

Peripheral Metabolic Effects of Endocannabinoids and Cannabinoid Receptor Blockade

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Key Words

Anandamide · 2-Arachidonoyl glycerol · CB1 receptors · Adipose tissue · Liver · Rimonabant

Summary

The endocannabinoid system consists of endogenous arachidonic acid derivatives that activate cannabinoid receptors. The two most prominent endocannabinoids are anandamide and 2-arachidonoyl glycerol. In obesity, increased concentrations of circulating and tissue endocannabinoid levels have been described, suggesting increased activity of the endocannabinoid system. Increased availability of endocannabinoids in obesity may over-stimulate cannabinoid receptors. Blockade of cannabinoid type 1 (CB1) receptors was the only successful clinical development of an anti-obesity drug during the last decade. Whereas blockade of CB1 receptors acutely reduces food intake, the long-term effects on metabolic regulation are more likely mediated by peripheral actions in liver, skeletal muscle, adipose tissue, and the pancreas. Lipogenic effects of CB1 receptor signalling in liver and adipose tissue may contribute to regional adipose tissue expansion and insulin resistance in the fatty liver. The association of circulating 2-arachidonoyl glycerol levels with decreased insulin sensitivity strongly suggests further exploration of the role of endocannabinoid signalling for insulin sensitivity in skeletal muscle, liver, and adipose tissue. A few studies have suggested a specific role for the regulation of adiponectin secretion from adipocytes by endocannabinoids, but that has to be confirmed by more experiments. Also, the potential role of CB1 receptor blockade for the stimulation of energy expenditure needs to be studied in the future. Despite the current discussion of safety issues of cannabinoid receptor blockade, these findings open a new and exciting perspective on endocannabinoids as regulators of body weight and metabolism.

Endocannabinoids: Recent History of an Ancient Biological System

Exogenous cannabinoids contained in hemp (*Cannabis sativa*) extracts and plant parts have been used for medical and recreational purposes for thousands of years. The principal chemical compound, Δ^9 -tetrahydrocannabinol (Δ^9 -THC), was identified in 1964. The history of the endocannabinoid system (ECS), however, is much more recent. Milestones of endocannabinoid research are summarized in table 1. Endocannabinoids are lipophilic molecules that act as cannabinoid receptor agonists. The arachidonic acid esters N-arachidonoyl ethanolamine (anandamide or AEA) and 2-arachidonoyl glycerol (2-AG) are the two most important and mostly studied endocannabinoids.

Endocannabinoids are not stored in cells but are acutely produced ‘on demand’ if intracellular Ca^{2+} concentrations rise. In neurons, neurotransmitter activation may stimulate endocannabinoid synthesis and release by membrane depolarization. In the brain, endocannabinoids released from postsynaptic neurons may act as retrograde signals to modulate presynaptic neurotransmitter secretion. Mechanisms of endocannabinoid synthesis in peripheral, non-neural cells have not been studied in detail, but the enzymes are most likely the same as in neurons, and thus the role of intracellular Ca^{2+} as a signal might be the same. Metabolic stress or cellular damage also leads to endocannabinoid synthesis. Endocannabinoids are rapidly degraded by intracellular enzymes such as fatty acid amide hydrolase (FAAH) and monoglyceride lipase (MGL).

The cannabinoid receptors (CB1 and CB2) are encoded by two different genes on human chromosomes 6q14-q15 (*CNRI*) and 1p36.11 (*CNR2*). Both are seven-transmembrane domain $G_{i/o}$ protein-coupled receptors, and typical intracellular events following cannabinoid receptor activation are decreased cyclic adenosine monophosphate (cAMP) synthesis, increased K^+ efflux, and decreased Ca^{2+} influx [26]. CB1 is highly expressed in most brain regions, lower expression levels

Table 1. Milestones of endocannabinoid research related to metabolic regulation

Year	Discovery
1964	Identification of Δ^9 -THC [1]
1988	Identification of cannabinoid binding sites in the brain [2]
1990	Cloning of rat CB1 receptors [3]
1991	Cloning of human CB1 receptors [4]
1992	Identification of anandamide as the first endocannabinoid [5]
1993	Cloning of human peripheral CB2 receptors [6]
1994	Development of rimonabant as the first CB1 antagonist [7]
1995	Identification of 2-AG as a second endocannabinoid [8]
1996	Identification of FAAH as the anandamide degrading enzyme [9]
1998	First description of weight reduction by rimonabant [10]
1999	Genetic inactivation of CB1 receptors (CB1 ^{-/-} mice) [11, 12]
2001	Discovery of endocannabinoids as retrograde neurotransmitters [13]
2003	First finding of peripheral metabolic effects of endocannabinoids [14, 15] Cloning of DAGL as 2-AG synthesizing enzymes [16]
2004	Identification of NAPE-PLD as anandamide synthesizing enzyme [17]
2005	First publications of RIO (Rimonabant in Obesity) Trials [18, 19] Protection of CB1 ^{-/-} mice from liver steatosis [20]
2006	Activation of the ECS in human visceral obesity [21, 22] Further publications of RIO (Rimonabant in Obesity) Trials [23, 24] EMA approves rimonabant as an anti-obesity drug in Europe
2007	Publication of a rimonabant mortality study in obese diabetic rats [25] FDA advisory panel voted against approval of rimonabant in the USA

are found in several peripheral tissues. CB2 is predominantly localized on peripheral immune cells, but has recently been also localized to the brain stem [27]. Exploration of CB1 receptor-mediated effects was stimulated by the discovery of the specific inverse agonist/antagonist SR141716 (rimonabant), and the development of CB1 knockout (CB1^{-/-}) mice. Using these tools, the physiology and pathophysiology of the ECS, as well as pharmacological means to target the ECS, have extensively been studied during the last decade [28–31]. Beside the peripheral metabolic effects of the ECS that will be described in detail in this review, mounting evidence suggests that endocannabinoid signalling plays a critical role in pathophysiological cardiovascular conditions such as hypotension with hemorrhagic shock [32] or liver cirrhosis [33], ischemic/reperfusion injury [34], acute heart failure [35], cardiovascular aging [36] and atherosclerosis progression [37].

The Endocannabinoid System in Obesity

One of the important findings related to metabolic endocannabinoid research is the fact that the ECS is inadequately activated in obesity. CB1 receptor expression is changed, and endocannabinoid levels are increased in several organs of obese

animals (e.g. adipose tissue, liver, pancreas) and in adipose tissue and the circulation of obese human subjects [15, 20, 38, 39]. The mechanisms of ECS activation in obesity are not clear as yet, but pathophysiological stimulation of cannabinoid receptors may in part explain obesity-associated metabolic changes and also offers an explanation for the successful use of the CB1 receptor inverse agonist/antagonist rimonabant for weight reduction and metabolic disorders of the obese [40].

The most intriguing data came from two human studies [21, 22] that measured adipose tissue distribution by imaging techniques and insulin sensitivity or glucose tolerance by the euglycemic-hyperinsulinemic clamp technique or an oral glucose load. Of importance, both studies investigated obese subjects within a reasonable range of body weight and did not focus on morbidly obese subjects. In a comparison of lean and obese men and women with either subcutaneous or visceral adipose tissue accumulation (n = 60 in total), 2-AG plasma concentrations were clearly correlated with visceral adipose tissue mass, whereas obese subjects with subcutaneous adipose tissue accumulation were not different from the lean control group with regard to 2-AG plasma concentration. Anandamide was higher in women than in men, but no relationship with obesity or body fat distribution was found in this study. Furthermore, the expression of CB1 and FAAH genes in both subcutaneous and visceral adipose tissue depots were down-regulated with visceral obesity [21].

Similar findings were reported in a study of 62 well-characterized men. The men in the highest tertile of 2-AG blood levels presented with markedly increased visceral adipose tissue mass despite having nearly the same BMI compared to the lower two tertiles of plasma 2-AG. Anandamide, in contrast, was negatively correlated with visceral fat mass [22]. Both studies found correlations of increased plasma 2-AG with markers of the metabolic syndrome (e.g. free fatty acids, triglycerides, HDL cholesterol, adiponectin) and decreased insulin sensitivity as represented by enhanced glucose, and insulin responses during an oral glucose load.

Rimonabant in Obesity: More to the Picture than Meets the Eye

The Rimonabant in Obesity (RIO) program included 6,600 overweight and obese subjects in four randomized, placebo-controlled 1-year (RIO-Lipids, RIO-Diabetes) or 2-year (RIO-Europe, RIO-North America) studies. All subjects were advised to reduce caloric intake by 600 calories daily throughout the placebo run-in and treatment period. The primary end point in all trials was weight reduction. Data on side effects of rimonabant can be found in the original reports [18, 19, 23, 24] and in a meta-analysis [41]. Rimonabant, 20 mg/day, enabled patients to lose 5 kg of body weight and 4 cm of waist circumference more than patients taking placebo. Reductions of weight and waist circumference were less pronounced in dia-

betic patients taking metformin or sulfonylureas, but still significantly larger than in the placebo group. Weight loss was accompanied by favorable changes in triglycerides (−14% in addition to results with placebo), high-density lipoprotein (+8%), and fasting insulin (−4 μU/ml in non-diabetic patients). In non-diabetic patients, rimonabant reduced the number of patients fulfilling the National Cholesterol Education Program's Adult Treatment Panel III criteria for the metabolic syndrome by 30% more than in those taking placebo. In RIO-Lipids, rimonabant reduced small-dense low-density lipoprotein particles, and a significant reduction in insulin secretion was seen following an oral glucose load. High-sensitive C-reactive protein and adiponectin was decreased by 30% and increased by 40%, respectively. In RIO-Diabetes, the absolute change of hemoglobin A_{1c} (HbA_{1c}) compared with placebo was −0.7%, independent of the concomitant oral hypoglycemic drug.

Metabolic changes and weight loss lasted as long as rimonabant was taken. Once patients were re-randomized to placebo after 1 year rimonabant treatment in RIO-North America, the rimonabant-related treatment effects were lost and body weight rose to the level of the ever-placebo treated group. This finding clarifies the chronic nature of obesity and obesity-associated metabolic disease. Using the placebo data as a calibrator for analysis of co-variance (ANCOVA) of the favorable changes in lipid parameters, fasting insulin, HbA_{1c}, and adiponectin, 50% were calculated to be due to weight loss, whereas the other 50% were due to rimonabant-specific effects independent of weight loss. These calculations await experimental verification, but they suggest peripheral metabolic effects of endocannabinoids.

A Closer Look on Weight Reduction by CB1 Receptor Blockade

Δ⁹-THC is used as an adjunct treatment in HIV and cancer patients because of antiemetic and orexigenic properties [42]. Injection of anandamide into the ventromedial hypothalamus of rats increased food intake [43]. Peripheral administration of anandamide increased food intake of rats even during periods of satiety [44]. These orexigenic effects were mediated by central CB1 receptors. Hunger, satiety, and food intake are predominantly regulated in hypothalamic nuclei [45]. Imaging studies identified several brain regions with high CB1 density in humans [46], and ultrastructural analyses demonstrated presynaptic expression of CB1 receptors on many hypothalamic neurons in the mouse brain [47]. Thus, endocannabinoids may act as modulators of the main orexigenic and anorexigenic neuropeptides, as described in other brain regions and other neural functions [13]. Furthermore, other brain regions than the hypothalamus are also involved in the regulation of food intake by endocannabinoids, thus homeostatic and hedonistic mechanisms are important for the orexigenic effects of endocannabinoids [48]. Leptin, one of the main anorexigenic

hormones that is secreted by adipocytes and acts via central leptin receptors, is able to reduce endocannabinoid levels in the hypothalamus [49]. Electrophysiological measurements confirmed the interactions between leptin and endocannabinoid signalling in hypothalamic neurons [50].

Confirming the pharmacological studies of endocannabinoid administration, CB1^{−/−} mice were lean and resistant to diet-induced obesity [51]. Body weight and body fat mass of CB1^{−/−} mice were approximately 24 and 60% lower compared to wild-type mice. The lean phenotype was associated with changes in the balance between orexigenic and anorexigenic peptides. In CB1^{−/−} mice, hypothalamic expression of the anorexigenic peptide corticotrophin-releasing hormone (CRH) was increased, whereas expression of the orexigenic peptide cocaine- and amphetamine-related transcript (CART) was decreased [14]. Recent studies in mice also demonstrated that CB1 receptor activation increased CART expression in hypothalamic nuclei and the nucleus accumbens, again suggesting that CART is a downstream mediator of orexigenic endocannabinoid actions [52]. Similar to genetic inactivation of CB1 receptors, rimonabant reduced food intake and body weight in animals [10, 53].

Although the opposing acute effects of endocannabinoids or CB1 receptor inactivation/blockade on food intake have been well described, the regulation of long-term energy balance by the ECS is not understood. In young CB1^{−/−} mice, decreased food intake contributed to the lean phenotype, but pair-fed older wild-type mice were heavier than CB1^{−/−} mice [14]. Rimonabant treatment of diet-induced obese mice over 6 weeks resulted in 20% body weight reduction, but reduced food intake was only seen during the first week of treatment. Surprisingly, body weight tended to fall throughout the studies despite normalization of food intake after the first week of treatment [53, 54]. In obese leptin-deficient mice, rimonabant also decreased body weight and food intake. Pair-feeding experiments, however, revealed that the reduction of food intake was not sufficient to reduce body weight of untreated, pair-fed obese mice [55]. Thus, reduced food intake is only a transient phenomenon of CB1 receptor blockade or genetic inactivation, and fat mass reduction must be due to additional, most likely peripheral metabolic mechanisms. Unfortunately, human studies that examined food intake during rimonabant treatment are not available. Energy expenditure studies that should be able to explain the lack of efficiency of pair-feeding to mimic rimonabant effects on body weight are also missing.

The Endocannabinoid-System in Adipose Tissue

Components of the ECS in Adipose Tissue

Human and rodent adipocytes express all genes necessary to synthesize and degrade anandamide and 2-AG [14, 38, 39, 56–58]. For example, MGL is an enzyme that was initially cloned

from an adipocyte cDNA library because the release of the last fatty acid from the glycerol backbone is an important step in adipocyte lipolysis [59]. A special case must be made for CB2 receptors. Some authors described CB2 expression in adipocytes [38, 60], whereas others did not identify them in mature adipocytes [39, 56, 58, 61]. Several explanations might be considered: first, anti-CB2 receptor antibodies were used in one study [60], but in the meantime it is rather clear that nearly all available antibodies against CB2 receptors are of low specificity. Second, species differences must be considered, as one study examined adipogenesis of the mouse clonal preadipocyte cell line 3T3-F442A [38], whereas most other studies used isolated mature human adipocytes. Third, the difference between isolated adipocytes and adipose tissue must be considered because in whole adipose tissue preparations several other cell types with known CB2 expression are included [62]. Thus, small quantities of CB2 mRNA in such samples do not reflect adipocyte expression but most likely originate from monocyte and lymphocyte infiltration of adipose tissue especially of obese donors [58]. Receptor binding studies revealed that only CB1 is present in membranes of isolated mature human adipocytes [56].

Endocannabinoids, Adipogenesis, and PPAR γ

The differentiation process during which preadipocytes change their gene expression pattern, increase in size due to triglyceride accumulation, and start to secrete adipokines is called adipogenesis. The expression of ECS genes and the synthesis of endocannabinoids increased during adipogenesis [38, 39, 58, 61, 63]. Adipogenesis itself was stimulated by synthetic cannabinoid receptor agonists and anandamide in several murine preadipocyte cell lines. Some authors suggested a direct adipogenic role of CB1 receptor activation [38], but a more likely explanation might be that several endocannabinoid compounds activate the master regulator of adipogenesis, PPAR γ (peroxisome proliferator-activated receptor gamma) [64, 65]. Whereas PPAR γ activation did not influence synthesis of endocannabinoids in immature 3T3-F442A adipocytes [38], CB1 gene expression was inhibited and FAAH gene expression was increased by PPAR γ activation in immature *in vitro* differentiated human adipocytes [58]. CB1 receptor stimulation increased PPAR γ gene expression in mouse and human adipocytes, but these data were obtained during active *in vitro* adipogenesis. Thus it is unclear whether a direct effect was observed or if PPAR γ expression increased because this gene was activated during adipogenesis anyway [38, 58].

Endocannabinoids, Lipogenesis, and Glucose Uptake in Adipocytes

Lipogenesis is a complex biochemical process which enables adipocytes (and other cells) to store triglycerides. Lipogenesis

involves both glucose and fatty acid metabolism. Glycolysis following glucose uptake provides the glycerol backbone, whereas fatty acids are transported into cells after they were cleaved from circulating triglyceride-rich lipoproteins by lipoprotein lipase (LPL) activity. In rodent adipocytes, a considerable amount of glucose is also used for *de novo* synthesis of fatty acids, whereas in human adipocytes this is only observed *in vitro*, but not *in vivo* under physiological situations. CB1 agonists promoted lipoprotein lipase activity in isolated mouse adipocytes [14].

A recent report described stimulation of insulin-mediated glucose uptake in murine adipocytes by anandamide via CB1 receptors that was mediated by stimulation of inducible nitric oxide synthase expression and nitric oxide formation by adipocytes [61]. In human adipocytes, CB1 activation increased basal, but not insulin-stimulated glucose uptake by stimulation of GLUT4 translocation and increased intracellular Ca²⁺ availability [58]. Both effects were dependent on activation of the PI3-kinase, thus CB1 and insulin receptors converge on the same pathways with respect to glucose uptake into human adipocytes. Maybe the insulin effect on glucose uptake was too strong in these experiments to allow additive effects of co-incubation with CB1 agonists.

Lipogenic effects of endocannabinoids in adipose tissue are also partly due to inhibition of AMP-activated kinase (AMPK) [66]. Indirect hints to lipogenic properties of endocannabinoids came from rimonabant studies. Here, rimonabant inhibited *in vitro* lipid accumulation of mouse preadipocytes [67] and induced several changes in gene expression in diet-induced obese mice that might translate to reduced adipocyte size and weight loss [54]. These gene expression studies await confirmation by physiological studies. In human subcutaneous and visceral adipocytes, however, no relationship was found between the quantity of CB1 mRNA expression and variables of either lipogenesis (triglyceride synthesis) or lipolysis (fatty acid release) [68].

Endocannabinoids, Adiponectin, and Leptin

In the RIO-Lipids trial, the increase of the insulin-sensitizing and anti-inflammatory adipokine adiponectin was calculated to be partly independent of weight loss [19]. Thus, the influence of the ECS on adiponectin was studied by some investigators. Increased adipose tissue expression and circulating levels of adiponectin were described in obese Zucker rats treated with rimonabant [15, 25]. The same results were obtained in diet-induced obese rats, but in contrast to the obese Zucker rat model, pair-feeding revealed that the increase of adiponectin in this experiment was solely explained by weight loss due to decreased food intake [69]. *In vitro*, CB1 activation reduced and rimonabant stimulated adiponectin mRNA expression and secretion by murine adipocytes [38, 67, 70]. Again, this CB1-mediated effect might be related to decreased AMPK activity

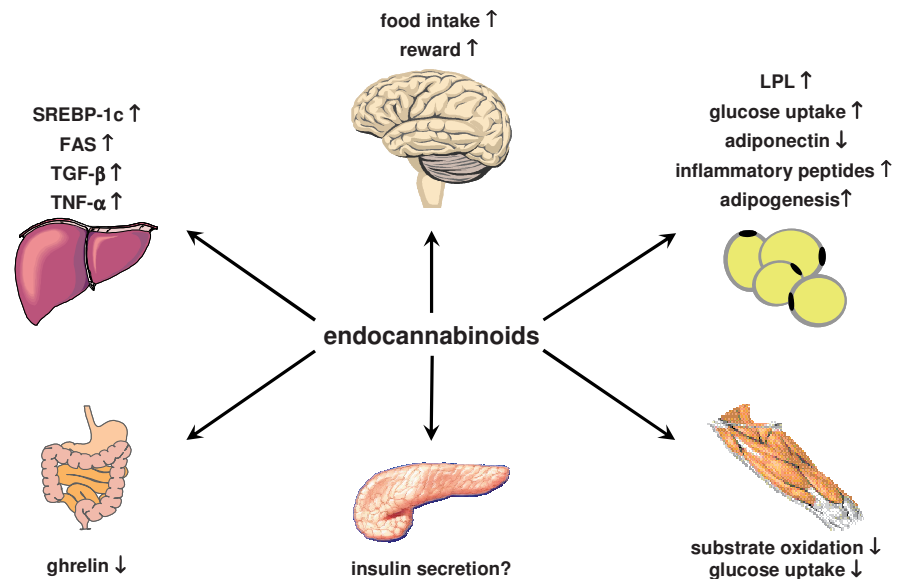


Fig. 1. Possible peripheral metabolic effects of endocannabinoids. The findings are derived from in vivo and in vitro experiments in animals and humans. Some of the effects are deduced only from cannabinoid receptor blockade studies. Abbreviations: FAS = Fatty acid synthase; LPL = lipoprotein lipase, SREBP-1c = sterol-regulated element binding protein 1c; TGF- β = transforming growth factor β , TNF- α = tumor necrosis factor α .

[66]. In clear contrast, however, CB1 receptor activation did not stimulate adiponectin expression in vitro in mature human adipocytes [58], and no correlation was found between CB1 expression levels and adiponectin in human adipocytes isolated from biopsy samples [68]. Leptin is another important secreted product of adipocytes. Leptin inhibits endocannabinoid synthesis, and leptin deficiency is accompanied by increased activation of the ECS in the brain and uterus [49, 71]. The negative regulation of endocannabinoid formation by leptin has also been described in human and rodent adipocytes [38, 57]. In contrast, leptin expression was not influenced by CB1 stimulation or blockade in human primary adipocytes [58].

The Endocannabinoid System in the Liver

Although restricted in number, the most intriguing data on peripheral effects of the ECS are currently available for the liver. CB1 receptor activation stimulated lipogenesis in mouse hepatocytes via the transcription factor SREBP-1c, which regulates fatty acid synthase and other lipogenic enzymes [20]. Inhibition of AMPK in hepatocytes may contribute to the lipogenic actions of endocannabinoids [66]. CB1 $^{-/-}$ mice were protected against the development of liver steatosis and fibrosis when fed a high-fat diet because important genes of lipogenesis (e.g. fatty acid synthase, acetyl-CoA carboxylase-1) were down-regulated in hepatocytes of CB1 $^{-/-}$ mice [20]. The same result was obtained by treating obese Zucker rats with rimobabant [72]. In this study, amelioration of hepatomegaly, liver steatosis, and hepatocyte damage was associated with decreased local and circulating tumor necrosis factor α levels, increased adiponectin levels, and improved lipid profile of the animals. Importantly, pair-feeding in a second group of untreated obese Zucker rats prompted few hepatoprotective ef-

fects, again suggesting that not reduced caloric intake per se but local peripheral mechanisms are operational for the metabolic effects of CB1 blockade.

Liver cirrhosis in humans was accompanied by strongly enhanced CB1 receptor gene expression [73], whereas in mice with toxic liver disease and resulting cirrhosis both anandamide and 2-AG tissue concentrations were increased 3- to 6-fold [74]. Furthermore, genetic inactivation or blockade of CB1 receptors decreased fibrotic activity of hepatic myofibroblasts in animal models of toxic liver damage. A possible antifibrotic mechanism of CB1 blockade in this study was decreased hepatic expression of transforming growth factor β in treated and knockout mice. Concurrent anti-inflammatory effects of hepatic CB2 receptor stimulation may also have contributed to the effects of CB1 receptor blockade [73].

The Endocannabinoid System in the Pancreas

The data on the ECS in the pancreas are preliminary at present, limited in number and contradictory, most likely because of species differences, the use of cell lines, and the quality of antibodies used (as discussed before). Pancreatic islets express ECS genes and synthesize endocannabinoids. Formation of 2-AG was stimulated by glucose and insulin, and inhibited by leptin in the RIN-m5F rat insulinoma β -cell line [38]. In mouse islets, CB1 expression was restricted to pancreatic α -cells that produce glucagon, whereas CB2 was detected in α - and β -cells. Anandamide and 2-AG via CB2 receptors reduced Ca^{2+} oscillations and insulin secretion in mouse islets [75]. In contrast to mouse islets, the rat insulinoma β -cell line expressed both cannabinoid receptors [38]. In these cells, cannabinoid receptor stimulation increased intracellular calcium availability and in-

sulin secretion [38, 76]. In rat islets, CB1 and CB2 were expressed on both α - and β -cells [77]. Activation of CB1 receptors in wild-type rats decreased glucose tolerance [78]. Furthermore, administration of CB2 agonists improved glucose tolerance and treatment with a CB2 antagonist also decreased glucose tolerance [77]. As concomitant treatment with a CB1 antagonists also improved glucose tolerance, the authors hypothesized that this effect is indirectly mediated by enhanced CB2 receptor signalling. Recent data in obese Zucker rats suggested that rimonabant treatment preserves pancreatic islet integrity and β -cell mass in this model of type 2 diabetes [25]. Together with metabolic and hemodynamic improvements, and a clear amelioration of renal pathology (decreased sclerosis and albuminuria), rimonabant treatment was associated with a strong decrease of mortality in the obese Zucker rat model.

The Endocannabinoid System in Skeletal Muscle

Only few reports investigated the ECS in skeletal muscle physiology. CB1 expression was found to be the same in myotubes grown in vitro from a small number of lean and obese human donors [79]. Both FAAH and MGL are also expressed in minor quantities in human skeletal muscle biopsies (unpublished data). AMP-kinase did not mediate the insulin-desensitizing effect of cannabinoids in rat skeletal muscle [66], but blockade of CB1 receptors in human skeletal muscle myotubes enhanced AMP-kinase mRNA expression [79]. High-fat feeding enhanced CB1 expression in skeletal muscle of mice. Treating genetically obese mice with rimonabant increased glucose uptake and oxygen consumption in the soleus muscle, which may lead to increased thermogenesis and enhanced insulin sensitivity [55]. In line with these observations, we reported a correlation between increased levels of circulating 2-AG and decreased insulin sensitivity during euglycemic-hyperinsulinemic clamp in 60 middle-aged human subjects [21]. Increased glucose and insulin responses to an oral glucose load in men with the highest circulating 2-AG levels were also described [22].

Endocannabinoids and Gastrointestinal Hormones

The influence of endocannabinoids on feeding behavior can to some extent also be explained by peripheral interactions with gastrointestinal hormones. CB1 receptors and receptors for gastrointestinal hormones such as cholecystokinin (CCK), ghrelin, and orexins are co-localized on vagal nerve terminals projecting from the gastrointestinal tract to the nucleus of the solitary tract [80, 81]. CB1 receptors are up-regulated by fasting and down-regulated by feeding, and this reaction was blocked by CCK antagonists. Thus, the anorexigenic action of CCK may in part mediated by down-regulation of orexigenic CB1 receptors. Activation of ghrelin receptors also prevents

the down-regulation of vagal CB1 receptors [80, 81]. Vice versa, the response of circulating ghrelin to fasting was diminished by rimonabant, suggesting that CB1 receptors are directly involved in ghrelin secretion. In contrast, glucagon-like peptide 1 was not influenced by rimonabant in these studies [82]. In the brain, the orexigenic effects of centrally administered ghrelin were abolished by rimonabant. Thus, ghrelin possibly increased endocannabinoid production in the hypothalamus in this study [83]. Other interactions between the ECS and gastrointestinal hormones, functions and diseases have been described, but are outside of the focus of this review [84].

Conclusion: Many Questions – Few Answers

The marketing of rimonabant was an important step forward because for the first time in nearly a decade a drug with weight-reducing actions successfully passed clinical development. Furthermore, rimonabant as a tool broadened the view on metabolic regulation and the regulation of food intake by introducing the ECS to metabolic research. However, several open questions remain. First, a strong discussion of the risk profile of the drug had started. At present this discussion is only based on the published data from the RIO trials, and no new insights have been added, but blockade of this widespread system clearly warrants a high level of pharmacovigilance. Given the many promising peripheral effects of CB1 receptor blockade, the development of antagonists not entering the central nervous system may offer an important improvement in terms of safety because, then, side effects caused by blockade of central CB1 receptors should be minimized. Several drugs affecting the ECS are currently in development, e.g. FAAH inhibitors, but most of them will directly or indirectly activate cannabinoid receptors in order to modulate chronic inflammation and pain disorders. Again, the development of drugs with only peripheral actions to avoid central side effects is preferred [85, 86].

Second, the peripheral metabolic effects described in this review (fig. 1) were extracted from a rather limited number of studies and were mostly obtained in animal models and cellular systems that are far away from the pathophysiology of human metabolic disorders and human obesity. Studies need to be done that address the effects of rimonabant on food intake in humans and on energy expenditure in humans and animals. The pharmacology of the ECS as such is complex, and the pharmacology of compounds currently available to target the ECS is even more complex. Thus, some of the possible endocannabinoid effects are only deduced from data of cannabinoid receptor blockade studies. In conclusion, several inconsistencies and uncertainties must currently be considered when the peripheral metabolic actions of the ECS are discussed. Hopefully, the development of newer drugs acting on the ECS will help to better define the promising peripheral metabolic effects of CB1 receptor blockade.

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