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The hepatic cannabinoid 1 receptor as a modulator of hepatic energy state and food intake

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The cannabinoid 1 receptor (CB_1R) has a well-established role in appetite regulation. Central CB_1R antagonists, notably rimonabant, induced weight loss and improved the metabolic profile in obese individuals, but were discontinued due to psychiatric side-effects. The CB_1R is also expressed peripherally, where its effects include promotion of liver fat accumulation, which consumes ATP. Type 2 diabetes in obese subjects is linked to excess liver fat, whilst there is a negative correlation between hepatic ATP content and insulin resistance. A decreased hepatic ATP/AMP ratio increases food intake by signals via the vagus nerve to the brain. The hepatic cannabinoid system is highly upregulated in obesity, and the effects of hepatic CB_1R activation include increased activity of lipogenic and gluconeogenic transcription factors. Thus, blockade of hepatic CB_1Rs could contribute significantly to the weight-reducing and insulin-sensitizing effects of CB_1R antagonists. Additionally, upregulation of the hepatic CB_1R may contribute to chronic liver inflammation, fibrosis and cirrhosis from causes including obesity, alcoholism and viral hepatitis. Peripheral CB_1R antagonists induce weight loss and metabolic improvements in obese rodents; however, as there is evidence that hepatic CB_1Rs are predominately intracellular, due to high intrinsic clearance, many drugs may not effectively block these receptors and therefore have limited efficacy. Hepatoselective CB_1R antagonists may be effective at reducing hepatic steatosis, insulin resistance and bodyweight in obese, diabetic patients, with far fewer side-effects than first-generation CB_1R antagonists. Additionally, such compounds may be effective in treating inflammatory liver disease, such as non-alcoholic steatohepatitis, reducing the likelihood of disease progression to cirrhosis or cancer.

Hepatic energy state and its effects on food intake

The cellular energy state is defined by adenine nucleotide levels. Healthy cells maintain a ratio of ATP to ADP of the order of 10:1. Cellular concentrations of ADP typically remain constant, while ATP and AMP levels deviate in reciprocal directions [1].

Hepatic energy state influences food intake [2, 3]. For instance, infusion of various lipids and carbohydrates into the hepatic portal vein of rodents was found to suppress food intake more effectively than administration of the same nutrients into the jugular vein [2]. Supporting results were found when injecting the fructose analogue 2,5-anhydro-D-mannitol (2,5-AM) into the portal vein. In the liver, 2,5-AM is phosphorylated at the 1 and 6 positions, but not metabolized further, thus lowering the levels of free intracellular phosphates, which reduces the generation of ATP, whilst increasing its degradation by disinhibiting adenosine deaminase. The net result is a lower hepatocellular ATP concentration, which increases feeding [4]. Pretreatment with sodium phosphate prevents the decrease in liver ATP levels and the increase in feeding [5]. Administration of the amino-acid analogue L-ethionine, which reduces ATP production by trapping the adenosine moiety of ATP, also increased food intake [6]. Also, liver fructose-1,6-bisphosphatase, which is upregulated in obesity and affects hepatic gluconeogenesis, has recently been demonstrated to regulate appetite and adiposity in mice [7].

Increased fatty acid oxidation in the liver reduces food intake, whereas inhibition of fatty acid oxidation increases it. Ablation of the hepatic branches of the vagus nerve prevented these effects. The consequences for feeding are

probably not due to fatty acid synthesis or oxidation *per se*, but rather due to the effects of fatty acid metabolism on liver energy production [2].

How changes in hepatocellular energy state lead to a signal that affects food intake has not been fully elucidated. However, *in vitro* studies suggest that reduced ATP production may affect sodium pumps, increasing the intracellular sodium concentration [8]. 2,5-Anhydro-D-mannitol also increases the calcium concentration in hepatocytes, which is a typical step in many signal transduction pathways [9]. Hence, intracellular sodium and/or calcium levels may play a role in signalling between hepatocytes and neurons.

The impact of the hepatic energy state on feeding has also been demonstrated in ruminants [3], suggesting that the liver influences feeding in many different species. Thus, from studies such as these, the liver has been recognized as an important organ in appetite and bodyweight regulation, reflecting the strong influence that hepatic energy state has on feeding behaviour, with signals being carried from the liver to the brain by the vagus nerve [2].

Hepatic energy metabolism is impaired in patients with type 2 diabetes. Due to a markedly lower ATP production, such patients were found to have 42% lower hepatic ATP turnover (i.e. nucleotide phosphorylation rate, measured with ^{31}P magnetic resonance spectroscopy) than control subjects. There was a strong negative correlation of ATP turnover to hepatic fat content and insulin resistance [10]. These findings are consistent with earlier studies showing that demonstrated 40% reduced mitochondrial oxidative phosphorylation in elderly, insulin-resistant patients with fatty liver compared with young, healthy control subjects [11] and that individuals with type 2 diabetes have less hepatic ATP and inorganic phosphate (P_i) than control subjects, with ATP and P_i content being negatively related to insulin resistance [12].

Taken together, these findings suggest that increased fatty acid synthesis and decreased fatty acid breakdown in the liver cause fat accumulation and a lowered hepatic energy state, marked by a low ATP : AMP ratio. In response to the low energy state, the liver generates signals that are carried via the vagus nerve to the brain, leading to increased food intake. This may lead to fatty liver, obesity and/or type 2 diabetes. Hence, targeting a main mediator of fatty liver and decreased hepatic energy state would counteract this chain of events. Studies of recent years suggest that the hepatic cannabinoid 1 receptor (CB_1R) may be such a target.

The role of cannabinoid receptors in fatty liver

Endocannabinoids are lipid ligands that bind to cannabinoid receptors. The best characterized of these receptors are the cannabinoid 1 receptor and the cannabinoid 2 receptor [13]. Although traditionally associated with the central nervous system (CNS), cannabinoid receptors in hepatocytes are increasingly being recognized as key mediators of fatty liver [14] and associated insulin resistance [15–17] caused by high-fat diet [18], viral hepatitis [19–21] and ethanol intake [22]. Additionally, cannabis smoking has been shown to be a risk factor for hepatic steatosis [23] and, in a separate study, patients with non-alcoholic fatty liver disease were found to have a 34.2 ± 9.7-fold increase in the amount of hepatic CB_1R mRNA compared with patients without liver pathology [24].

One of the downstream effects of CB_1R activation is to increase the expression of the transcription factor sterol regulatory element-binding protein 1c (SREBP-1c), which controls the expression of a number of key lipogenic genes [25]. Among the more important effects of SREBP-1c are the upregulation of acetyl-CoA carboxylase 1 and fatty acid synthase (key enzymes of fatty acid synthesis) and inhibition of carnitine palmitoyltransferase I, which controls the rate-limiting step of β-oxidation [26]. See reference [27] for a more detailed review of the steps leading from CB_1R activation to steatosis and Figure 1 for a simplified representation of the key mediators of this process.

Activation of CB_1Rs, causing fatty liver by a combination of increased lipogenesis and reduced fatty acid oxidation, depletes ATP, which decreases the cellular ATP : AMP ratio. Additionally, CB_1R activation has been demonstrated to increase activation of the endoplasmic reticulum stress-associated liver-specific transcription factor cyclic AMP

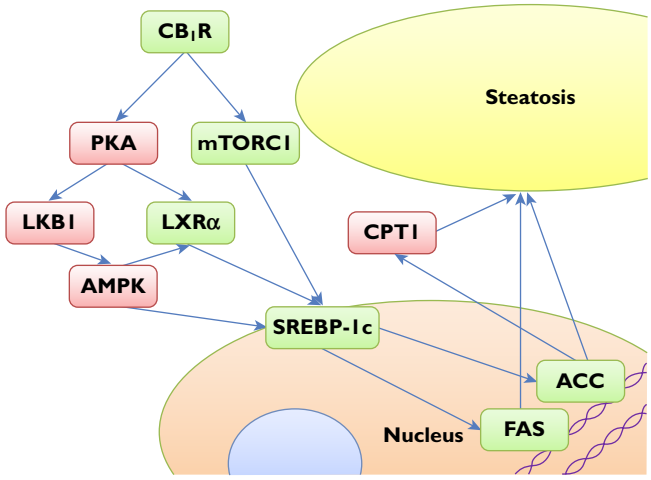

Figure 1

Central mediators of steatosis activated by CB_1R. Green boxes indicate increased activity; red ones decreased activity. Abbreviations are as follows: ACC, acetyl-CoA carboxylase; AMPK, AMP-activated protein kinase; CB_1R, cannabinoid 1 receptor; CPT1, carnitine palmitoyltransferase I; FAS, fatty acid synthase; LKB1, liver kinase B1; LXRα, Liver X receptor alpha; mTORC1, mammalian target of rapamycin complex 1; PKA, protein kinase A; SREBP-1c, sterol regulatory element-binding protein 1c

response element-binding protein H [16, 17, 24], which regulates expression of gluconeogenic genes and, at least in part, accounts for the CB_1R-induced reduction in insulin sensitivity and raised glucose levels, whilst further exacerbating the reduced hepatic energy status.

In a healthy cell, the low ATP : AMP ratio would activate AMP-activated protein kinase (AMPK) [1], which then inhibits anabolic pathways and promotes catabolic ones, in part by reducing SREBP-1c activity and expression [28]. In contrast to this, AMPK is less sensitive to stimulation by AMP in the livers of rats with ethanol-induced fatty liver [29, 30]. Also, obese diabetic and nondiabetic individuals have decreased AMPK activity in skeletal muscle in response to exercise [31], as do mice made obese by a high-fat diet [32]. Therefore, in these diseases, the stimulatory effects of increased AMP on AMPK appear to be counteracted. In other studies, CB_1R activation has been shown to counteract the effects of high AMP on AMPK [27], with steatosis as the net effect.

Liver-specific knockout of the CB_1R made mice resistant to high-fat diet-induced steatosis, although overall adiposity and weight gain were not affected in this situation [18]. However, knockout of genes that control important metabolic regulators may lead to compensatory antenatal changes affecting appetite; thus, the knockout does not always have the same effects as inhibition of the corresponding metabolic regulators in adults. For example, deletion of the gene for acetyl CoA carboxylase 2 did not improve glucose homeostasis in *db*/*db* mice [33], but a small-molecule acetyl CoA carboxylase 2 inhibitor has been reported to do so [34]. Acetyl CoA carboxylase 2 is a key regulator of fatty acid oxidation, but adaptation during fetal development and/or early life may compensate for reduced hunger signalling to the brain in acetyl CoA carboxylase 2 null mice. The finding that liver-specific CB_1R knockout mice did not become resistant to diet-induced obesity may have been due to similar compensatory mechanisms.

Nevertheless, studies with liver-specific CB_1R knockout mice demonstrated that the hepatic CB_1R contributes significantly to steatosis or metabolic disturbances that lead indirectly to steatosis. Also, treatment of cultured mouse liver explants with rimonabant increased fat oxidation [35], showing that hepatic CB_1R inverse agonism reduced expression of genes for prolipogenic enzymes. Studies such as these demonstrate that the effects of CB_1R inhibition on liver fat are mediated peripherally, independently of the nervous system.

Based upon evidence from the peripherally selective CB_1R antagonist JD5037, it has recently been proposed that reversal of obesity-related leptin resistance accounts for the hypophagic effect of peripheral CB_1R inverse agonism and that this is achieved by normalization of the hyperleptinaemia of diet-induced obese mice through the suppression of leptin secretion in adipose tissue and increased leptin clearance via the kidney [36]. However, whilst it seems likely that activation of the CB_1R is an important regulator of diet-induced leptin resistance [36], which probably contributes to the development of obesity, this is due to the inability of the high levels of leptin, normally associated with obesity, to reduce appetite effectively, rather than to the high leptin levels themselves [37]. Thus, leptin resistance is an additional mechanism by which peripheral CB_1R activation may exacerbate the development of obesity.

The efficacy of hepatic or peripheral CB_1R inhibition is also suggested by data in a clinical trial with 20 mg rimonabant daily for the treatment of abdominal obesity and dyslipidaemia. According to regression analysis, 72% of the improvement in high-density lipoprotein-cholesterol could not be explained by the weight loss alone [38]. Hence, peripheral effects of rimonabant probably contribute to its metabolic benefits. However, although hepatic CB_1Rs presumably contribute to this efficacy, it is not clear exactly how effectively receptors in the liver are blocked at doses used clinically. This is because, as will be discussed in the next section, hepatic CB_1Rs, which are highly upregulated in obesity and a number of inflammatory liver conditions, appear to be mainly intracellular and, whilst functional activity of intrahepatic CB_1Rs has not been confirmed, studies in other cell types have demonstrated intracellular receptors to be highly important for CB_1R-mediated effects [39–43]. Thus, the efficacy of a CB_1R antagonist drug may be influenced by how well it maintains exposure inside hepatocytes, in the vicinity of high levels of metabolizing enzymes.

Cytosolic localization, functional activity and hyperactivation of CB_1R in hepatocytes

The main direct evidence for intracellular localization of CB_1Rs in hepatocytes is from immunostaining experiments with rodent [19, 44–48] and human [19, 48, 49] liver tissue samples. Although this does not provide definitive proof for intracellular localization of upregulated hepatic CB_1Rs, due to the absence of information from classical biochemical tests (such as preparing heavy organelle, light organelle and cytosolic fractions from cells by directed Förster resonance energy transfer assays on binding partners) or by ultrastructural analyses using immune-electron microscopy, the pattern of staining in the published images, particularly in articles by van der Poorten *et al*. [19] and Floreani *et al*. [44], nevertheless strongly indicates cytosolic localization.

These suppositions are also consistent with emerging evidence that the hepatic CB_1R may become upregulated in response to elevated intracellular endocannabinoid levels as part of a hyperactivation of the cannabinoid system. For instance, of the two most-studied endocannabinoids, 2-arachodonylglycerol has been shown to

mediate binding of the retinoic acid receptor subunit γ to the regulatory domain for the CB_1R [50], whilst anandamide has also been demonstrated to increase CB_1R mRNA [35] Furthermore, it has also been proposed that CB_1R upregulation may in turn lead to increased endocannabinoid synthesis [48]. Elevated cellular endocannabinoid levels may be caused by faster synthesis or increased uptake from the systemic circulation or reduced activity of the intracellular metabolizing enzymes; in particular, monoacylglycerol lipase and fatty acid amide hydrolase (FAAH) are important for regulating levels of 2-AG and AEA, respectively. For instance, whilst FAAH is primarily expressed in the liver, human *FAAH* gene mutations are associated with increased bodyweight and obesity [19, 51], whilst *FAAH*$^{-/-}$ mice have altered energy homeostasis, demonstrated by decreased oxygen consumption and hyperinsulinaemia, with adipose, skeletal muscle and hepatic insulin resistance [52].

Whilst high cannabinoid concentrations can be maintained for hours insde hepatocytes, rapid fluid flow through hepatic vessels and the space of Disse washes away extracellular cannabinoids. It would therefore seem reasonable to assume that elevated endocannabinoid levels in the liver can be maintained only if they are intracellular. It thus seems likely that the resulting elevated endocannabinoid levels will have maximal effect if they act through intracellular receptors. These suppositions are consistent with observations made in other cell types; for instance, in retinal epithelial cells high glucose was shown to downregulate the expression of FAAH 1 mRNA and protein in ARPE-19 cells, whilst upregulating CB_1R mRNA and protein. Furthermore, overexpression of FAAH 1 and treatment with the CB_1R antagonist AM 251 blocked high-glucose-induced internalization of CB_1R in HEK 293 cells and ARPE-19 cells, expression of CB_1R in the cytosolic fraction, as well as generation of reactive oxygen species (ROS) and lipid peroxide formation [53].

Although the precise mechanism by which endocannabinoid levels are elevated in hepatocytes is not fully elucidated, there is evidence in other cell types implicating oxidative stress; for instance, in hepatic stellate cells, acetaldehyde, H_2O_2, as well as 2-arachodonylglycerol and tetrahydrocannabinol, alone or in combination with acetaldehyde, were shown to induce *CB1* mRNA expression *in vitro* [47]. This may explain the pathogenesis of CB_1R-mediated liver disease, because circulating ROS have been shown to be elevated due to hyperlipidaemia [54], high-fat [55] or carbohydrate [56] diets. However, given that elevated ROS may be caused by CB_1R activation [57], it is necessary to distinguish between cause and effect before drawing firm conclusions about what stimulus induces upregulation of the cannabinoid system in hepatocytes. Nevertheless, the concept that elevated ROS leads to upregulation and activation of hepatic CB_1R is intriguing, because this might imply a positive feedback loop whereby hepatic CB_1R activation leads to increased food intake, which in turn leads to increased ROS production and further increased CB_1R activation, but this remains to be proved.

In summary, we postulate that localized hyperactivation of the cannabinoid system occurs internally in various cell types, possibly in response to inflammatory stimuli, such as elevated ROS, to activate specific cellular processes. In this context, considerable evidence implicates the cannabinoid system as a key mediator of apoptosis in many cell types [58], including immune cells [59] and many forms of cancer cells [60, 61], whilst, in neuronal cells, intracellular CB_1R activation has been shown to play a role in stabilizing lysosomes against amyloid toxicity, thereby improving cell viability and conferring neuroprotection [62]. Anandamide activation of the hepatic cannabinoid system does not induce apoptosis in hepatocytes [63], possibly due to elevated levels of glutathione, which protects against elevated ROS, and high levels of FAAH [64]. Moreover, in the light of a recent report implicating the hepatic CB_1R as a regulator of transcription for enzymes involved in M-phase progression and as an important mediator in the early phase of liver regeneration [48], it may seem reasonable to assume that hepatic cannabinoid system upregulation has a physiological role as part of the mechanism inducing cell replication.

Understanding of the effects of alterations in the expression level of cannabinoid receptors in the pathogenesis of many chronic diseases is emerging [65], and the key role played by aberrant chronic activation of the hepatic CB_1R in the development of inflammatory liver conditions, including non-alcoholic fatty liver disease [18], non-alcoholic steatohepatitis [66], alcoholic steatohepatitis [57] and fibrosis/cirrhosis [46, 67, 68], is well documented. Recent evidence links development of fibrosis to increased hepatocyte apoptosis [69] mediated by NADPH oxidase [70, 71] due to cell damage caused by chronic inflammation, which is, at least in part, attributable to CB_1R activation. Thus, it is expected that, as well as reducing obesity, liver fat and insulin resistance, peripheral CB_1R antagonists may additionally have significant clinical benefit in the treatment and prevention of fibrosis in many forms of inflammatory liver disease.

Pharmacological applications

Challenges in the development of peripheral CB_1R antagonists

Rimonabant is a CB_1R inverse agonist that was designed to target the central nervous system. In obese patients, the drug caused weight loss and improved several metabolic risk factors [72]. However, rimonabant was removed from the market because of its psychiatric side-effects, most significantly depression, anxiety and suicidal ideation [73]. To avoid these side-effects while retaining the benefits of the

drug, some groups have sought peripherally restricted CB_1R antagonists, hoping that drugs with an improved risk–benefit profile will be approved for the treatment of conditions believed to be mediated by peripheral CB_1Rs, such as obesity, steatosis and insulin resistance [74]. Indeed, peripheral CB_1R antagonists with promising efficacy have been reported, such as AM6545, currently in preclinical testing [75], and TM38837, in early clinical development [76].

A good peripheral CB_1R antagonist should not reach concentrations in the CNS that cause adverse reactions at doses giving significant efficacy. The brain is separated from the systemic circulation by the blood–brain barrier, which consists of endothelial cells differing from those in the rest of the body by having extensive tight junctions, sparse pinocytotic transport and no fenestrations [77]. Free drug concentrations are equal on both sides of a physiological membrane at steady state. Thus, for drugs with normal permeability, the free concentration in the brain will resemble the free concentration in plasma during long-term treatment, unless active transport moves drug into or out of the brain, or the drug is metabolized in the endothelial cells of the blood–brain barrier. It is the free drug that exerts the physiological effects [78]; thus, a peripherally selective drug needs to be a substrate for an active (energy-consuming) process that limits long-term brain exposure. Ideally, the drug should not have high permeability, because this counteracts the active transport. Several methods are available to estimate the free drug concentration in the brain interstitial fluid and immediately near the target receptor [79].

ATP-binding cassette transporters are ATP-dependent integral membrane proteins that translocate solutes across cellular membranes [80]. These transporters are present in several tissues; those relevant to this article move solutes across the blood–brain barrier, the blood–cerebrospinal fluid barrier and the cell membrane of hepatocytes. Among these transporters, permeability glycoprotein 1 (P-gp) is particularly important for transporting xenobiotics out of the brain across the blood–brain barrier [81]. Permeability glycoprotein 1 is an efflux pump with broad substrate specificity, implicated in limiting the access of drugs to the brain parenchyma [80]. Permeability glycoprotein 1 also reduces absorption from the gastrointestinal tract and transports some drugs and metabolites from hepatocytes into the bile [82].

Taranabant is a CB_1R inverse agonist that is not a P-gp substrate [83]; it caused psychiatric side-effects, even at low doses [84]. In contrast, AM6545 and JD5037 are CB_1R inverse agonists that are actively transported by P-gp [85]. Thus, P-gp-mediated extrusion of AM6545 and JD5037 reduces their concentration in the brain, lessening the risk of psychiatric side-effects. JD5037 has high CB_1R binding affinity (K_i = 0.35 nM) [36], which suggests that effective doses in humans would be low. JD5037 reduces appetite and weight in mice with diet-induced obesity and does so as effectively as a central CB_1R inverse agonist, SLV319 [86]. Publications have not reported the ratio of free concentration of JD5037 in brain to free concentration in plasma and have not clarified whether effects on hepatic CB_1Rs contribute in a relevant manner to the efficacy of JD5037.

Several peripheral CB_1R antagonists are under development [76], with researchers reporting often extensive studies to demonstrate reduced CNS exposure compared with first-generation drugs, such as rimonabant. However, a compound similar to rimonabant but without CNS exposure might be expected to have reduced *in vivo* efficacy, in comparison to rimonabant, with limited anticipated clinical effectiveness. Such an argument assumes that, at doses used clinically, rimonabant achieves high levels of receptor occupancy, both centrally and peripherally; thus, close to maximal efficacy is observed. However, centrally mediated effects upon food intake may arise at very low occupancy [87, 88], and dose limitations, due to psychiatric adverse events, might mean that the full peripheral effect is not realized. Furthermore, the efficiency with which these compounds block receptors in the liver, which may vary considerably between compounds, has generally not been considered, and this may significantly affect the observed efficacy of a particular compound. The liver has a major role in regulating whole-body energy and lipid homeostasis, whilst CB_1Rs in hepatocytes are believed strongly to influence enzymes controlling energy usage and storage. Thus, considering the cytosolic distribution of CB_1Rs, we propose that the ability of CB_1R antagonist drugs to maintain high unbound concentrations in hepatocytes, where they are vulnerable to metabolic clearance, may be a key determinant of *in vivo* and clinical efficacy, perhaps of greater importance than the degree of CNS exposure. Moreover, a strategy of specifically blocking CB_1Rs in the liver of patients with metabolic syndrome may have been overlooked as a therapeutic mechanism. Such a strategy would negate adverse events from CNS exposure as well as reduce side-effects from blockade of other peripheral receptors (such as those in the gut), thus providing an opportunity for safe, effective drugs.

Strategies to increase hepatoselectivity

Although G protein-coupled receptors are generally transported to the cell membrane [89], as mentioned previously, several studies have demonstrated that most CB_1Rs in various tissues are present and active in intracellular compartments, such as endosomes, lysosomes and mitochondria [39–42, 44]. Thus, a hepatoselective CB_1R antagonist should maintain a high unbound concentration in the cytosol of hepatocytes relative to the plasma and other tissues. In this context, it should be noted that a high apparent total (free and bound) concentration in the liver does not necessarily indicate good exposure. If the drug

maintains an adequate unbound concentration in the interstitial space, lipophilic compounds (such as rimonabant and taranabant) will be strongly associated with cell membranes and thus may attain apparent high concentrations in whole liver even though cytosolic free concentrations are low.

Strategies that have been employed to achieve pharmacological hepatoselectivity include producing molecules with the following characteristics: (i) they are actively transported into liver cells by liver-specific transporters, such as organic anion transporting polypeptide (OATP) transporters or organic cation transporters; (ii) they are conjugates with liver-targeting substances, such as bile acids; and (iii) they undergo metabolic activation in the liver [90].

The OATPs are believed to play an important role in drug disposition [82]. The hepatic specificity of pravastatin, a hydrophilic HMG-CoA reductase inhibitor, largely depends on OATPs [91, 92]. Pfefferkorn *et al*. report designing a glucokinase activator optimized for active liver uptake via members of the OATP family, leading to a greater than 50-fold higher substrate distribution in the liver than in the pancreas [93]. Likewise, Oballa *et al*. have designed a stearoyl-CoA desaturase inhibitor that accumulates to a higher concentration in the liver than in the skin [94].

A hepatoselective drug should be designed to have low passive permeability to reduce diffusion out of hepatocytes. To maintain a high intrahepatic concentration, it should also be metabolically stable and not a substrate for transporters that move compounds into the bile. Rimonabant and other lipophilic inverse agonists of CB_1R may not be particularly metabolically stable in hepatocytes, and little is known about how intrahepatic free concentrations vary with plasma concentrations *in vivo*.

Summary

Overall, the references of this review suggest that a CB_1R inverse agonist that achieves sufficient selectivity for hepatocytes may provide efficacy with no or negligible psychiatric side-effects. In patients with overexpression of CB_1R inside hepatocytes, as implicated in many aspects of the metabolic syndrome, a hepatoselective CB_1R antagonist could improve ATP turnover and hepatic energy state, which may in turn reduce food intake. In these patients, the hepatoselective CB_1R antagonist could ameliorate hepatic steatosis, insulin resistance, dyslipidaemia and inflammatory liver disease, as well as promote significant weight loss. We propose that long-term metabolic benefits could exceed those from rimonabant 20 mg daily in obese diabetic patients with hepatic steatosis, as well as in patients with various forms of inflammatory liver disease from other causes.

Competing Interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no support from any organization for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years; no other relationships or activities that could appear to have influenced the submitted work.

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