Review

Effects of CB1 antagonist on the control of metabolic functions in obese type 2 diabetic patients

M. Lafontana\textsuperscript{a}, P.V. Piazza\textsuperscript{b,*,} J. Girard\textsuperscript{c}

\textsuperscript{a}Institut Louis-Bugnard IFR31, institut de médecine moléculaire de Rangueil-I2MR, Inserm-UPS U858, BP 84225, 31432 Toulouse cedex 04, France
\textsuperscript{b}Inserm Bordeaux Neuroscience Research Center (U862), University of Bordeaux-II, 146, rue Leo-Saignat, 33077 Bordeaux, France
\textsuperscript{c}Département endocrinologie, métabolisme et cancer, institut Cochin Inserr U567 CNRS UMR8104, université René-Descartes, 24, rue du Faubourg Saint-Jacques, 75014 Paris, France

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Abstract

Clinical reports (RIO trials) have shown that chronic administration of a CB-cannabinoid receptor antagonist (rimonabant) provides improvements of disturbed metabolic parameters observed in overweight and obese patients with type 2 diabetes. The production of endocannabinoid and the expression of CB1-cannabinoid receptors are largely distributed in the different organs aside from the brain. It is now clearly established that endocannabinoids act both through orexigenic effects and peripheral metabolic effects in various tissues involved in the control of metabolism and energy expenditure (i.e. adipose tissue, liver, gastrointestinal tract, skeletal muscle and pancreas). This review will consider: i) the disturbances of glucose and lipid metabolisms in obese type 2 diabetics; ii) an overview of the pharmacological properties of rimonabant and iii) the various mechanisms involved in tissues and organs to explain the therapeutic efficacy of rimonabant. A special attention will be paid to its utilization in obese type 2 diabetics. The emerging concept of endocannabinoids acting as metabolic regulators is the more likely explanation of the success of rimonabant treatments in phase III studies.

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Résumé

Effets d’un antagoniste des récepteurs CB1 sur les fonctions métaboliques des patients obèses diabétiques de type 2.

Diverses études cliniques (Études RIO) ont montré que l’administration d’un antagoniste des récepteurs des cannabinoïdes (rimonabant) améliorait divers paramètres métaboliques défavorables rencontrés chez des patients en surpoids ou des patients obèses atteints de diabète de type 2. En dehors du système nerveux, la production d’endocannabinoïdes et l’expression des récepteurs CB1 des cannabinoïdes ont été décrites dans de nombreux organes et tissus. Il est maintenant bien établi que les endocannabinoïdes agissaient à la fois par une action sur la prise alimentaire et plusieurs autres actions métaboliques dans les tissus impliqués dans le contrôle du métabolisme et de la dépense énergétique (i.e. tissu adipeux, foie, tractus gastro-intestinal, muscle squelettique et pancréas). Cette revue qui commence par une synthèse des perturbations du métabolisme glucidique et lipidique rencontrées chez les diabétiques de type 2, abordera les propriétés pharmacologiques du rimonabant et ses divers mécanismes d’action dans les tissus et organes qui sont susceptibles d’expliquer son efficacité thérapeutique. Une attention toute particulière sera dévolue à son utilisation chez l’obèse avec un diabète de type 2. Le concept émergent d’une action des endocannabinoïdes comme régulateurs métaboliques permet probablement d’expliquer le succès d’un traitement par le rimonabant dans les études de phase III rapportées à ce jour.

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* Corresponding author.
E-mail address: piazza@bordeaux.inserm.fr (P.V. Piazza).

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1. Introduction

The clinical importance of the endocannabinoid system in the control of energy status and metabolic equilibrium is supported by a number of clinical reports (RIO trials) showing that chronic administration of a CB-cannabinoid receptor antagonist (rimonabant) provides improvements of disturbed metabolic parameters observed in overweight and obese patients with type 2 diabetes (T2D) [1–5]. When compared with currently marketed antiobesity drugs, the weight loss (7.4 kg) observed after a 2-year administration of rimonabant (20 mg/day) in the RIO-North America Study is significant and there was no weight regain during the second year of treatment. An increase in plasma high-density lipoprotein (HDL)-C and a decrease in plasma triglyceride (TG) levels were also observed with rimonabant 20 mg/day relative to placebo. When administered to type 2 diabetic patients poorly controlled by metformin and/or sulfonylureas, in combination with diet and exercise, rimonabant promotes a reduction in body weight and in HbA1c levels, and an improvement of other cardiovascular and metabolic risk factors. A very important aspect of rimonabant action is that 50% of its metabolic effects are independent from the weight lost. This observation, combined with relevant preclinical data that will be summarized below, indicates that CB1 blockers can improve cardiovascular risk factors by a direct metabolic action.

The production of endocannabinoids and the expression of CB1-cannabinoid receptors are largely distributed in the different organs out off the brain. It is now clearly established that endocannabinoids act both through orexigenic effects and peripheral metabolic effects in various tissues involved in the control of metabolism and energy expenditure (i.e. adipose tissue, liver, gastrointestinal tract, skeletal muscle, endothelial cells and pancreas) (Fig. 1). The first results revealing the impact of cannabinoids on energy homeostasis and weight control originate from investigations performed on chronic marijuana smokers under clinically-controlled conditions. Consumption of marijuana, in addition to its behavioral effects, mediated by central mechanisms, and assessed by a transient and positive impact on calorie intake, was postulated to influence energy metabolism in the long-term. A continuous weight gain of the patients involved in the protocol was observed, although appetite stimulation and caloric intake leveled rapidly [6]. The existence of independent effects between the cannabinoid control of appetite and the peripheral actions controlling the energy status was postulated. More recently, experiments in rats fed a standard high carbohydrate diet [7] or in mice submitted to an obesity-promoting high fat diet [8] and treated chronically with the selective CB1-receptor antagonist, rimonabant, also supported the idea that factors other than food intake regulation were involved in the weight-reducing effects of CB1 antagonists. In fact, the metabolic action of rimonabant does not entirely result from weight loss and 50% of effects independent of weight loss result from changes in metabolism. The reducing effect on food intake was limited to the first week of administration of rimonabant while decrease in body weight was maintained over the entire period of treatment (5 weeks) [8]. The effect of chronic treatment with rimonabant was also studied in mice with established obesity (5-month high fat diet). The impact on food intake was transient whilst a sustained decrease in body weight associated with improvements in serum biochemical parameters and lipid profiles (i.e. decrease in leptin, insulin, glucose, TGs and low-density lipoprotein (LDL)-cholesterol levels) was observed [9]. The administration of another CB1 antagonist (AM-251) to diet-induced obese mice, has now confirmed all the results obtained with rimonabant [10]. Therefore, despite the waning of the anorectic effects, rimonabant, leads to a significant and sustained reduction in body weight that was not explained by food-intake regulation only. The lack of CB1-receptor in mutant KO mice (CB1–/– mice) causes hypophagia and body fat reduction as well as resistance to high fat diet. Pair-fed older wild-type mice are heavier than the CB1–/– mice. These results suggest that putative peripheral metabolic factors may be the cause of the lean phenotype [11,12]. Moreover, studies in obese rodents and obese patients suggest that endocannabinoid formation is enhanced in obesity, perhaps because endocannabinoid degradation is decreased [5]. CB1 receptor

![Fig. 1. Stimulation of cannabinoid CB-1 receptors by endocannabinoids or synthetic CB-1 agonists promotes various metabolic disturbances. Overfeeding induces an activation of central and peripheral CB-1 receptors followed by metabolic disorders summarized in the diagram.](image-url)
mediated effects in the major tissues involved in the regulation of metabolism are summarized in Fig. 1.

This review will be organized as follows: i) disturbances of glucose and lipid metabolisms in obese type 2 diabetics; ii) overview of the pharmacological properties of rimonabant and iii) the mechanisms of the therapeutic efficacy of rimonabant with a special attention to its utilization in obese type 2 diabetics.

2. Disturbances of glucose and lipid metabolisms in obese type 2 diabetics

T2D is associated with a two to four increased risk of cardiovascular disease [13]. Much of this increased risk derives from a typical dyslipidemia that is part of insulin resistance and that is exacerbated by insulin deficiency (development of overt hyperglycemia).

2.1. Dysregulation of glucose metabolism in T2Ds

Obese patients with T2D are characterized by two major anomalies: 1- a decrease in the effects of insulin on target tissues: skeletal muscle and liver (insulin resistance) and 2- a decrease in quantitative and qualitative (early phase and pulsatility) insulin secretion (Fig. 2). T2D is also associated with an increased glucagon secretion. Insulin resistance is associated with an increase in glucose production by the liver and a decrease in insulin-stimulated glucose uptake by skeletal muscle and adipose tissue. Insulin resistance results from a defect in insulin signaling secondary to an abnormality of adipose tissue metabolism. The transition from a pre-diabetic state to T2D is characterized by three major changes. The first one is a decrease in the functional mass of \( \beta \)-cells which does not allow an increased release of insulin to compensate for insulin resistance. We do not know if this loss of the functional mass of \( \beta \)-cells is genetically determined or if it is acquired (glucolipotoxicity). This transition is crucial for the development of T2D. The second one is an increased glucose production by the liver, probably secondary to the excessive secretion of glucagon and to an enhanced release of free fatty acids (FFA) by adipose tissue, in particular by visceral adipose tissue. The third one is a decrease in insulin-stimulated glucose uptake by skeletal muscles, largely linked to the presence of obesity and to an enhanced release of FFA and an abnormal release of adipokines by adipose tissue. In particular, a decreased secretion of adipokines with insulin-like properties (adiponectin), and an increased secretion in adipokines with anti-insulin properties (TNF-alpha, IL-6 and resistin) is observed. These anomalies have been largely described in different recent reviews and will not be developed in the present paper [14–19].

2.2. Dyslipidemia in T2Ds

Dyslipidemia of T2D is characterized by several qualitative and quantitative lipoprotein abnormalities [20]. The most common cluster of lipid abnormalities is the combination of both fasting and postprandial high TG levels, low HDL-cholesterol levels, and an increase in small dense LDL particles.

2.2.1. Role of insulin resistance in the overproduction of very low-density lipoproteins (VLDL)

Overproduction of VLDL is a central feature of the diabetic dyslipidemia and this accounts for much of the low HDL and abnormal LDL levels. As indicated in Fig. 3, T2D and insulin resistance are associated with an increase in the three main sources of TG for VLDL assembly: 1- FFA flux from adipose tissue to the liver [21], 2- hepatic uptake of VLDL and chylomicron remnants [22], and 3- de novo lipogenesis from glucose [23–26]. In obese hyperinsulinemic ob/ob mice, hepatic lipogenesis is increased despite resistance to insulin action on hepatic glucose metabolism [27]. Hepatic lipogenesis is tightly controlled by the sterol response element-binding protein 1c (SREBP-1c) [28] and the carbohydrate response element-binding protein (ChREBP) [29,30]. SREBP-1c gene expression is stimulated by insulin whereas ChREBP is increased in response to an enhanced hepatic glucose metabolism. The elevated level of SREBP1c [27] and ChREBP levels [31] observed in the liver of insulin resistant rodents are responsible for the over expression of key lipogenic enzymes and of the high lipogenesis rate (Fig. 4). However, it seems that liver resistance to the inhibition of VLDL production by insulin
diabetics have a reduced catabolism of VLDL and IDL [20], increase in VLDL assembly in the liver. In addition, type 2 diabetes results in decreased activity of LPL. As insulin is an activator of LPL, the reduced LPL activity in T2D is secondary to insulin resistance or deficiency [32-34].

2.2.2. Role of insulin resistance in the postprandial chylomicron metabolism

Following digestion of dietary nutrients, the small intestine absorbs fatty acids (FA) and cholesterol from the diet and incorporates them, as TG, into chylomicrons which are secreted into the lymph. ApoB48 and microsomal TG transfer protein (MTP) are required for the assembly and secretion of chylomicrons by the small intestine (for a review see [22]). Increased ApoB48 secretion has been demonstrated in insulin resistant states. As they travel through the lymph and subsequently enter the circulation by way of the thoracic duct, chylomicrons acquire other apolipoproteins, in particular apoC-II, a required activator of lipoprotein lipase (LPL). This enzyme hydrolyzes chylomicrons TG and allows the uptake of FA by adipose tissue before FA can be reincorporated into TG for storage in the adipocyte.

Chylomicron and chylomicron-remnant metabolism are significantly altered in type 2 diabetics [35]. The association of apoB48 with dietary lipids to form chylomicrons is increased and the clearance of TG is modestly decreased since LPL is decreased in T2D [36]. Additionally there is an increase in secretion of VLDL that competes with chylomicrons for LPL-mediated TG hydrolysis. Furthermore, in insulin resistant states there is also an increase in apoCIII, which inhibit LPL. Hepatic lipase which hydrolyzes remnants is increased in insulin resistant states contributing to low HDL levels.

Postprandial hyperlipidemia is common in insulin resistant individuals because of the reduction of LPL activity which although modest is sufficient to reduce the clearance of chylomicrons and VLDL. This is associated with an increased hepatic uptake of chylomicrons and VLDL remnant enriched in TG in insulin resistant individuals. The uptake of TG-enriched remnants stimulates VLDL assembly and secretion.

2.2.3. Role of insulin resistance in the generation of small dense LDL

Plasma LDL levels are generally normal in T2D but there are important modifications of their metabolism. They have a reduced turnover associated with a decrease of their catabolism. LDL enriched in TG and depleted in cholesterol ester (CE) are present in plasma in insulin resistant states. This is largely derived from the increase of cholesteryl ester transfer protein (CETP) activity in response to high TG-rich lipoproteins. This enzyme is associated with plasma lipoproteins and mediates the exchange of the TG of VLDL or chylomicrons for the CE of HDL, thereby creating a TG-enriched CE-depleted LDL particle. High levels of plasma FFA stimulate the exchange of CE and TG between LDL and VLDL. The TG in LDL are then hydrolyzed by LPL or hepatic lipase, generating the small dense LDL. The increase in hepatic lipase in insulin resistant state can therefore hydrolyze more efficiently TG in HDL than in VLDL or chylomicrons. The small dense LDL has reduced affinity for LDL receptor and has an increased susceptibility to oxidation. Oxidized LDL are rapidly taken up by macrophages leading to foam cell formation. Increased glycation of LDL (apoB) in T2D is associated with an increased oxidation and a reduction of their uptake via the LDL receptor.

2.2.4. Role of insulin resistance in the generation of low levels of HDL cholesterol

The low levels of HDL cholesterol in T2D are due to an increased catabolism of HDL particles. One of the main reasons for increased catabolism of HDL is the increased pool of VLDL which drives through the CETP the transfer of TG to HDL leading to the formation of LDL particles rich in TG which becomes a very good substrate for hepatic lipase. An increased activity of hepatic lipase has been observed in T2D and is due to the insulin resistant state. In addition, HDL is glycated in T2D and there is a correlation between plasma glucose levels and glycation of ApoA1. ApoA1 glycation reduces HDL binding to its receptors and thus impairs HDL-mediated cholesterol efflux and reverse cholesterol transport.

2.2.5. Adipokines and dyslipidemia

Obesity and T2D are associated with low plasma adiponectin concentrations [37,38] and plasma adiponectin has been shown to be negatively correlated with TG concentrations and positively correlated with HDL levels [39,40]. Recently plasma adiponectin has been shown to be positively correlated with VLDL ApoB catabolism, independently of insulin sensitivity [41]. This suggests a possible action of adiponectin on lipid metabolism independent of its effect on insulin sensitivity. Adiponectin may decrease plasma TG by enhancing FFA oxidation [42] or by stimulation of LPL [43] via an increased expression of PPAR-alpha in the liver and adipose tissue [42]. In contrast, plasma resistin and TNF-alpha are increased in T2D but no correlation was found with TG-rich lipoprotein metabolism (for a review see [20]).
3. Pharmacological properties of rimonabant

Rimonabant (SR141716), (N-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide) belongs to the family of 1.5-diarylpyrazoles. It is a potent and selective antagonist of CB1-cannabinoid receptors which prevents the occurrence of different in vitro and in vivo CB1-receptor mediated events [44]. In vitro, rimonabant displayed nanomolar affinity for the rodent and human CB1-receptor while metabolites of rimonabant have no affinity for CB1-receptors. In vivo, rimonabant interacted with the brain mouse CB1-receptors. Rimonabant has no affinity for any other receptors and channels investigated in vitro to date. Rimonabant antagonized all the classical behavioral effects induced by cannabinoid receptor agonists (WIN 55212-2 and Δ9-tetrahydrocannabinol-Δ9-THC). Rimonabant induced a withdrawal syndrome in rats which were treated with Δ9-THC.

Pharmacokinetics of rimonabant was characterized by a large volume of distribution. It is rapidly absorbed with $C_{\text{max}}$ generally observed 1–3 hours after administration. Over the dose range investigated (10–60 mg), plasma $C_{\text{max}}$ values ranged from 50 to 300 ng/ml. The apparent half-life of rimonabant in plasma generally ranged from 6 to 10 days when measured following a single or 21 daily administrations in healthy normal weight subjects. Following administration, less than 5% of the dose was excreted in the feces suggesting the primary clearance pathways are either metabolic or biliary. The repeated dose pharmacokinetics of rimonabant was similar between females and males and age has no major effect on the pharmacokinetics of rimonabant. The time for steady state attainment after once-daily doses was shorter in Japanese (median = 7 days) than in the Caucasians (median = 13 days). Obese subjects with BMI > 30 kg/m² exhibited a longer terminal half-life (mean = 16 days) compared with healthy subjects with BMI = 18–28 kg/m² (mean 6–9 days). Based on in vitro studies, the primary pathway of metabolism is de-esterification. Oxidative metabolism is primarily mediated by CYP3A4. Inhibitors of CYP3A4 have minimal effects on the pharmacokinetics of rimonabant. Drug interactions with rimonabant due to induction or inhibition of cytochrome P450 enzymes are not likely and rimonabant had no effect on the steady state pharmacokinetics of digoxin or oral contraceptives.

4. Mechanisms explaining the therapeutic efficacy of rimonabant

Rimonabant suppresses CB1-receptor mediated effects in a number of target organs involved in the control of lipid and energy storage, for example in adipose tissue and liver. Rimonabant also suppresses CB1-mediated effects in the gastrointestinal tract, skeletal muscle and pancreatic β-cells. The mechanisms by which rimonabant exerted its metabolic effects have been mainly explored in rodents. Studies performed in human tissues are scarce.

4.1. Effect of rimonabant on lipid storage and adipocyte secretions

The adipose tissue is one of the peripheral targets of rimonabant. The effect of the drug on this tissue can be explained by two major mechanisms: 1) a reduction of effect of endocannabinoids secreted locally by the adipocytes on lipogenesis (conversion of glucose into FA) and TG storage and 2) the enhancement of the release of a major insulin-sensitizing hormone of the adipocyte, adiponectin, the release of which is normally inhibited by endocannabinoids.

CB1-receptors and the enzyme involved in endocannabinoid degradation, the fatty acid amide hydrolase (FAAH) have been identified in human adipocytes [45–47]. Weakly expressed in fat cell precursors, CB1-receptors and FAAH (mRNAs) are expressed at a high level in mature adipocytes [45]. This point requires further validations with CB1-receptor protein determinations. Gene expression analysis in paired samples of visceral and subcutaneous adipose tissue of obese and non-obese patients revealed that mRNA expression of both CB1-receptors and FAAH was higher in visceral than in subcutaneous fat depots. No sex-related differences were detected but the expression of both genes was higher in adipose tissue of lean compared with adipose tissue of obese subjects. Increased levels of 2-AG were associated with higher CB1-receptor expression in the visceral adipose tissue of obese subjects. Although the mechanisms regulating CB1-receptor expression in human and rodent adipocytes are unknown, these observations suggest that CB1-receptors are not down-regulated by the excess of the endocannabinoids in the visceral fat deposit. This is surprising since generally receptor expression is down-regulated by an excess of endogenous ligands. This aspect is discussed in the companion paper by Piazza et al. This suggests that endocannabinoid production is regulated by a feed-forward mechanism. Expression of CB1-receptors and FAAH (mRNA) was decreased after calorie restriction and weight loss in subcutaneous adipose tissue of obese patients. However, these parameters were not modified by calorie restriction in control non-obese subjects [45]. In conclusion, visceral fat accumulation is an important correlate of an activated endocannabinoid system and the possible over-activity of CB1-dependent pathways in visceral fat will certainly explain the beneficial effect of the CB1 antagonist (rimonabant) on visceral adipose tissue. Some precautions should be taken for interpreting the present results since gene expression (mRNA) data and correlation analysis must be confirmed by analysis of protein concentrations.

Systemic endocannabinoid levels, anandamide (AEA) and 2-arachidonoylglycerol (2-AG), are increased in postmenopausal women with uncomplicated obesity and levels of 2-AG and AEA are also increased in T2D [47]. Another study has revealed that systemic 2-AG is increased in obese subjects and a significant correlation was found between circulating 2-AG and visceral fat extent [48]. Fat depot-specific differences in the levels of 2-AG have been observed. Higher levels of 2-AG were found in the visceral, but not subcutaneous, adipose tissue of obese male subjects [47]. Higher systemic levels of
endocannabinoids are associated with decreased CB1-receptor and FAAH gene expression in subcutaneous adipose tissue [45]. Reduced FAAH gene expression in the adipose tissue of obese women, if confirmed by studies on FAAH activity determinations, suggests that increment of systemic endocannabinoid levels could be a consequence of FAAH hypoactivity. It is necessary to clarify the part played by adipose tissue FAAH in systemic cannabinoid inactivation. Investigations of the relationship between a FAAH cDNA polymorphism (385 A/A [P129T]) and obesity-related disorders have revealed that the homozygous FAAH 385 A/A genotype group of patients (compared with heterozygote and wild-type groups) was significantly associated with overweight and obesity. Existence of FAAH missense polymorphism may be a risk factor in overweight/obesity-related to endocannabinoids. Such a result could be considered as an indirect evidence for the role of the endocannabinoids in overweight/obesity [49]. Correlations studies between anthropometric and metabolic parameters with the peripheral cannabinoid system revealed percent body fat and circulating 2-AG levels, as determinants of visceral CB1-receptor gene expression and only circulating 2-AG as a predictor of visceral fat FAAH gene expression [48].

4.1.1. Rimonabant and TG storage in adipose tissue

The CB1-receptor agonist HU-210 promotes adipocyte differentiation and stimulates lipogenesis [47], both effects being blocked by rimonabant. Detailed mechanisms of action were explored in rodents. In mice fed a diet inducing obesity, oral treatment with rimonabant at 10 mg/kg per day for 40 days induced a significant weight reduction, leading to morphological changes in white adipose tissue similar to the one of lean animals. Increased expression of genes involved in FA oxidation, glucose metabolism and energy expenditure was observed in adipose tissue. It must be noted that the major alterations in gene expression levels related to obesity in white and brown adipose tissues were mostly reversed in rimonabant-treated obese mice. Changes induced by rimonabant were accompanied by a significant adipose tissue remodeling with a potent depletion of adipocyte fat content [50]. Moreover the treatment also contributed to a limitation of the inflammatory conditions associated with obesity (i.e. reduced expression of acute-phase genes and gene products expressed in macrophages) commonly observed in such animals [51].

In cultured adipocytes from C57Bl6N mice, a CB1-agonist (WIN-55,212) increased the activity of LPL (heparin-releasable LPL). The effect was suppressed by rimonabant treatment [11]. This suggests that blockade of CB1-receptors with rimonabant could limit LPL activity and reduce both plasma TG hydrolysis and FFA uptake by the adipocyte and the concomitant esterification of FFA to TGs. In agreement with this proposal is the significant correlation existing between CB1-receptor expression in visceral adipose tissue and the sterol regulatory element-binding protein-1c (SREBP-1c) mRNA levels, a transcription factor involved in the expression of genes (FAS, ACC) controlling the lipogenic pathway [48]. Further investigations are required to define the rate of TG synthesis and to establish the physiological role of endocannabinoids and the mechanisms of CB1-receptor-stimulated lipogenesis in human adipocytes.

The stimulation of CB1-receptors in adipocytes inhibits AMP-activated protein kinase (AMPK) [52]. As, the inhibition of AMPK has been shown to stimulate lipogenesis and to inhibit FA oxidation, the overall effect of stimulation of CB1-receptors in adipocytes is to favor TG deposit. In contrast, the activation of AMPK inhibits lipogenesis, stimulates FA oxidation while the effects of AMPK on hormone-sensitive lipase and lipolysis activation remain controversial [53–55]. Thus, the blockade of CB1-receptors by rimonabant treatment should promote activation of AMPK. The suppression of cannabinoid-dependent inhibitory effects on AMPK by rimonabant should inhibit lipogenesis. Obviously, these results must be confirmed in human adipocytes.

In mice brown fat cells differentiated in vitro, CB1-receptor stimulation inhibits expression of the protein involved in the control of thermogenesis, UCP-1 [56]. Blockade of such effects, by rimonabant, although interesting in rodents, could be of limited interest for clinical purposes since the role of brown fat is a matter of debate in humans. Using whole body calorimetry and oxygen consumption at thermoneutral temperature in ob/ob mice, it was demonstrated that chronic treatment with rimonabant increased thermogenesis [57]. The tissue responsible for the thermogenic effect was not identified and the putative incidence of the chronic treatment on the expression of uncoupling proteins was not explored.

4.1.2. Rimonabant, CB1-receptors and adipocyte secretions

In addition to direct CB1-dependent effects on lipogenesis, stimulation of CB1-receptors in adipocytes could also affect lipid metabolism through the release of adipocyte cytokines. Two major adipocyte proteins (i.e. leptin and adiponectin) are known to exert a number of metabolic effects in various target tissues (mainly skeletal muscle and liver). Although the role of adiponectin is not yet totally elucidated, adiponectin possesses both insulin-sensitizing and anti-inflammatory effects [58]. Clinical studies have revealed the strong relationship existing between the appearance of an insulin resistant state and a decrease in plasma adiponectin levels [59,60]. Adiponectin levels are more closely related to visceral adipose tissue than total adiposity [61,62]. Increment of plasma adiponectin exerts insulin-sensitizing effects in skeletal muscle and liver and counteracts insulin resistance. Adiponectin is specifically secreted by adipocytes and different complexes built up from a low molecular weight (LMW) trimer form, to mild molecular weight (MMW) hexamers consisting of two trimers to high molecular weight (HMW) forms consisting of up to 18 molecules. All three isoforms are present in serum [63]. Recent studies suggest that the HMW adiponectin is a better biomarker of insulin resistance than is the commonly used measure of total adiponectin. Glucose tolerance is better positively correlated with the levels of HMW complexes in serum than with the total adiponectin [64]. It is becoming obvious that the determination of the various isoforms of circulating adiponectin will
be necessary to optimum interpretation of clinical studies. In addition to circulating adiponectin determinations, delineation of the expression level of adiponectin receptors in target tissues will also become useful [65]. The ubiquitously expressed adiponectin receptor, AdipoR1, possesses high affinity for the globular form of adiponectin whereas AdipoR2 expressed in skeletal muscle and liver exhibits intermediate affinity for the globular and the full length adiponectin.

Stimulation of CB1-receptors by HU210, a pharmacological agonist, or by the endocannabinoids secreted by the adipocyte, reduced adiponectin expression (mRNA levels) in 3T3-F442A adipocytes while CB1-receptor blockade with rimonabant increased the expression of adiponectin and its secretion in the culture medium [47,66,67]. If occurring in obese humans who have an increased production of 2-AG in adipose tissue, CB1-mediated inhibition of adiponectin release could contribute to the initiation of the insulin resistance observed in the obese.

Acute and chronic administrations of rimonabant promoted adiponectin expression in adipose tissue of Zucker rat and mice. The increased expression in adipose tissue is followed by an increase of circulating adiponectin [66,67]. Unfortunately, local adipose tissue levels of endocannabinoids were not determined in these studies. Chronic treatment with rimonabant in mice with established obesity (5-month high fat diet) modestly, but significantly, (18%) increased plasma adiponectin levels. It is difficult to establish if this increase of plasma adiponectin is responsible for the improvement in serum biochemical parameters and lipid profiles (e.g. reduction of plasma insulin and glucose levels, increase in the HDLc/LDLc ratio) [9]. Additional studies are needed to determine if CB1-dependent changes in adiponectin are responsible for the observed metabolic effects.

A weight loss-independent increment of plasma adiponectin has been reported in patients treated with rimonabant; it could be explained by an action at the adipocyte level [2]. Nevertheless, there was no correlation between circulating 2-AG levels and adiponectin levels in the 60 patients studied by Bluher’s group [48]. Additional studies are required to determine if changes in adiponectin are truly related to endocannabinoid-dependent effects in human visceral adipocytes. Adiponectin predicts glucose tolerance and plasma HDL-C levels in a manner which is partly independent from the contribution of visceral adiposity [62]. Recently, a CB1-receptor agonist (WIN 55,212-2) has been reported to stimulate visfatin, expression and to inhibit adiponectin expression in murine adipocytes differentiated in vitro [56]. Visfatin (also known as pro-B cell colony-enhancing factor) is a recently identified adipokine possessing insulino-mimetic properties and linked to several inflammatory disease states which has been linked to adiposity and the metabolic syndrome [68]. These results merit further investigations and additional studies are required to determine if this observation could have some relevance in humans.

4.2. Effect of rimonabant on liver function

CB1-receptors have been identified in mouse liver. Higher CB1-receptor levels were detected in Kupffer cells and lower levels in endothelial cells as well as in hepatocytes, particularly in the perivascular area [69]. Increased CB1-receptor level was observed in liver from mice kept on a high fat diet. Conversely, CB1-receptors are weakly expressed in normal liver in humans and they are essentially located along sinusoidal walls in normal liver. However, a marked increase in CB1-receptor expression was observed in liver diseases such as cirrhosis. They were distributed in non-parenchymal cells distributed along the fibrotic septa in spindle-shaped cells, inflammatory cells and ductular proliferating cells. Liver fibrogenic cells within fibrotic septa is the prominent cell type expressing CB1-receptors in human liver [70]. Liver fibrogenic cells in their myofibroblast phenotype and hepatic myofibroblasts as well as fully activated hepatic stellate cells expressed CB1-receptors in humans and mice [70,71].

Rimonabant pre-treatment counteracted CB1-mediated increase in lipogenesis in rodent liver. Acute in vivo activation of CB1-receptors with the CB1-agonist HU210, in wild-type mice fed a regular chow, increases the expression of lipogenic genes including the lipogenic transcription factor SREBP-1c and the enzymes involved in FA synthesis such as fatty acid synthase (FAS) and acetyl-CoA carboxylase-1 (ACC-1). The effect is not observed in CB1−/− mice; it is a CB1-receptor mediated effect. De novo FA synthase (lipogenesis) was observed in the liver of C57Bl/6J mice after CB1-agonist administration; it is not observed in CB1−/− mice. Wild-type (CB1+/+) mice on high fat diet become obese and develop a fatty liver while CB1−/− mice, although having a similar caloric intake, remained unaffected by the high fat diet. The basal rate of lipogenesis is markedly increased in mice on the high fat diet (3 weeks following the beginning of the diet), before appearance of a marked weight gain; this increment is suppressed by a pre-treatment with rimonabant. At the same time, hepatic levels of AEA were greatly elevated in animals on the high fat diet with no difference in hepatic levels of 2-AG. Although to a lesser extent AEA levels also increased in CB1−/− mice. Higher levels of AEA could be explained by a dramatic reduction of FAAH activity and the stable activity of the enzyme involved in AEA synthesis (i.e. N-acetyl transferase-NAT) [69].

Taken together, the results of these experiments in mice indicate that obesity induced by feeding a high fat diet is leading to the induction of liver AEA production (activation of the endocannabinoid system) which stimulates lipogenesis, via CB1-receptor. In vivo, hepatic CB1-receptor stimulation contributes to the activation of lipogenic pathways while CB1-blockade with rimonabant suppresses the effect. The excess production of AEA initiated by the high fat diet seems to be essentially under the control of FAAH since NAT activity was unaffected. One of the common complications of obesity is the appearance of non-alcoholic steatohepatitis (NASH) and the occurrence of a dyslipidemia characterized by an increase in serum TGs and a decrease in HDL-C levels. Confirmation of the above results obtained in mice is required in humans. If confirmed, endocannabinoids produced consecutively to a high fat diet and CB1-receptors may play an important role in
the initiation of hepatic lipid disorders. Rimonabant will be useful to limit fat accumulation in liver. Improvements in TG and HDL-C described in rimonabant-treated patients are explainable by this action.

In addition to the results obtained in mice on lipid metabolism, liver CB1-receptors benefit of a larger interest since they are probably involved in other liver diseases. A profibrogenic role of CB1-receptors was suspected from results obtained in CB1−/− mice which exhibit reduced susceptibility to fibrosis. A recent study has revealed another important role of liver CB1-receptors. CB1-receptors are up-regulated in the liver of cirrhotic individuals and expressed in liver fibrogenic cells. Treatment with a CB1-receptor antagonist reduces matrix remodeling currently associated with acute liver injury and decreases the fibrogenic response associated with chronic liver injury [70]. When comparing the results of this study and the previous one, it is shown that CB1-receptor antagonists could also be, in addition to the prevention of lipid infiltration, promising agents for the treatment of liver fibrosis which could appear after a NASH in some obese and type 2 diabetic patients.

4.3. Impact of rimonabant on gastrointestinal function

Endocannabinoids distribution along the gastrointestinal tract is complex. Their levels are apparently modulated by the nutritional status. AEA levels are increased in the small intestine of rats in response to food deprivation and decreased after refeeding. Systemic blockade of the endocannabinoid system with rimonabant is promoting reduction of food intake in rats deprived of food for 24 hours and in rats partially satiated [72]. Endocannabinoids also appear to be involved in modulating the orexigenic effect of ghrelin [73]. The inhibition of gastrointestinal motility and secretion is mediated by CB1-receptors located on the terminals of both intrinsic and extrinsic submucosal neurons [74]. CB1-receptors are also expressed by cholinergic neurons controlling intestinal motility and in some sensory terminals of vagal and spinal neurons. Administration of natural cannabinoid agonists is known to reduce gastric emptying and intestinal motility in humans and rodents. The effects are mimicked by a CB1-agonist. Rimonabant blocks the effects of CB1-agonists on intestinal motility [75]. The gastrointestinal effects of rimonabant are explainable by the existence of an endocannabinoid tonus in gastrointestinal tract. The clinical impact of the endocannabinoid system in the gastrointestinal tract on nutrient absorption and metabolic regulations remains largely unexplored. From current knowledge of rimonabant effect on ghrelin and gastrointestinal tract motility, rimonabant treatment could interfere with nutrient absorption.

When administered to mice with chemically induced enteritis, cannabinoids reduce inflammation and fluid accumulation in the gut [76,77]. Increased levels of AEA and 2-AG as well as increased expression of CB1-receptors have been detected in the inflamed gut. Endocannabinoids inhibit leukocyte infiltration promoted by the chemical treatment. Since AEA acts not only through CB1-receptors, the nature of the effect remains to be elucidated. However, CB1-receptors are probably required for protection from inflammation since stronger chemically-induced inflammatory responses was described in mice lacking CB1-receptors [78]. Treatment of mice with rimonabant mimicked the inflammatory phenotype of CB1−/− mice. Cannabinoids have been shown to suppress TNF-alpha release from macrophages and mast cells [79]. In another model of gastrointestinal tract disease which is indomethacin-induced ulcer formation, rimonabant prevented ulcer formation and inhibited the LPS-induced increase in TNF-alpha levels [80]. If confirmed in humans, these results suggest that modulation of the physiological activity of the endogenous cannabinoid system and CB1-receptors function could interfere with inflammatory gastrointestinal tract and attention must be paid in patients at risk.

4.4. Effect of rimonabant on skeletal muscle

Skeletal muscle plays a major role in metabolic regulation. The insulin sensitivity of skeletal muscle is critical in the regulation of glycemic control. Chronic treatment of ob/ob mice with rimonabant stimulated energy expenditure and improved glucose uptake by the isolated soleus muscle [57]. An effect of rimonabant on efferent sympathetic activity from hypothalamic areas and uncoupling proteins expression in brown adipose tissue cannot be excluded to explain the thermogenic effect. This chronic treatment with rimonabant also improves insulin-induced glucose uptake in the isolated soleus muscle. Cannabinoid CB1-receptor mRNA is expressed in the skeletal muscle of mice, its expression is higher in the high-fat induced obese mouse [12]. However, it is not known if the effect of chronic treatment with rimonabant results from a direct effect of rimonabant on skeletal muscle CB1-receptors or an indirect effect due to the increase in adiponectin secretion (see Section 3.1.2). Additional studies are also required to determine if this observation could have some relevance in humans.

4.5. Effect of rimonabant on pancreatic β-cells

CB1- and CB2-receptors (mRNAs and Western immunoblots) as well as enzymes for endocannabinoid biosynthesis and metabolism are expressed in islets of Langerhans of mice and rats and in rat insulinoma RIN-m5F β-cells, a commonly used model of pancreatic β-cells. CB1-receptors are more abundant in alpha-cells (glucagon-secreting cells) than in β-cells, but they were equally distributed in islet β-cells. In β-cells cultured at high glucose concentration (25 mM for 24 hours), the stimulation of cannabinoid receptors by HU-210 enhanced insulin release. This effect was suppressed by rimonabant but not by a CB2-antagonist. It should be noticed that high concentrations of glucose increased the levels of AEA and 2-AG in β-cells. Whatever the interest of these preliminary results in rodent and rodent cell lines, further studies will be...
necessary to establish the impact of CB1-receptor antagonists on human pancreatic β-cells under physiological conditions.

5. Conclusions

Rimonabant is the first of a new class of selective cannabinoid receptor-1 (CB-1) antagonists used for the treatment of obesity and metabolic disorders. In several randomized, double-blind clinical trials in overweight or obese adults with or without T2D, orally administered rimonabant, once-daily, improved lipid and glucose metabolism parameters and regulated food intake and energy balance. A significantly greater proportion of rimonabant recipients achieved a clinically significant weight loss target of > 5% or > 10% of initial weight than placebo-treated patients. Extent of weight and waist circumference reduction was significantly greater in rimonabant-treated than in placebo. Rimonabant was also associated with improvements in glycemic control and reduction in glycated hemoglobin, an effect partly independent of the effects of weight loss. Improvements in other cardiometabolic risk factors (i.e. increases in HDL-C and decreases in TG levels) were significantly greater with rimonabant than with placebo. Exploration of mechanisms of action were performed in animal models and expanded towards patients whenever possible. Antagonism of cannabinoid CB-1 receptors reduces food intake only acutely. However, the long-term effects on metabolic regulation and weight reduction appear to be mediated by peripheral effects related to adipose tissue, liver, skeletal muscle and pancreas physiology. For example, in adipose tissue, antagonism of CB-1 receptors increases secretion of adiponectin and decrease LPL activity, whilst in the liver, it reduces the expression of lipogenic enzymes and FA synthesis. Many questions presently remain unanswered concerning mechanistic aspects. Additional studies are also required to determine if the results obtained in cell systems and rodent models have the same relevance in humans. Nevertheless, the emerging concept of endocannabinoids acting as metabolic regulators is the more likely explanation of the success of rimonabant treatments in phase III studies.

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