BJP British Journal of Pharmacology

DOI:10.1111/j.1476-5381.2011.01352.x
www.brjpharmacol.org

Themed Issue: Cannabinoids in Biology and Medicine, Part I

REVIEW

The case for peripheral CB_1 receptor blockade in the treatment of visceral obesity and its cardiometabolic complications

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Keywords
diet-induced obesity; metabolic syndrome; endocannabinoids; anandamide; 2-arachidonoylglycerol; peripheral CB_1 receptors

Received
2 December 2010
Revised
21 February 2011
Accepted
28 February 2011

In this review, we consider the role of endocannabinoids and cannabinoid-1 (CB_1) cannabinoid receptors in metabolic regulation and as mediators of the thrifty phenotype that underlies the metabolic syndrome. We survey the actions of endocannabinoids on food intake and body weight, as well as on the metabolic complications of visceral obesity, including fatty liver, insulin resistance and dyslipidemias. Special emphasis is placed on weighing the relative importance of CB_1 receptors located in peripheral tissues versus the central nervous system in mediating the metabolic effects of endocannabinoids. Finally, we review recent observations that indicate that peripherally restricted CB_1 receptor antagonists retain efficacy in reducing weight and improving metabolic abnormalities in mouse models of obesity without causing behavioural effects predictive of neuropsychiatric side effects in humans.

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Abbreviations
2-AG, 2-arachidonoylglycerol; AM6545, 5-(4-[4-Cyanobut-1-ynyl]phenyl)-1-(2,4-dichlorophenyl)-4-methyl-*N*-(1,1-dioxo-thio-morpholino-)-1*H*-pyrazole-3-carboxamide; ApoE, apolipoprotein E; CAMkinase, Ca^{2+}/calmodulin-dependent protein kinase; CB_1 receptor, cannabinoid-1 receptor; TG, triglyceride

Endocannabinoids are key mediators of the thrifty phenotype

Endocannabinoids are lipid mediators generated on demand in the cell membrane from membrane phospholipid precursors, which then act on cannabinoid receptors in the same or adjacent cells (Pacher *et al.*, 2006). The two most widely studied endocannabinoids are arachidonoyl ethanolamide (anandamide) and 2-arachidonoylglycerol (2-AG), which are ubiquitous as they are present not only in central and peripheral neurons but also in parenchymal cells of various tissues. The well-known effects of smoked marijuana provided early give-aways as to the biological functions of its endogenous counterparts. A case in point is the 'munchies', which had prompted a study that provided evidence for endocannabinoids acting via cannabinoid-1 (CB_1) receptors (drug/target nomenclature as outlined in Alexander *et al.*, 2008) being part of the leptin-regulated central neural appetitive circuitry as orexigenic mediators (Di Marzo *et al.*, 2001). This accounted for the reported ability of CB_1 receptor blockade to inhibit food intake in rodents (Colombo *et al.*, 1998; Simiand *et al.*, 1998; Williams and Kirkham, 1999; Freedland *et al.*, 2000). Together, these findings provided the impetus for testing such compounds as potential treatment for obesity. Paradoxically, subsequent epidemiological studies indicated

that although chronic regular marijuana use was associated with increased caloric intake, body/mass index was unchanged (Rodondi *et al.*, 2006) or even reduced in marijuana users (Smit and Crespo, 2001), which may be related to the unexplored role of some of the other cannabinoids present in marijuana that do not interact with CB_1 receptors.

Indeed, the first-in-class CB_1 antagonist rimonabant proved effective not only in reducing body weight, but also in improving the associated insulin resistance and dyslipidemias in obese/overweight people with the metabolic syndrome (Despres *et al.*, 2005; Van Gaal *et al.*, 2005; Pi-Sunyer *et al.*, 2006; Scheen *et al.*, 2006). In additional human studies, chronic rimonabant treatment reduced intra-abdominal and liver fat in abdominally obese subjects with atherogenic dyslipidemia (Despres *et al.*, 2009), and was also effective in improving glycemic control either as a monotherapy in drug-naïve diabetic patients (Rosenstock *et al.*, 2008) or in type II diabetics receiving insulin (Hollander *et al.*, 2010).

Such findings, along with other 'energy conserving' effects of cannabinoids such as hypothermia, hypomotility, reduced sympathetic tone and promotion of sleep (Pacher *et al.*, 2006), could suggest that endocannabinoids are key mediators of the 'thrifty' phenotype, which helped evolutionary survival during periods of starvation. However, in our contemporary society of abundant food supplies coupled with a sedentary lifestyle, this phenotype has become the main culprit in the epidemic spread of obesity and its metabolic complications. Indications that blocking endocannabinoid action by rimonabant improves most, if not all, components of the metabolic syndrome, suggest that increased endocannabinoid 'tone' may be its unifying pathogenic feature (Di Marzo and Matias, 2005). The alternative explanation that rimonabant's effects reflect inverse agonism rather than enhanced endocannabinoid 'tone' is unlikely in view of the finding that treatment of obese mice with a neutral CB_1 antagonist produced comparable metabolic benefits (Tam *et al.*, 2010). The correlation of circulating levels of 2-AG with visceral fat mass and indicators of insulin resistance (Bluher *et al.*, 2006; Matias *et al.*, 2006; Di Marzo *et al.*, 2009) also fits into such a concept, although the relationship between CB_1 receptor activation and the very low plasma levels of endocannabinoids is unclear.

CB_1 expression and anandamide levels positively correlated with visceral adipose mass in obese-hypertensive patients (Bordicchia *et al.*, 2010). Furthermore, adipocyte-derived substances inhibit insulin signalling in human skeletal muscle via CB_1 activation (Eckardt *et al.*, 2009). Together, such findings would favour the viewpoint that the metabolic syndrome or 'syndrome X' represents a true diagnostic entity, rather than coincident but mechanistically unrelated pathologies (Reaven, 2007).

Development of CB_1 antagonists for the pharmacotherapy of the metabolic syndrome

Although appetite reduction was the original rationale for developing CB_1 antagonists for the treatment of obesity, it had soon become clear that reduced food intake is not the only – and may not even be the primary – mechanism of weight reduction, at least in preclinical models of obesity. In mice with diet-induced obesity (DIO), tolerance develops rapidly to the reduction in food intake, but not to the decrease in body weight induced by chronic treatment with rimonabant, suggesting food intake-independent effects on energy expenditure (Ravinet Trillou *et al.*, 2003). Although the mechanism(s) involved in the development of tolerance to the anorexigenic effect of CB_1 blockade are not known, the observation that endocannabinoids can both increase and decrease appetite via suppressing excitatory glutamatergic or striatal inhibitory GABAergic neurotransmission, respectively (Bellocchio *et al.*, 2010), may be relevant in this regard. Mice lacking CB_1 receptors are resistant to DIO and its metabolic consequences, despite similar caloric intake during the diet period (Ravinet Trillou *et al.*, 2004; Osei-Hyiaman *et al.*, 2005), which also points to energy metabolism being directly regulated by endocannabinoids. Indeed, endocannabinoids have been found to promote lipogenesis in adipose tissue (Cota *et al.*, 2003) and liver (Osei-Hyiaman *et al.*, 2005), whereas they inhibit fatty acid oxidation (Jbilo *et al.*, 2005; Herling *et al.*, 2008; Osei-Hyiaman *et al.*, 2008; Flamment *et al.*, 2009) and mitochondrial mitogenesis (Tedesco *et al.*, 2010). Treatment with a CB_1 antagonist has opposite effects and also promotes mitochondrial biogenesis (Tedesco *et al.*, 2008) and transdifferentiation of white to brown adipocytes (Perwitz *et al.*, 2010), which could all contribute to an increase in energy expenditure (Herling *et al.*, 2008; Osei-Hyiaman *et al.*, 2008; Tam *et al.*, 2010).

A key question that has arisen as a result of such findings is the relative importance of central versus peripheral CB_1 receptors involved in these effects. This question has considerable practical implications in light of observations that a small but significant fraction of individuals treated with rimonabant developed anxiety, depression and/or suicidal ideation (Christensen *et al.*, 2007). This had led not only to the eventual withdrawal of rimonabant from the market, but also discontinuation of the development of all CB_1 inverse agonists by Big Pharma and doubts about the therapeutic potential of CB_1 blockade (Jones, 2008). The neuropsychiatric, anhedonic side effects of global CB_1 blockade should not have been unexpected, given the fact that endocannabinoids and CB_1 receptors are obligatory components of the mesolimbic dopaminergic reward pathway that mediates both natural and drug reward (Gardner, 2002). A preclinical counterpart of this is the depression-like phenotype reported in rimonabant-treated rats (Beyer *et al.*, 2010), although in some rodent models of depression, rimonabant displayed antidepressant-like activity (Steiner *et al.*, 2008; Takahashi *et al.*, 2008). Additionally, CB_1 null mice were noted to have a reduced life span (Zimmer *et al.*, 1999), which may be related to the early onset of ageing-like changes that appear to be restricted to cognitive abilities and skin structure (Bilkei-Gorzo *et al.*, 2010).

If peripherally located CB_1 receptors do contribute to the metabolic benefit of CB_1 blockade, then limiting the access of CB_1 antagonists to the brain may improve their therapeutic index by reducing or eliminating the potential for CNS-mediated neuropsychiatric side effects, while retaining some or most of their metabolic actions. As for early ageing, there is no evidence that chronic pharmacological blockade, as opposed to life-long absence, of CB_1 causes similar effects.

Even so, a peripherally restricted CB_1 antagonist would be unlikely to influence cognitive functions.

Central versus peripheral sites of the metabolic actions of endocannabinoids

In considering the relative importance of central versus peripheral CB_1 receptors in metabolic regulation, there are some general considerations. First, CB_1 receptors are highly abundant in the mammalian, including human brain, but are also present at much lower, yet functionally relevant concentrations in many peripheral tissues involved in metabolic regulation, including adipose tissue, liver, skeletal muscle and pancreas (Pacher *et al.*, 2006). The abundance of CB_1 receptors in a given tissue is not a good predictor of their functional relevance, as increased efficiency of coupling can offset the effect of low receptor density. This is illustrated by the lack of correlation between CB_1 receptor density and CB_1-stimulated GTPγS labelling in various brain regions (Breivogel *et al.*, 1997).

Second, in peripheral tissues involved in metabolic regulation, the baseline level of CB_1 receptors is very low, but is markedly up-regulated of obesity, as seen in adipose tissue (Bensaid *et al.*, 2003; Jourdan *et al.*, 2010), liver (Osei-Hyiaman *et al.*, 2008; Jourdan *et al.*, 2010; Quarta *et al.*, 2010) and skeletal muscle (see Figure 1, page 76 in Pagotto *et al.*, 2006). This may be an important factor in the apparent increase in endocannabinoid 'tone' in obesity, which is also indicated by the ability of CB_1 antagonists to affect metabolic parameters under obese, but not under non-obese conditions. The situation is less clear in human obesity, where CB_1 expression was reportedly decreased in subcutaneous fat (Engeli *et al.*, 2005), but increased in visceral fat (Bordicchia *et al.*, 2010). In liver, CB_1 expression was reduced in the presence of steatosis in immortalized human hepatocytes (De Gottardi *et al.*, 2010), but showed a robust, >30-fold increase along with increased CB_1 immunoreactivity in liver tissue from 26 patients with non-alcoholic fatty liver disease, relative to samples of non-fatty livers (R. Bataller, pers. comm.). Although tissue levels of receptors and their ligands are usually inversely related, there are examples of an obesity-related parallel increase in endocannabinoid levels in the same tissues (Osei-Hyiaman *et al.*, 2005; 2008; Bordicchia *et al.*, 2010). This may be related to the 'autoinduction' of CB_1 expression by its own ligands (Borner *et al.*, 2007; Mukhopadhyay *et al.*, 2010).

Third, although the CNS obviously exerts regulatory control over both energy intake and peripheral energy metabolism, the neural networks are complex and involve interorgan communication via bidirectional connections among various peripheral tissues and the brain (Uno *et al.*, 2006; Imai *et al.*, 2008; Sabio *et al.*, 2008). This means that the activity in a specific circuit can be modulated, not only in the brain, but also at sensory afferent or autonomic efferent terminals in peripheral tissues. This is particularly relevant for regulation via CB_1 receptors, which are prominently expressed in peripheral terminals of sensory neurons (Burdyga *et al.*, 2004) as well as in peripheral sympathetic (Ishac *et al.*, 1996; Niederhoffer *et al.*, 2003) and parasympathetic terminals (Coutts and Pertwee, 1997).

Fourth, in mice with tissue-specific deletion of CB_1 receptors, which results in a lean phenotype, the observed changes may be secondary to the resistance of the animal to diet-induced weight gain rather than due to the tissue-specific loss

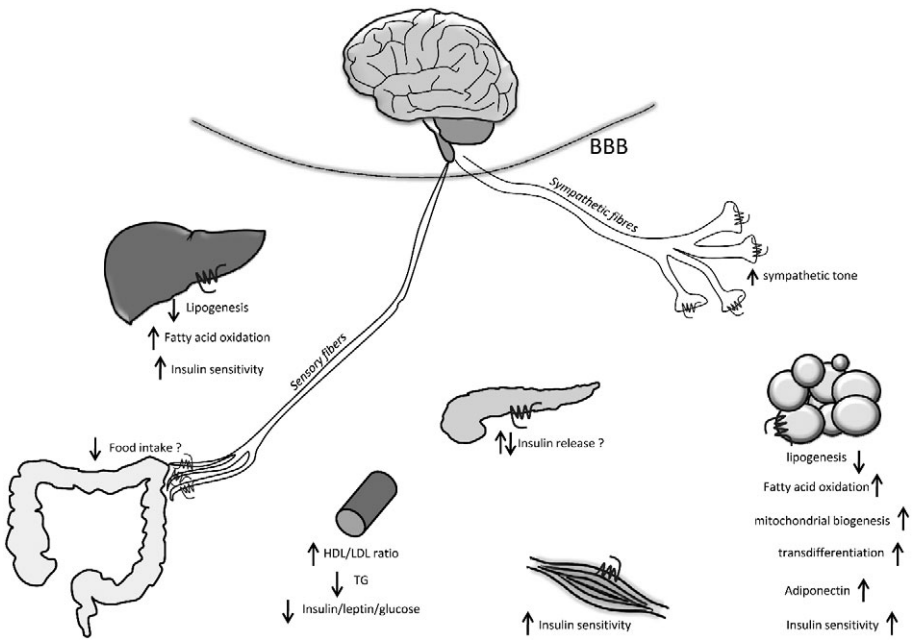

Figure 1

Therapeutically relevant effects of blockade of peripheral cannabinoid 1 (CB_1) receptors in visceral obesity/metabolic syndrome. BBB, blood/brain barrier; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; TG, triglyceride.

of CB_1 receptors. Indeed, this explanation may reconcile seemingly contradictory findings in two recent studies by the same group, where mice lacking CB_1 receptors in Ca^{2+}/calmodulin-dependent protein kinase (CAMkinase) IIα-expressing neurons, including peripheral sympathetic nerves, had a similar resistance to diet-induced obesity and metabolic complications (Quarta *et al.*, 2010) as mice with selective deletion of CB_1 receptors in adipocytes (Mancini *et al.*, 2010). With the above considerations in mind, let us examine available evidence regarding the potential sites of the metabolic actions of endocannabinoids, schematically illustrated in Figure 1.

Food intake

Endocannabinoids are part of the leptin-regulated neural circuitry in the hypothalamus (Di Marzo *et al.*, 2001), and have been implicated in the control of both the consummatory and appetitive aspects of food intake (Thornton-Jones *et al.*, 2005). Several hypothalamic sites, including the ventromedial nucleus (Jamshidi and Taylor, 2001) and the lateral hypothalamus (Jo *et al.*, 2005), as well as sites in the limbic forebrain (Kirkham *et al.*, 2002) and lower brainstem (Miller *et al.*, 2004) have been implicated in their orexigenic action. CB_1 receptors at peripheral sensory nerve terminals may also modulate food intake. In a study in rats, the food intake-reducing effect of rimonabant was abolished by capsaicin-induced sensory deafferentation (Gomez *et al.*, 2002). CB_1 receptors have been detected in the nodose ganglion, where cell bodies of sensory neurons projecting from the gut to the hypothalamus are located, and their expression was increased by fasting and reduced by re-feeding (Burdyga *et al.*, 2004), which is compatible with their involvement in the control of food intake. In contrast, selective vagal deafferentation by subdiaphragmatic vagotomy did not affect the anorexic effect of rimonabant (Madsen *et al.*, 2009). It is possible that the loss of the effect of rimonabant in capsaicin-treated animals (Gomez *et al.*, 2002) was due to elimination of non-vagal afferents by capsaicin (Madsen *et al.*, 2009). Interestingly, loss of the food intake-reducing effect of rimonabant in mice with selective knockout of CB_1 receptors in CAMkinase IIα-expressing neurons (Quarta *et al.*, 2010) may involve a similar mechanism, given the fact that CAMkinase IIα is prominently expressed not only in forebrain and peripheral sympathetic neurons (Quarta *et al.*, 2010), but also in sensory neurons (Carlton, 2002; Price *et al.*, 2005).

In another study, comparable reductions in food intake in mice were achieved at much lower levels of central CB_1 receptor occupancy using the CB_1 inverse agonist, SLV-319 (11% occupancy) than using rimonabant (65% occupancy), again suggesting a site of action outside of the brain (Need *et al.*, 2006).

Body weight

The reduction in body weight by chronic CB_1 blockade is due to reduced adipose tissue mass, which is unrelated to reduced energy intake, at least in a mouse model of diet-induced obesity (Ravinet Trillou *et al.*, 2004). In high fat-fed dogs that develop abdominal obesity similar to human visceral obesity, chronic rimonabant treatment reduced abdominal fat mass. As in mice, this was unrelated to the transient reduction in food intake and was not associated with any change in basal metabolic rate (Richey *et al.*, 2009), suggesting a peripheral mechanism. Rimonabant has been shown to increase sympathetic tone (Quarta *et al.*, 2010), which could result in decreased adipose mass due to increased β-adrenergic lipolysis. However, lipolysis in the high fat-fed dogs was unaffected by rimonabant (Richey *et al.*, 2009), which argues against a neural mechanism, at least in this model.

Insulin sensitivity

Similar to the effects on energy intake, there is evidence for both central and peripheral sites of action for the modulation of insulin sensitivity by endocannabinoids. The *in vivo* site of action of insulin itself is a matter of debate. Insulin suppresses hepatic glucose production and increases tissue uptake of glucose, but the question of whether its actions *in vivo* are primarily via receptors in target tissues (Michael *et al.*, 2000) or via insulin receptors in the brain, which would then influence target organ responses indirectly via neural pathways (Buettner *et al.*, 2005), has not been definitively settled.

Similarly, anandamide may affect hepatic insulin sensitivity via an action in the mediobasal hypothalamus, where it may mediate the insulin resistance induced by short-term overfeeding (O'Hare *et al.*, 2010). On the other hand, mice with liver-specific knockout of CB_1 receptors are protected from the insulin resistance induced by chronic exposure to a high-fat diet, even though they become as obese as wild-type mice on the same diet (Osei-Hyiaman *et al.*, 2008). This latter finding indicates that hepatic CB_1 receptors play a major role in the obesity-related, weight-independent component of insulin resistance. These two findings are not necessarily mutually exclusive: insulin resistance induced by chronic high-fat diet is associated with up-regulation of hepatic CB_1 receptors, and both changes are normalized by CB_1 antagonist treatment (Osei-Hyiaman *et al.*, 2008; Jourdan *et al.*, 2010; Quarta *et al.*, 2010). Short-term overfeeding may not be sufficient to induce up-regulation of hepatic CB_1 receptors, thus minimizing their involvement in insulin resistance. Insulin resistance in DIO rats was reversed by systemic but not intracerebroventricular administration of rimonabant, which also points to the dominant role of peripheral CB_1 receptors in this effect, in established obesity (Nogueiras *et al.*, 2008). The finding that a peripherally restricted CB_1 antagonist was equieffective with rimonabant in reversing insulin resistance in DIO mice (14 weeks on high-fat diet) indicates that central endocannabinoid mechanisms play minimal – if any – role in obesity-related insulin resistance (Tam *et al.*, 2010).

Recent preliminary observations indicate that in mice with adipocyte-specific deletion of CB_1 receptors, high-fat diet fails to induce glucose intolerance and insulin resistance (Mancini *et al.*, 2010). As pointed out above, these mice also remain lean and do not develop steatosis, so their resistance to diet-induced impairment in insulin sensitivity may be secondary to the absence of the obese phenotype, rather than a direct effect of adipocyte CB_1 receptors in the control of insulin sensitivity. One possible mechanism by which adipocyte CB_1 receptors may indirectly influence insulin sensitivity is via adiponectin. The adipocyte-derived protein, adiponectin is known to increase insulin sensitivity, and obesity is often associated with reduced plasma adiponectin levels.

Rimonabant increases plasma adiponectin levels in obese individuals (Despres *et al.*, 2005), or adiponectin synthesis and secretion in cultured rat adipocytes (Bensaid *et al.*, 2003), although CB_1 regulation of adiponectin production has not been documented in human adipocytes. This effect of rimonabant may contribute to the insulin-sensitizing action of CB_1 blockade, as suggested by the reduced effectiveness of rimonabant in reversing diet-induced insulin resistance in adiponectin knockout mice (Migrenne *et al.*, 2009; Watanabe *et al.*, 2009). Another possible peripheral mechanism involves the blockade of CB_1 receptors in adipose tissue macrophages. Macrophage infiltration into adipose tissue has been implicated in obesity-related insulin resistance (Weisberg *et al.*, 2003). Recent findings indicate that rimonabant inhibits the ability of lipopolysaccharide-activated macrophages to inhibit insulin signalling by reducing their production of the inflammatory cytokine tumour necrosis factor α and increasing the production of the anti-inflammatory cytokine interleukin-10 (Miranville *et al.*, 2010). CB_1 antagonists also counteract CB_1-mediated increases in reactive oxygen species production by macrophages (Han *et al.*, 2009), or the vascular inflammation that accompanies certain diabetic complications (El-Remessy *et al.*, 2011), although an elevation of pro-inflammatory cytokine levels in the CNS of rimonabant-treated rats has also been reported (Beyer *et al.*, 2010).

Hepatic steatosis

Activation of hepatic CB_1 receptors induces lipogenic gene expression (Osei-Hyiaman *et al.*, 2005; Ruby *et al.*, 2008; Son *et al.*, 2009; Jourdan *et al.*, 2010) and promotes *de novo* hepatic lipogenesis (Osei-Hyiaman *et al.*, 2005), whereas fatty acid oxidation in the liver is inhibited by CB_1 receptors (Osei-Hyiaman *et al.*, 2008). These effects, however, only modestly contribute to diet-induced accumulation of triglycerides (TGs) in the liver, because although liver-specific CB_1 knockout mice are partially protected from diet-induced steatosis (Osei-Hyiaman *et al.*, 2008), mice with transgenic re-expression of hepatic CB_1 receptors on a global CB_1 knockout background remain largely resistant to the steatotic effect of a high-fat diet (Tam *et al.*, 2010). The major source of hepatic TGs is likely to be fatty acids transferred from adipose tissue (Jourdan *et al.*, 2010), where they are generated through CB_1-mediated activation of adipocyte lipoprotein lipase (Cota *et al.*, 2003) and released via 'spillover' into the circulation (Miles *et al.*, 2004). This is also compatible with the recent finding that adipocyte-specific CB_1 knockout mice are resistant to diet-induced steatosis (Mancini *et al.*, 2010).

Plasma lipid profile

CB_1 antagonist treatment results in improved plasma lipid profile (Despres *et al.*, 2005; Van Gaal *et al.*, 2005; Pi-Sunyer *et al.*, 2006), which is likely mediated via peripheral CB_1 receptors. In a mouse model of acutely increased endocannabinoid tone, the increase in tissue 2-AG levels elicited by treatment with a monoacylglyceride lipase inhibitor was associated with elevated plasma TG and cholesterol levels, as well as an accumulation of apolipoprotein E (ApoE)-depleted TG-rich lipoproteins. These effects were absent in $CB_1^{-/-}$ and $ApoE^{-/-}$ mice and were reversed by rimonabant in wild-type mice, and could be attributed to reduced TG clearance mediated by peripheral CB_1 receptors (Ruby *et al.*, 2008). TG secretion was not affected in this acute model. However, when endocannabinoid tone is chronically increased, such as in DIO mice, a peripherally restricted CB_1 antagonist induced an acute increase in secretion of TG-rich very-low-density lipoproteins, which implicated hepatic CB_1 receptors in this effect (Tam *et al.*, 2010).

The therapeutic potential of peripherally restricted CB_1 antagonists

The likely contribution of peripheral CB_1 receptors to the metabolic effects of endocannabinoids coupled with the undisputed role of central CB_1 receptors in their hedonic effects has been the motivating factor for the development of second-generation CB_1 antagonists with limited brain penetration for the treatment of the metabolic syndrome. Compounds with high CB_1 potency (K_d < 10 nM) and low brain penetrance (plasma : brain ratio < 10%) have been reported (McElroy *et al.*, 2008; Receveur *et al.*, 2010; Tam *et al.*, 2010), with 5-(4-[4-Cyanobut-1-ynyl]phenyl)-1-(2,4-dichlorophenyl)-4-methyl-*N*-(1,1-dioxo-thio-morpholino-)-1*H*-pyrazole-3-carboxamide (AM6545) being the first such compound undergoing detailed pharmacological, metabolic and behavioural assessment in mouse models of obesity.

AM6545 is a structurally modified analogue of rimonabant. Its CB_1 binding affinity (K_d: 3.3 nM) and CB_1/CB_2 selectivity (~200-fold) is similar to that of rimonabant, but unlike the inverse agonist rimonabant, AM6545 is a neutral antagonist, as revealed by GTPγS binding assays (Tam *et al.*, 2010). AM6545 has very low brain penetrance (3–7% of plasma level following either acute or chronic administration compared with ~80% for rimonabant), due to reduced lipophilicity as well as P-glycoprotein-mediated extrusion from the brain. Unlike rimonabant, AM6545 does not affect behavioural responses mediated by CB_1 receptors in the brain, including catalepsy, hypomotility and the centrally mediated component of cannabinoid-induced hypothermia. It is also devoid of the anxiogenic effect of rimonabant, as tested in the elevated plus maze, and causes only a minor and transient reduction in food intake. At the dose used (10 $mg \cdot kg^{-1}$ i.p.), AM6545 completely blocked the anandamide-induced inhibition of upper gastrointestinal motility mediated by CB_1 on cholinergic terminals innervating the gut, indicating full occupancy of peripheral CB_1 receptors.

In DIO mice, chronic treatment with 10 $mg \cdot kg^{-1} \cdot day^{-1}$ AM6545 for 28 days caused a significant, 12% weight reduction without affecting caloric intake, as compared with a 21% reduction achieved by the same dose of rimonabant, the difference likely due to centrally mediated reduction of food intake by rimonabant. However, AM6545 is equieffective or only slightly less efficacious than rimonabant in improving glucose tolerance and insulin sensitivity, reversing fatty liver and improving the plasma lipid profile of DIO mice. Similar metabolic effects were observed in genetically obese *ob/ob* mice in which AM6545 did not affect body weight, indicating that the metabolic effects are weight-independent. These effects are due to CB_1 blockade in peripheral tissues, including the liver. The role of hepatic CB_1 receptors in glycemic control

is indicated by the finding that $CB_1^{-/-}$ mice with transgenic re-expression of CB_1 restricted to hepatocytes develop insulin resistance on a high-fat diet, which is reversed by AM6545 treatment.

The ability of AM6545 to reduce body weight in DIO, but not in leptin-deficient *ob*/*ob* mice suggests that this effect is due to the reversal of the peripheral-type leptin resistance that accompanies diet-induced obesity. Leptin is known to suppress lipogenic gene expression in adipose tissue independently of its anorectic effect. Therefore, the observation that AM6545 treatment suppressed lipogenic gene expression in visceral and subcutaneous fat of DIO, but not of *ob*/*ob* mice, is compatible with the role of endogenous leptin in these effects. Leptin was found to decrease endocannabinoid levels in adipose tissue (Matias *et al*., 2006; Buettner *et al*., 2008), which could be involved in its ability to reduce lipogenic gene expression in adipocytes.

More recently, we tested a highly potent CB_1 inverse agonist with very low brain penetrance. Preliminary – as yet unpublished – findings in our laboratory indicate that, similar to the neutral antagonist AM6545, the CB_1 inverse agonist is effective in reversing diet-induced hepatic steatosis, glucose intolerance and dyslipidemias in mice without causing behavioural effects that are normally seen following blockade of CB_1 receptors in the CNS. Relative to AM6545, the inverse agonist is much more efficacious in reducing body weight and in reversing insulin resistance, suggesting the importance of inverse agonism in these latter effects.

Conclusions

There is growing evidence for an important role of peripherally located CB_1 receptors in metabolic regulation, which has gained further support by the pharmacological profile of novel, peripherally restricted CB_1 antagonists. Compounds with limited brain penetrance retain efficacy in improving the hormonal/metabolic complications of obesity, but are devoid of behavioural effects that result from blocking CB_1 receptors in the brain. Among peripherally restricted compounds, CB_1 inverse agonists may offer distinct advantages over neutral antagonists, particularly as far as weight reduction and insulin sensitization are concerned. The improved therapeutic profile of such compound, due to the greatly reduced risk of neuropsychiatric side effects, warrants their clinical testing for the treatment of obesity and its metabolic complications, including fatty liver disease, insulin resistance and dyslipidemias.

Acknowledgements

Work in the authors' laboratory was supported by the National Institutes of Health (intramural funds from National Institute on Alcohol Abuse and Alcoholism to G. Kunos).

Conflict of interest

The authors declare that no conflict of interest exists.

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