis, and myocardial ischemia/reperfusion injury.
Activation of the ECS in inflammatory cells and cardiovascular tissues by bacterial endotoxin(s) has been implicated in cardiovascular collapse in various forms of shock (e.g., septic, hemorrhagic, and cardiogenic) and advanced liver cirrhosis (reviewed in [6]). In these conditions, treatment with CB1 antagonists prevented or reversed the hypotension and/or decreased myocardial contractility (reviewed in [6, 8]). In rat models of acute and chronic myocardial infarction, studies with CB1 agonists/antagonists yielded conflicting results [9, 10]. More recently, the role of the ECS was explored in a mouse model of doxorubicin(DOX)-induced heart failure [3]. Following doxorubicin administration, the tissue anandamide content, but not CB1/CB2 receptor expression, was elevated in the myocardium and also in cardiomyocytes exposed to DOX in vitro, suggesting activation of the ECS. Pretreatment of mice with CB1 antagonists (rimonabant and AM281) not only improved DOX-induced cardiac dysfunction, but also attenuated the DOX-induced cell death both in vivo and in vitro. This cytoprotective effect suggests that the cardioprotective effect of CB1 antagonists in various cardiac pathologies may extend beyond beneficial hemodynamic effects. In fact, CB1 antagonists exert various anti-inflammatory and cytoprotective effects in multiple unrelated preclinical disease models [11-17]. Furthermore, rimonabant also attenuates multiple inflammatory markers [e.g., tumor necrosis factor-α (TNF-α), C-reactive protein, etc.], plasma leptin and insulin levels, and increases plasma adiponectin in obese patients with metabolic syndrome and/or type 2 diabetes, thereby attenuating the development of cardiovascular risk factors associated with obesity/metabolic syndrome and diabetes [18-24]. On the basis of these studies, it was also suggested that rimonabant may have favourable effects in atherosclerosis. With this in mind, the results of the recent STRADIVARIUS clinical trial examining the effect of 18 months of rimonabant treatment on coronary disease progression in subjects with abdominal obesity/metabolic syndrome yielded somewhat disappointing results [19]. Rimonabant had no significant effect on the primary endpoint of coronary disease progression (the percent atheroma volume), however, it decreased the normalized total atheroma volume, which was the secondary endpoint [19]. The favourable effects of rimonabant on body weight and hormonal/metabolic parameters were similar to those observed in previous large-scale trials.

Paradoxically, the ECS may also be activated as a compensatory mechanism in various forms of hypertension to limit pathologically increased blood pressure and myocardial contractility [6]. In this case, the enhancement of endogenous cannabinoid tone by inhibition of the anandamide degrading enzyme fatty acid amide hydrolase (FAAH) can decrease blood pressure and myocardial contractility [6].

The role of myocardial CB2 receptors during ischemia/reperfusion and other cardiovascular pathologies is still vague. In contrast, activation of CB2 receptors in inflammatory cells and endothelium attenuates TNF-α-induced endothelial inflammatory response, chemotaxis, and adhesion of inflammatory cells to the activated endothelium, and consequent release of various proinflammatory mediators (key processes involved in the initiation and progression of atherosclerosis, restenosis, and reperfusion injury) [8, 25]. Activation of CB2 receptors in human coronary smooth muscle cells
decreases proliferation [5], which may have clinical implications for the treatment of atherosclerosis and restenosis.

**Priorities for Future Studies**

An increasing number of studies suggests that the beneficial effects of CB1 antagonists in various cardiomyopathies on contractile function may extend far beyond the simple inhibition of CB1-mediated cardiovascular depressive effects of pathologically overproduced endocannabinoids in these disease conditions. Future studies using both knockout mice and additional selective CB1/2 agonists/antagonists must explore the possible interactions of the ECS with oxidative/nitrosative stress and related inflammatory pathways in models of myocardial ischemia/reperfusion, cardiomyopathies, heart failure, and atherosclerosis. Additional prospective studies should also examine if CB1 antagonist treatment leads to reduction of clinical events related to coronary disease. Novel therapeutic strategies targeting development of peripherally restricted CB1 antagonists may improve the benefit/risk ratio for this class of compounds by decreasing psychiatric side effects.

**References**


ROLE OF THE ENDOCANNABINOID SYSTEM IN Atherosclerosis

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Introduction
Atherosclerosis is an inflammatory disease that involves vascular and immune cell types. Endothelial cells, smooth muscle cells, resident macrophages, as well as circulating leukocytes and platelets are the active players in the atherosclerotic inflammatory processes. Recently, basic research studies, animal models, and clinical trials have strongly suggested that the endocannabinoid system (ECS) is a crucial modulator of these cells in atherosclerosis.

State of the Art
The ECS comprises several endogenous agonists of the cannabinoid type 1 (CB1) and type 2 (CB2) receptors and their degrading enzymes, which are secreted on demand. Endocannabinoid activity is mainly mediated by the binding and activation of CB1 or CB2 receptors, which are differentially expressed in inflammatory cell types and organs. However, endocannabinoids can also exhibit immunomodulatory activities through pathways that are independent of “classical” cannabinoid receptors. For instance, they can activate transient receptor potential vanilloid type-1 receptors (TRPV1), peroxisome proliferator-activated receptor-α (PPAR-α), and the orphan G protein-coupled receptor GPR55. There is mounting evidence for immunomodulatory effects of endocannabinoids, suggesting their crucial role in atherosclerotic inflammatory processes. In particular, the endocannabinoid anandamide has been shown to reduce the pro-inflammatory effects of tumor necrosis factor-α in human coronary artery endothelial cells and the adhesion of THP-1 monocytes to human coronary artery endothelial cells in a CB1 and CB2-dependent manner. More recently, we have shown that the activation of CB2 receptors inhibits human monocyte migration in response to classical chemotactants, which are expressed in atherosclerotic plaques. Furthermore, endothelial cells, macrophages, or platelets themselves increase their endocannabinoid synthesis during atherosclerosis, thus triggering platelet activation. These cells are also able to metabolize anandamide. Although some studies have also shown a possible pro-thrombotic effect of endocannabinoids, the majority of in vitro experimental evidence supports their possible anti-inflammatory role in atherosclerosis. Several animal models have confirmed in vitro studies by showing that treatment with cannabinoid agonists reduced blood pressure and atherosclerosis progression in rodents. In spontaneously hypertensive rats, prevention of endocannabinoid anandamide degradation by an inhibitor of fatty acid amide hydrolase (FAAH) was shown to lower blood pressure and heart rate through reductions in both car-
diac contractility and vascular resistance. These effects were prevented by CB1 antagonists. These findings suggest that the ECS represents a therapeutic target for the treatment of hypertension, which is a major risk factor for atherosclerosis. A more recent study investigated the age-associated decline of cardiac function and changes in inflammatory gene expression, nitrative stress, and apoptosis in FAAH-/-mice as compared to wild type mice. Enhanced anandamide levels in the FAAH-/-animals were protective, which further supports the protective role of endocannabinoids in inflammatory disorders such as atherosclerosis. Nevertheless, direct experimental evidence supporting a direct role of the ECS in atherosclerosis is still missing.

Obesity is a metabolic disease and a major risk factor for atherosclerosis. The ECS plays a crucial role in obesity. In particular, overactivity of the ECS promotes excessive food intake and fat accumulation in animal models and humans. In rodents, pharmacologic blockade or genetic ablation of CB1 receptors reduces appetite and weight and prevents obesity and insulin resistance. CB1 blockade in rodents acts on adipocytes to increase adiponectin expression, on hepatocytes to decrease \textit{de novo} lipogenesis and increase fatty acid oxidation, and in the gastrointestinal tract to increase satiety. Clinical trials investigating treatment with rimonabant (a selective antagonist of the cannabinoid type 1 receptor) have suggested a beneficial effect of this drug in the management of obesity in humans. The first study on the efficacy of rimonabant against atherosclerosis and coronary artery disease (CAD) in obese subjects was published recently (in 2008). The Strategy to Reduce Atherosclerosis Development Involving Administration of Rimonabant - The Intravascular Ultrasound Study (STRADIVARIUS) was a prospective, multicentre, multinational, randomized, double-blind placebo-controlled 2-group, parallel-group study, involving 112 centres in North America, Europe, and Australia. Patients were randomly assigned to two groups (placebo vs. 20 mg/day rimonabant) and followed for 18 months. Although rimonabant ameliorated the normalized total atheroma volume (TAV, secondary endpoint), the study failed to show a decrease in percent atheroma volume (PAV, primary endpoint). These controversial results indicated that the use of rimonabant in the management of coronary disease in patients with central obesity or metabolic syndrome requires further investigation. However, the elevated incidence of adverse events in this study raises some doubts regarding the safety of rimonabant.

**Priorities for Future Studies**

Basic research, animal models, and clinical trials clearly show that the ECS is a crucial player in the modulation of inflammatory processes in atherosclerosis. In particular, the majority of the studies indicate that endocannabinoids are anti-inflammatory rather than pro-inflammatory agents. Further studies are needed to clarify a possible use of rimonabant, the selective antagonist of CB1 receptors, in acute and chronic events in atherosclerosis.
Introduction

In 1964, the structure of delta-9-tetrahydrocannabinol, the main psychoactive ingredient in marijuana, was identified [1]. The breakthrough was given impetus by the discovery of cannabinoid receptors and their endogenous ligands, the endocannabinoids. The endocannabinoid system (ECS) is a physiological system with an important regulatory role in numerous biological functions, both centrally and peripherally. In certain conditions it can become overactivated and induce a variety of metabolic disorders, i.e., obesity, type 2 diabetes, etc. The system has two receptor types, designated CB1 and CB2, as well as endogenous ligands and systems for their transport, synthesis, and degradation.

Discovery

In the late 80s and early 90s, scientists at Sanofi wondered if antagonists of “cannabis” could promote an inhibition of appetite and thus be a potential treatment of obesity. At around the same time, the human central cannabinoid receptor (the CB1 receptor) was cloned [2]. This was followed three years later by the characterization of a second cannabinoid receptor (CB2). A high throughput screening was then performed on the cannabinoid receptors. These studies led, after optimization, to the selection of SR141716 (rimonabant). Rimonabant was found to be highly selective for the central CB1 receptor (at nM levels), exhibiting only very weak activity (at mM levels) for the cloned peripheral CB2 re-

Key Points

- CB1 receptors may be involved in the motivational aspects of eating.
- Rimonabant can reduce the appetitive and rewarding properties of food and drink in animal models.
- An activation of peripheral metabolic processes contributes to the weight-reducing effect of rimonabant.
- Preclinical studies have shown the potential of rimonabant in the specific treatment of dyslipidemia, glycemia, and atherosclerosis and in reducing the risk of major cardiovascular events.
- Rimonabant in the RIO studies achieved clinically significant improvements in body weight, HbA1c levels in type 2 diabetics, and in lipid parameters.
- Subsequent clinical studies have demonstrated that rimonabant improves glycemic control in type 2 diabetes across a range of patient populations while improving weight loss and lipid parameters, thereby addressing multiple cardiometabolic risk factors commonly observed in type 2 diabetes.
- CB1 receptor antagonism with rimonabant represents a novel approach to the treatment of abdominal obesity and other cardiovascular and cardiometabolic risk factors.
The compound was then examined in an in vivo environment and, at low doses administered by the oral or intraperitoneal routes, it antagonized in a dose-dependent way the behavioural responses elicited by the cannabinoid receptor agonist WIN 55212-2 in rodents. In addition, other tests established the good bioavailability of the compound and its long duration of action.

**Preclinical Profile**

Numerous preclinical studies have shown that rimonabant selectively reduces the intake of palatable food or drink [4], and it is also able to diminish the intake of a high-fat diet preferred by the obese Zucker rats, without modifying the intake of a high-carbohydrate diet. Rimonabant has been studied in different models of obesity in rodents. The anti-obesity effect of rimonabant has also been studied in a model of obesity (DIO) induced by a high-fat diet in mice. In this model, rimonabant administered at a dose of 10mg/kg/day during 6 weeks produced a marked reduction in eating, which was transitory during the first weeks of treatment. Afterwards this effect tended to disappear. However, rimonabant produced a marked loss in body weight that was maintained up to the end of the 6-week treatment [5]. The initial loss in body weight was probably due to the reduction in food intake observed at the beginning of treatment. However, the fact that this effect persisted even when the mice increased their food intake to normal levels suggested a metabolic effect involving the peripheral tissues.

It has been demonstrated that the CB1 receptors are also expressed in the adipose tissue of rodents and humans and also in the cultured mouse 3T3 F442A adipose cells. This expression is increased in the adipose tissue of obese Zucker rats compared to nonobese rats [6]. Rimonabant at a dose of 10mg/kg/day for 10 days in Zucker rats stimulated the expression of adiponectin mRNA in the adipose tissue of the obese animals. This effect was mediated by the blockade of the CB1 receptors, as rimonabant did not modify the expression in the adipose tissue of CB1 knockout mice. These results demonstrate that rimonabant, in blocking the adipocyte CB1 receptors, regulates the expression of this adiponectin protein. The increase in the levels of adiponectin could therefore be partially responsible for the peripheral metabolic effects of rimonabant. A follow-up study in Zucker rats demonstrated that rimonabant had a definite hepatoprotective effect with a reduced hepatomegaly associated with reduced elevated plasma levels of enzyme markers of hepatic damage as well as reduced local hepatic tumor necrosis factor-α levels indicative of steatohepatitis [7].

Further long-term studies in obese Zucker rats that developed chronic renal failure showed that rimonabant preserved renal function and increased survival [8]. When rimonabant was administered to Zucker diabetic rats, it prevented the development of hypoglycemia and improved β-cell function, while maintaining a normal profile of insulin secretion with a much lower impact on body weight compared to rosiglitazone treatment [9].

In addition, rimonabant positively modulates the lipid profile, reduces circulating neutrophils and monocytes, attenuates platelet activation as well as the release of proatherosclerotic chemokines, reducing cardiovascular risk in the process [10].
Clinical Studies
The preclinical studies confirmed the potential of rimonabant as a CB1 receptor antagonist as well as its potential therapeutic application in the treatment of obesity. Initial phase II clinical studies confirmed the potential of the compound in reducing body weight in obese patients. In 4 large randomized phase III clinical studies in obese or overweight patients (RIO program in over 6,600 patients) [11-14], rimonabant has shown the ability to reduce body weight and waist circumference, increase HDL cholesterol levels, reduce triglyceride and HbA1c levels and other cardiometabolic risk factors, and is thus considered a novel treatment in conjunction with diet and exercise for obese and overweight patients with cardiometabolic diseases such as type 2 diabetes or dyslipidemia.

After the RIO program, different clinical studies were initiated to assess the therapeutic potential of the compound in the treatment of type 2 diabetes and other cardiovascular risk factors. These included the SERENADE study, where the drug was found to improve glycemic control in newly diagnosed naive type 2 diabetic patients [15]. More recently, the ARPEGGIO study demonstrated an improvement in glycemic control in a difficult population of type 2 diabetic patients inadequately controlled by insulin alone, with a significant decrease in HbA1c levels being observed [16]. In the ADAGIO-Lipids study, rimonabant was active against certain biomarkers indicative of atherogenic dyslipidemia in abdominally obese patients, e.g., fatty acid index, alanine transaminase, high-sensitivity C-reactive protein, and adiponectin [17]. Further studies included the STRADIVARIUS study, where the effect of rimonabant over 18 months on the progression of atherosclerosis was measured by IVUS endpoints. Rimonabant failed to show a statistically significant effect on the primary endpoint, but did show encouraging results across 4 IVUS endpoints [18].

Current/Future Clinical Studies
In the cardiovascular area, as a follow-up to the STRADIVARIUS study, the AUDITOR study will specifically look for an effect on the inhibition of atherosclerosis progression as assessed by carotid artery intima-media thickness (CIMT) in overweight patients. In addition, the ongoing RAPSODI study will assess the efficacy and safety of long-term administration of rimonabant to delay the onset of type 2 diabetes in patients with a pre-diabetic status. Finally, the CRESCENDO study (~17,000 patients), due to be completed in 2011, will see whether rimonabant treatment can reduce cardiovascular events in abdominally obese patients with high cardiovascular risk factors.

References


ABDOMINAL OBESITY, DYSLIPIDEMIA, INSULIN RESISTANCE, TYPE 2 DIABETES AND ATHEROSCLEROSIS: WHO IS THE RIGHT PATIENT TO BE TREATED WITH CB1 RECEPTOR ANTAGONISTS?

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Introduction
The health hazards of abdominal obesity were documented several decades ago when, in 1947, a French physician by the name of Dr. Jean Vague published in Presse Médicale the results of his clinical observations on the “android” type of obesity (“apple shape”) [1]. Vague was the first to suggest that android obesity was the high-risk form of obesity. In contrast, he proposed that “gynoid” obesity (often found in women) was rather benign [1]. Thus, Vague was the first to foresee the importance of upper body, abdominal obesity as a phenotype frequently observed in individuals with cardiovascular disease, type 2 diabetes and hypertension. Results from epidemiological studies that began to be published in the early eighties confirmed the increased risk of adverse cardiovascular outcomes associated with such a form of overweight/obesity. Most of these studies assessed the absolute or relative amount of abdominal fat using crude anthropometric indices such as waist circumference or the waist-to-hip circumference ratio [2-6]. Very recently, the importance of abdominal obesity beyond overall general adiposity as a risk factor for total mortality has been confirmed in the largest prospective study ever conducted on the topic. Results of the EPIC

Key Points
- Abdominal obesity is the high-risk form of obesity.
- CB1 receptor antagonism can induce weight loss, loss of abdominal fat and improvements in the cardiometabolic risk profile.
- CB1 receptor antagonists have been shown to decrease intra-abdominal (visceral) and liver fat.
- Developing the “right drug for the right patient” is an important challenge inherent to compounds that are labelled “weight loss drugs”.
- Whether reducing abdominal obesity can reduce the risk of cardiovascular disease still remains to be determined.
study provided robust evidence that waist circumference predicted mortality beyond body mass index [7]. Studies that have directly measured abdominal fat using imaging techniques such as computed tomography have demonstrated that among abnormally obese individuals, those characterized by a selective excess of intra-abdominal (visceral) fat accumulation have the most atherogenic and diabetogenic metabolic profile (often referred to as the metabolic syndrome) compared to subjects with a selective excess of subcutaneous fat [8-10]. In addition, intra-abdominal fat – as a reflection of overall ectopic fat – may be the link between obesity and cardiovascular disease [11].

**State of the Art**

As abdominal obesity is an emerging modifiable risk factor for type 2 diabetes and cardiovascular disease, a pharmacological approach targeting the excess abdominal fat depot (which most of the time accompanies features of the metabolic syndrome) could be relevant to optimally reduce the cardiovascular disease risk of patients with intra-abdominal obesity. In this regard, the evidence of an overactivation of the endocannabinoid system (ECS) in obesity, particularly abdominal obesity [12-14], and the published results of the phase III program (Rimonabant In Obesity; RIO) to be conducted with the first CB1 blocker developed, rimonabant, may open new possibilities for targeting abdominal obesity and related abnormalities [15-18]. Rimonabant works centrally to reduce food intake through antagonism of the cannabinoid receptor (CB1), but there is now evidence that it also acts peripherally in key tissues involved in carbohydrate and lipid metabolism such as the liver and adipose tissue [19-22]. For instance, CB1 blockade with rimonabant has been shown in animals to reduce liver lipogenesis and to stimulate adiponectin gene expression and protein secretion by fat cells [19, 22]. These findings are particularly relevant for the management of the metabolic abnormalities of intra-abdominal obesity.

Because of the designs requested by regulatory authorities, initial studies with rimonabant have mainly focused on weight loss and on its effect on cardiometabolic risk factors in patients selected only on the basis of their excess body weight. However, the RIO-Lipids study was specifically designed to test the effect of rimonabant in higher-risk patients: those who were not only overweight/obese (body mass index: 27–40 kg/m²) but who also

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**Table:** Effects of the cannabinoid-1 receptor antagonist rimonabant on anthropometric and cardiometabolic risk variables.
had an atherogenic dyslipidemia (triglyceride levels between 1.7–7.9 mmol/l and/or choles-
terol/HDL cholesterol >5 for men or 4.5 for women) [15]. As for all four phase III studies with ri-
monabant, patients of the RIO-Lipids trial were asked to reduce their caloric intake by 600 kcal/day
during a 4-week run-in period, which they did as they lost about 2 kg of body weight and their waist
circumference was reduced by 2 cm. After the run-in period, the baseline characteristics of these
dyslipidemic patients were assessed and they were then randomized and exposed either to placebo
(n=342) or treatment with rimonabant 5 mg (n=345) or 20 mg (n=346) daily for 12 months. By the
end of the study, patients treated with rimonabant 20 mg had a significantly greater body weight loss
compared with the placebo group; this was accompanied by a significantly greater decrease in waist
circumference. In addition, this substantial loss of abdominal fat was, as expected, accompanied by
significant improvements in the plasma lipoprotein-lipid profile, which included a reduction in
triglycerides (p<0.001) and an increase in HDL cholesterol levels (p<0.001) among patients treated
with rimonabant 20 mg. Although there was no change in LDL cholesterol levels with rimonabant
therapy, the group treated with rimonabant 20 mg showed an increase in LDL particle size
(p=0.008) relative to the placebo group, whereas the proportion of small LDL particles decreased
compared to the placebo group (p=0.007). In addition, plasma adiponectin levels increased by 58%
(p<0.001) over baseline in the rimonabant 20 mg group, and this difference could not be entirely ex-
plained by weight loss. For instance, patients in the placebo group who had a ≥10% weight loss had
an increase in adiponectin levels of slightly >2 μg/ml whereas patients treated with rimonabant had
an increase in adiponectin levels of >3 μg/ml. These results provided the first evidence in a clinical
trial that CB1 blockade with rimonabant could have a direct effect on the production of adiponectin
by adipose tissue beyond what could be explained by weight loss. Thus, this peripheral effect of ri-
monabant on adipose tissue metabolism could help explain, at least partly, the drug’s well docu-
mented effect on cardiometabolic risk markers beyond what can be explained by weight loss, a con-
sistent finding in the phase III RIO program.

One of the four phase III studies with rimonabant (RIO-Diabetes) was performed in over-
weight/obese patients with type 2 diabetes who were treated either by sulphonylurea (about 1/3) or
with metformin (about 2/3) therapy [17]. In addition to confirming the robust effect of rimonabant
on plasma lipids and some other markers of cardiometabolic risk, the study revealed that CB1 an-
tagontism with rimonabant could significantly improve glycemic control (HbA1c levels) beyond the
effect mediated by weight loss. Such a glucose-lowering effect of rimonabant was found irrespective
of background anti-diabetic therapy. A recent study (SERENADE) has also confirmed the cardiome-
tabolic benefits and glucose-lowering effects of rimonabant in drug-naive patients with type 2 dia-
abetes [23]. As type 2 diabetes is the ultimate manifestation of intra-abdominal obesity and of ectopic
fat deposition, these effects of rimonabant on markers of abdominal obesity, glycemic control and
cardiometabolic risk variables make this drug an interesting option for the global management of pa-
tients with type 2 diabetes.

The results of published studies with rimonabant are quite consistent and indicate that rimonabant 20
mg/day produces a significant decrease in body weight as well as a substantial mobilization of ab-
dominal adipose tissue as indicated by a considerable reduction in waist circumference. Moreover,
these benefits were found to be maintained over two years in the RIO-Europe trial [24]. Overall,
these results suggest that rimonabant therapy could be useful for the management of clustering cardiovascular disease risk factors in high-risk abdominally obese patients through its marked effects on both abdominal adiposity and related metabolic risk factors. In this regard, a recent 1-year imaging trial (ADAGIO-Lipids) has confirmed that rimonabant can induce a significant loss of both intra-abdominal and liver fat [25]. Key cardiometabolic effects of rimonabant are summarized in the Table.

**Safety**

Antagonism of the ECS clearly produces significant improvements in several markers of cardiometabolic risk. Of course such benefits have to be weighed against the side effects of the drug. Main side effects of the drug have been nausea, dizziness, some gastrointestinal side effects as well as anxiety, mood changes and depression symptoms [26]. Regarding the latter, further analyses from pooled studies as well as more recent trials (such as STRADIVARIUS) have indicated that although the relative risk of depression associated with rimonabant was about 1.7, the absolute risk was largely dependent upon past/present history of depression [27]. On that basis, although regulatory authorities had recommended that rimonabant should not be prescribed in patients with a history of depression, the challenge of ensuring that the right patient is treated with this CB1 antagonist has led the European Medicines Agency (EMEA) to recommend the withdrawal of the drug from the market until further evidence of a favourable benefit/risk ratio becomes available.

**Futures Studies/Perspectives**

Based on the results published or available with rimonabant, we would like to propose that the best patient for rimonabant therapy is an abdominally obese, insulin-resistant patient with an atherogenic dyslipidemia or an abdominally obese patient with type 2 diabetes. Of course, these two categories of high-risk patients should exclude those for whom there is evidence of past depression episodes or susceptibility to depression. Whether it will ever be possible to develop proper treatment algorithms to make sure that the right patient is treated with rimonabant is uncertain at this stage. However, the discovery of the ECS and of its profound impact on body fat distribution, ectopic fat deposition and carbohydrate and lipid metabolism has been a remarkable breakthrough. It is hoped that this body of knowledge will be properly used to treat the right patient with the right drug.

**References**


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