Role of the endocannabinoid system in food intake, energy homeostasis and regulation of the endocrine pancreas

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ABSTRACT

The endocannabinoid system (ECS) is a signalling cascade consisting of CB1 and CB2 receptors, and enzymes for the synthesis and degradation of endogenous ligands for these receptors. Central CB1 receptors have been most widely studied since they play key roles in energy homeostasis and rimonabant, a CB1 receptor antagonist, was used clinically to treat obesity. Less is known about CB2 receptors, but their abundant expression by lymphocytes and macrophages has led to suggestions of their importance in immune and inflammatory reactions. More recently, it has become apparent that both CB1 and CB2 receptors are more widely expressed than originally thought, and the capacity of endocannabinoids to regulate energy balance also occurs through their interactions with cannabinoid receptors on a variety of peripheral tissues. In general, pathological overactivation of the ECS contributes to weight gain, reduced sensitivity to insulin and glucose intolerance, and blockade of CB1 receptors reduces body weight through increased secretion of anorectic signals and improved insulin sensitivity. However, the notion that the ECS per se is detrimental to energy homeostasis is an oversimplification, since activation of cannabinoid receptors expressed by islet cells can stimulate insulin secretion, which is obviously beneficial under conditions of impaired glucose tolerance or type 2 diabetes. We propose that under normal physiological conditions cannabinoid signalling in the endocrine pancreas is a bona fide mechanism of regulating insulin secretion to maintain blood glucose levels, but that energy balance becomes dysregulated with excessive food intake, leading to adipogenesis and fat accumulation through enhanced cannabinoid synthesis.

Contents

1. Introduction

Type 2 diabetes mellitus (T2DM) is an increasingly common, chronic disorder of fuel storage and metabolism. It has been estimated that 285 million people world-wide will have diabetes in 2010 and that number is predicted to rise to 438 million by 2030 (Diabetes Atlas, 4th Edition, 2009). The chronic hyperglycaemia associated with T2DM leads to the development of devastating secondary complications in the macro- and micro-vascular systems, and these complications are responsible for reduced quality of life, greatly increased morbidity, premature mortality and considerable health-care costs. T2DM is a heterogeneous disorder in which a combination of genetic susceptibility and environmental factors, particularly obesity, generates a pathology in which the insulin-producing pancreatic β-cells are unable to secrete sufficient insulin to meet the demands of insulin-resistant target tissues. The subsequent failure of liver, muscle and fat to regulate fuel homeostasis leads to hyperglycaemia and dyslipidaemia which, in turn, have further deleterious effects on β-cell function and survival, exacerbating the progression of the disease. The metabolic dysfunctions associated with T2DM therefore involve the functions of many tissues and organs, including the regulation of food intake by the central nervous system, absorption of nutrients from the gastrointestinal tract, and their uptake and storage in liver, skeletal muscle and adipose tissue. In this review we will consider the roles of the endocannabinoid system in these processes, paying particular attention to the β-cells of the endocrine pancreas because of their key regulatory role in fuel homeostasis and because they offer an excellent pharmacological target for the development of new therapies for T2DM.

2. The endocannabinoid system

The physiological effects of *Cannabis sativa* have been known for centuries and it is now clear that they arise from the interaction between Δ^9-tetrahydrocannabinol (THC), the main active constituent of cannabis, and the so called cannabinoid receptors. Three subtypes of cannabinoid receptor have been identified to date: CB1 (Matsuda et al., 1990), CB2 (Munro et al., 1993) and GPR55, which has been classified as a novel cannabinoid receptor (Begg et al., 2005; Baker et al., 2006; Mackie & Stella, 2006; Ryberg et al., 2007). Despite each receptor being highly conserved across species, the amino acid sequences of the cannabinoid receptors show a relatively low level of homology among the receptor subtypes. Thus, CB1 and CB2 receptors only share 44% overall resemblance (68% at the active site) and GPR55 shows a mere 13.5% and 14.4% similarity to CB1 and CB2 receptors respectively (Begg et al., 2005; Lauckner et al., 2008). All three cannabinoid receptors belong to the G protein-coupled receptor (GPCR) superfamily and are expressed by numerous cell populations.

The CB1 receptor, initially identified in rat brain, is one of the most abundantly expressed GPCRs in the central nervous system, particularly in regions such as the cerebral cortex, hippocampus, basal ganglia and cerebellum (Lopez de Jesus et al., 2006). It is also expressed peripherally in organs including the testes, prostate, heart, lung and bone marrow (Howlett et al., 2002). The CB2 receptor, characterised first in human HL60 leukaemic cells, is mainly expressed in immune tissues and cells, such as the marginal zone of the spleen, macrophages, lymph nodes and microglia, with lower expression in non-immune cells (Munro et al., 1993; Walter et al., 2003; Van Sickle et al., 2005). More recently, both central and peripheral mRNA expression of the third cannabinoid receptor, GPR55, has been reported in mice (Ryberg et al., 2007). The wide distribution of cannabinoid receptors is consistent with the suggested physiological functions of the ECS, such as modulation of alertness, cognition, immunosuppression, locomotion and satiety (Dewey, 1986).

Two phospholipid-derived compounds, N-arachidonoyl ethanolamine (anandamide, AEA), extracted from porcine brain, and 2-arachidonoylglycerol (2-AG), from canine intestine, were characterised as having cannabimimetic properties and identified as the endogenous ligands of cannabinoid receptors (Devane et al., 1992; Mechoulam et al., 1995; Sugiura et al., 1995). They are thus known as endocannabinoids. A number of studies have shown that 2-AG and AEA are produced locally in peripheral tissues including adipocytes, hepatocytes, skeletal muscle, the gastrointestinal tract and endocrine pancreas (Sugiura et al., 2000; Di Marzo et al., 2009). They are synthesised on demand by diacylglycerol lipases (DAG-lipases) and N-acyl-phosphatidylethanolamine phospholipase D (NAPE-PLD) respectively, and degraded once the stimulation ceases via the enzymatic activities of monoacylglycerol lipase (MGL) and fatty acid amide hydrolase (FAAH; Prescott & Majerus, 1983; Goparaju et al., 1999). These biosynthetic and degrading pathways enable the system to exert its regulatory activity with spatial and temporal specificity. Collectively, the endocannabinoid system (ECS) is composed of the cannabinoid receptors, their endogenous ligands and the enzymes that are responsible for endocannabinoid metabolism (Fig. 1).

AEA has a greater affinity towards CB1 receptors than 2-AG and lacks CB2 receptor potency, so is therefore generally regarded as an endogenous CB1 receptor ligand (Di Marzo, 1998). However, the content of AEA is ~800 times less than that of 2-AG in rat brain, so endogenous levels of AEA are not considered sufficiently high to activate CB1 receptors in the brain (Sugiura & Waku, 2000; Sugiura et al., 2000). To the contrary, 2-AG, which has been extracted and identified in numerous tissue types, activates both CB1 and CB2 receptors. The relatively higher tissue content of 2-AG may compensate for its lower CB1 receptor potency and it has been suggested that it provides a housekeeping level of endocannabinoid for the maintenance of receptor activity (Sugiura & Waku, 2000). Thus, 2-AG is considered the major endocannabinoid in mammalian physiology. In addition, over the years, several other compounds including 2-arachidonoyl-glyceryl-ether (noladin ether) (Hanus et al., 2001), O-arachidonoyl-ethanolamine (virodhamine) (Porter et al., 2002), N-arachidonoyl-dopamine (Huang et al., 2002) and oleamide (Leggett et al., 2004) have also been reported to exert cannabimimetic activities. These lipid mediators are believed to act, at least in part, through cannabinoid receptors or by inhibiting endocannabinoid degrading enzymes to exert corresponding physiological effects (Smart et al., 2002; Bradshaw & Walker, 2005).

3. Cannabinoid receptor coupling

There is little information available on signalling downstream of GPR55, but it is known that the conventional cannabinoid receptors are coupled to $G_{i/o}$-proteins and their activation can result in inhibition of adenylate cyclase (AC) activity and decreased Ca^{2+} influx through voltage-gated Ca^{2+} channels (VGCCs). The reduction in cyclic AMP generation following inhibition of AC can be dissociated from the decreased Ca^{2+} influx since inhibition of Ca^{2+} currents still occurred in the presence of an exogenous cyclic AMP analogue capable of activating protein kinase A (Mackie et al., 1993). Cannabinoid-induced inhibition of VGCCs is reported to be secondary to decreased excitability following activation of a pertussis toxin (PTX)-sensitive inwardly rectifying K^+ conductance (Mackie et al., 1995). The inhibition of Ca^{2+} channel activity appears to be restricted to CB1 receptors since it was not observed in cells over-expressing CB2 receptors (Felder et al., 1995).

In contrast to the observations that CB1 receptors are coupled to inhibition of Ca^{2+} influx, there are reports that cannabinoids increase intracellular Ca^{2+} ([Ca^{2+}]$_i$). Thus, the endocannabinoid 2-AG induced elevations in [Ca^{2+}]$_i$ in neuroblastoma-glioma hybrid NG108-15 cells via CB1 receptor activation (Sugiura et al., 1996) and in human HL60 cells through CB2 receptor stimulation (Sugiura et al., 2000). Increased Ca^{2+} was also detected in N18TG2 neuroblastoma cells following cannabinoid receptor activation (Sugiura et al., 1997), but not in C6 glioma cells (Sugiura et al., 1997) or CHO cells transfected with CB1 or CB2 receptors (Felder et al., 1992). It therefore appears that the effects of cannabinoid receptor agonists on [Ca^{2+}]$_i$ depend on the cell type and
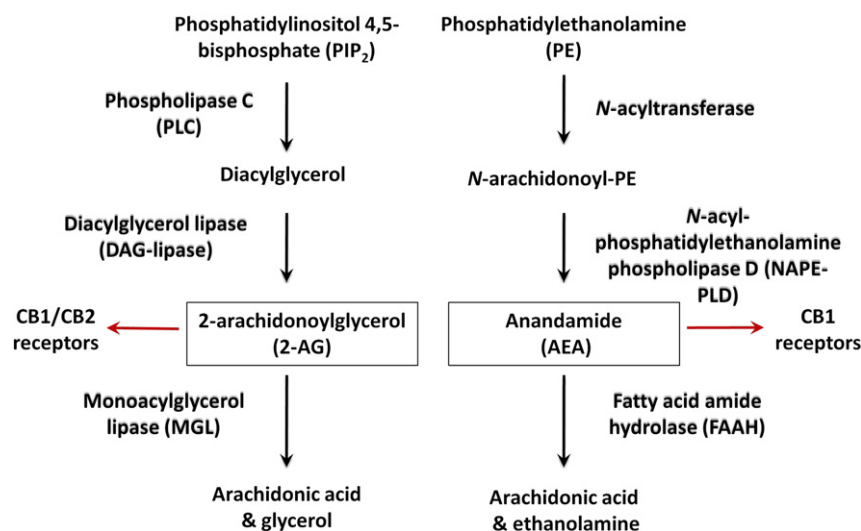
Fig. 1. Metabolic pathways regulating 2-AG and anandamide synthesis and degradation. The figure illustrates the primary pathways involved in 2-AG (left) and anandamide (right) synthesis and degradation. 2-AG is produced on demand via the action of DAG-lipase from diacylglycerol, a downstream product of phospholipase C-mediated degradation of PIP$_2$, and it is hydrolysed by MGL to arachidonic acid and glycerol. Similarly, anandamide is synthesised from a phospholipid precursor, N-arachidonoyl-PE, which is generated from phosphatidylethanolamine (PE) through the catalytic activity of N-acyltransferase. The main enzyme involved in anandamide formation is a phospholipase D (NAPE-PLD) and the degradation of the endocannabinoid to arachidonic acid and ethanolamine is catalysed by FAAH.

receptor subtype under investigation and the experimental conditions being used. The elevations in Ca^{2+} in response to cannabinoid receptor activation may be via influx through L-type VGCCs following their phosphorylation by PKA or PKC (Rubovitch et al., 2002), or mobilisation from intracellular stores since the 2-AG-induced increase in Ca^{2+} was inhibited by a phospholipase C (PLC) inhibitor (Sugiura et al., 1997). A CB1 receptor-activated G$_{q/11}$-coupled Ca^{2+}-sensitive PLC pathway, which exerted a positive impact on [Ca^{2+}]$_i$ without affecting cyclic AMP levels, has been identified in CB1 receptor-expressing HEK 293 cells and cultured hippocampal neurons (Lauckner et al., 2005).

Both CB1 and CB2 receptors stimulate the p42/p44 mitogen-activated protein kinase (MAPK) cascade in a PTX-sensitive manner, indicating that this effect is via G$_{i/o}$ (Bouaboula et al., 1995, 1996). CB1 receptor activation also leads to activation of p38 MAPK in rodent hippocampus, an effect that is abolished in CB1 knockout mice (Derkinderen et al., 2001), and p38 MAPK activation was also observed in Jurkat leukaemia cells via CB2 receptors (Herrera et al., 2005). The precise mechanism by which MAPKs are activated has not been established, but there is evidence that cannabinoids activate the p42/p44 MAPK upstream kinase Raf, possibly through regulating phosphatidylinositol 3-kinase (PI 3-K)-mediated protein kinase B (PKB) activity (Sanchez et al., 1998, 2003; Lipina et al., 2010). The stimulation of p42/p44 MAPK activities by cannabinoids is reported to increase proliferation of cancer cells and it has been speculated that this may contribute to cancer progression (Hart et al., 2004). Conversely, p38 MAPK activation is associated with apoptosis through increased caspase activity (Herrera et al., 2005), and the pro-apoptotic function of cannabinoids has led to them being considered for development as anti-cancer agents (Guzman, 2003).

Although most studies have demonstrated that CB1 and CB2 receptor agonists inhibit AC activity and stimulate MAPK cascades, and that the receptor subtypes possess similar biochemical properties (reviewed by Demuth & Molleman, 2006; Howlett et al., 2002), differences do exist in downstream signalling via CB1 and CB2 receptors. Thus, CB1 receptor coupling to G$_s$-proteins has been reported in PTX-treated CHO cells transfected with human CB1 receptors (Bonhaus et al., 1998) and striatal neurons (Glass & Felder, 1997) or human embryonic kidney (HEK) 293 cells (Jarrahian et al., 2004) overexpressing dopamine D$_2$ receptors. These studies suggest that the preferential coupling of CB1 receptors is to G$_i$ to inhibit AC, but under conditions of reduced availability of G$_i$, such as following

expression of G$_i$-coupled D$_2$ receptors or where G$_{i/o}$ have been ADP-ribosylated by PTX treatment, this coupling may be compromised allowing transduction via G$_s$. Similar stimulatory effects on cyclic AMP levels have not been observed in CB2 receptor-expressing CHO cells following PTX treatment (Glass & Felder, 1997). Stimulation of Ca^{2+} influx by the cannabinoid agonist desacetyl-levonantradol in N18TG2 cells may be via G$_s$-coupled AC activation since it was abolished in the presence of a PKA inhibitor (Rubovitch et al., 2002). As for other GPCR families it seems that the intracellular signalling cascades activated by cannabinoid receptor agonists differ between different cell types. A schematic summary of the key signalling elements downstream of CB1 and CB2 receptor activation is shown in Fig. 2.

4. Regulation of food intake via CB1 receptors

THC-induced changes in human feeding behaviour and body weight were first investigated in the late 1970s, when stimulatory effects of THC on appetite and food intake were demonstrated (Greenberg et al., 1976). The endocannabinoids AEA and 2-AG were also reported to promote hyperphagia (Kirkham et al., 2002), confirming the key involvement of the endocannabinoid system in the regulation of food intake. Furthermore, since the mid 1980s, the U.S. Food and Drug Administration has approved the use of THC (known as Dronabinol) as an orexigenic drug for the treatment of chemotherapy-induced nausea and weight loss in cancer patients, AIDS-associated anorexia in HIV-affected individuals and in Alzheimer's disease (Pagotto et al., 2006). Conversely, in 2006 the CB1 receptor antagonist rimonabant (known as Acomplia) was approved for clinical use in the UK as an anti-obesity agent. Despite being withdrawn from the market due to its adverse psychological effects in 2008, improvement in metabolic parameters including lipid profile and insulin sensitivity was identified following its administration (Scheen & Paquot, 2009). Thus, clinical use of rimonabant, an antagonist of the endocannabinoid system, fuelled further investigation on the role of endocannabinoids in the regulation of food intake and fuel homeostasis.

4.1. Central effects of CB1 receptors on food intake

CB1 receptors are found in the olfactory bulb, cortical regions (neocortex, pyriform cortex, hippocampus and amygdala), and several parts of the basal ganglia, thalamic and hypothalamic nuclei,
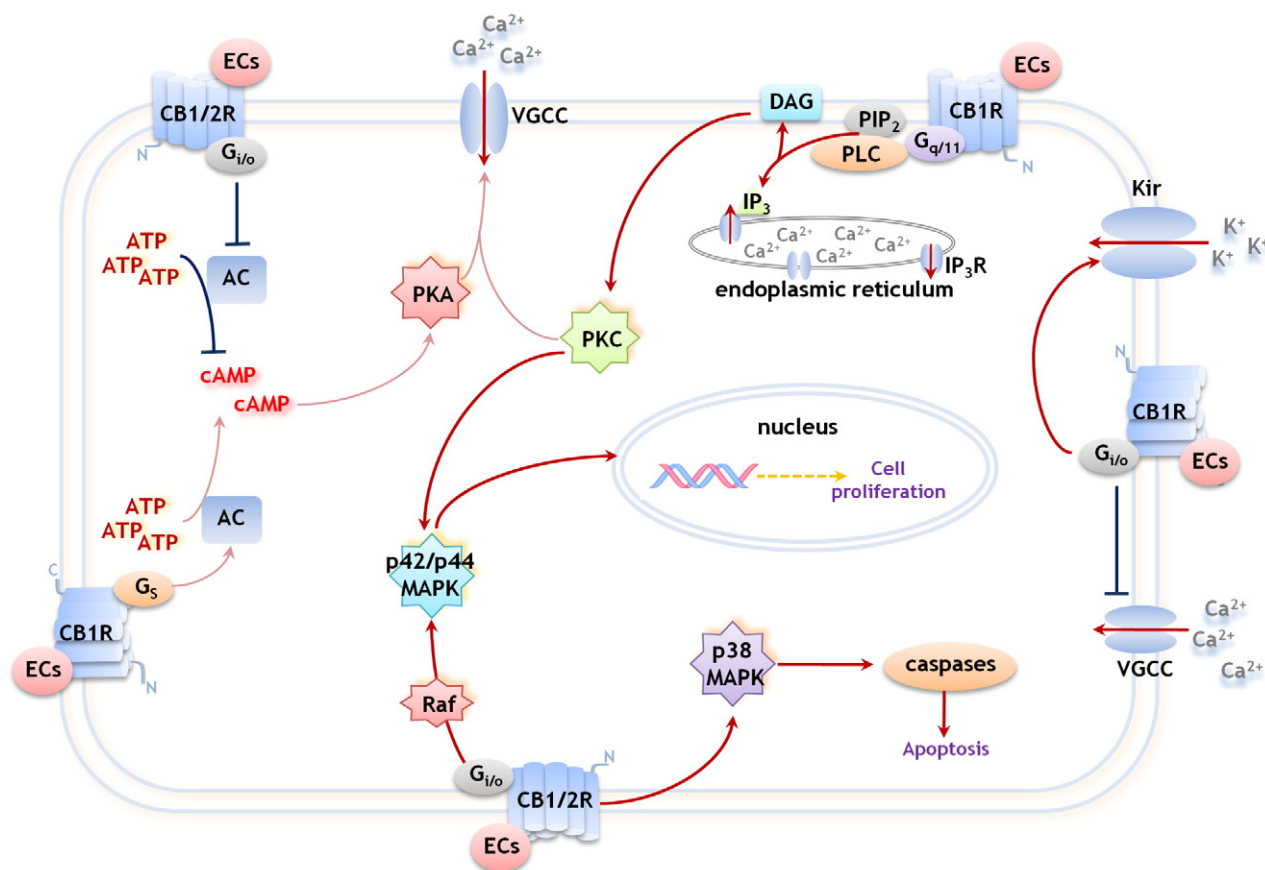
Fig. 2. Signalling pathways downstream of cannabinoid receptor activation. The schematic shows the main pathways activated by CB1 and CB2 receptors following binding of endocannabinoids (ECs). A red solid arrow shows CB1 receptor-induced activation of an inwardly rectifying potassium channel (Kir) current that decreases excitability, resulting in inhibition of voltage gated calcium channels (VGCC) and inhibition of Ca^{2+} influx, as shown by the solid blue line. Red solid arrows also demonstrate activation of p38 and p42/p44 mitogen activated protein kinases (MAPKs) following CB1/2 receptor activation. The p42/p44 isoforms of MAPK can stimulate cellular proliferation, while p38 MAPK activates caspases to induce apoptosis. The solid blue line downstream of CB1 and CB2 receptor-evoked $G_{i/o}$ activation indicates inhibition of adenylate cyclase (AC) and subsequent reductions in cyclic AMP (cAMP). The lighter pink lines demonstrate that under certain circumstances CB1 receptors may stimulate AC via G_s and the increased cyclic AMP can activate PKA, which may phosphorylate VGCCs to allow Ca^{2+} influx (pink lines). Another pathway through which Ca^{2+} may be elevated is via CB1 receptor activation of phospholipase C (PLC) via $G_{q/11}$ to generate inositol 1,4,5-trisphosphate (IP$_3$) and diacylglycerol (DAG) through phosphatidyl bisphosphate (PIP$_2$) hydrolysis (solid red arrows). IP$_3$ mobilises Ca^{2+} from the endoplasmic reticulum following binding to IP$_3$ receptors (IP$_3$R) and DAG activates protein kinase C (PKC) which may phosphorylate VGCCs to allow Ca^{2+} influx. PKC may also activate p42/44MAPKs to induce cell proliferation.

cerebellar cortex and brainstem nuclei (Cota, 2007). Importantly, CB1 receptors are abundant in CNS areas involved in reward/reinforcement circuitry, leading to the proposal that endocannabinoid levels increase with prolonged interval between meals, thus amplifying signals for hunger and consequently encouraging feeding (Gardner & Vorel, 1998). Indeed, several reports have shown a gradual elevation of 2-AG and AEA between meals (Williams & Kirkham, 2002), which was supported by the observation of increased 2-AG during fasting and its subsequent decline after feeding (Kirkham et al., 2002). In contrast, in the same experiments where hypothalamic 2-AG levels correlated with feeding, the local concentration of 2-AG in the cerebellum of those animals remained unchanged, implying regional differences of cannabinoid activity. It also confirmed the nature of 'on demand' synthesis of the endocannabinoids.

CB1 receptors are reported to co-localise with other receptors in the CNS whose activities are essential in the processes of feeding and satiety (Spanagel & Weiss, 1999). For example, the dopaminergic system, which is involved in reward regulation, interacts with CB1 receptors (Melis et al., 2004). In addition, co-localisation of dopamine receptors (D1 and D2) and CB1 receptors was reported in mouse hippocampus (CB1 and D2), striatum and olfactory tubercle (CB1, D1 and D2) (Hermann et al., 2002), and a correlation between limbic forebrain endocannabinoid and dopamine levels and feeding of

palatable food believed to stimulate activity in the region was also demonstrated (Gardner & Vorel, 1998; Bisogno et al., 1999).

As well as stimulating food intake the endocannabinoids inhibit the release of other appetite regulatory neurotransmitters and neuropeptides such as opioids (Gallate & McGregor, 1999; Corchero et al., 2004), serotonin (Rowland et al., 2001) and γ-aminobutyric acid (GABA) (Jo et al., 2005), mainly via the activity of CB1 receptors in the hypothalamus (Di Marzo et al., 2009). Not surprisingly then, the activity of the ECS is subject to regulation by several hormones, including cholecystokinin (CCK), ghrelin, glucocorticoids and leptin, which are also produced and act peripherally and thus co-ordinate central–peripheral communication to maintain energy balance (Di Marzo, 2009).

5. Peripheral effects of cannabinoid receptors on energy homeostasis

In addition to controlling food consumption and satiety via the CNS, the ECS also regulates energy expenditure in peripheral organs. Several studies have demonstrated that the effects of CB1 receptor antagonism on appetite and metabolism are not limited to the central role of CB1 receptor blockade. Thus, rimonabant-induced weight loss in rats persisted independently of decreased food intake (Colombo

et al., 1998) and resistance to diet-induced obesity has also been reported in mice that lack functional CB1 receptors, with no significant alteration in energy intake (Ravinet Trillou et al., 2004). Moreover, pair-feeding experiments with CB1 receptor-deficient and wild type mice further demonstrated that reductions in food intake and body fat observed in adult CB1 receptor-deficient mice could be attributed in part to peripheral mechanisms (Cota et al., 2003). These observations suggest that the peripheral effects of rimonabant are responsible, at least in part, for the positive impact of CB1 receptor blockade on the regulation of metabolism and energy expenditure. In fact, it is now widely acknowledged that the ECS also acts directly to regulate peripheral processes such as gastric emptying, lipogenesis and glucose uptake (Cota, 2007) through cannabinoid receptors expressed by the gastrointestinal (GI) tract, adipose tissue and skeletal muscle. In this way, signals from these peripheral organs can be collectively converged and fed back centrally, allowing the brain to constantly monitor the metabolic state of an organism (Flier, 2004). The ECS therefore functions via CB1 receptors as a communicative circuit that correlates central and peripheral signalling mechanisms, which ultimately controls energy expenditure and food intake as outlined later and summarised in Fig. 3.

5.1. Gastrointestinal tract

Identification of CB1 receptors throughout the GI tract in mice suggested a regulatory role for this receptor in gut function (Casu et al., 2003). It is now known that CB1 receptors are mainly localised in the intrinsic (enteric) and extrinsic neurons, where the release of neurotransmitters is modulated, indicating their involvement in gut-neuronal signalling (Duncan et al., 2005). Indeed, ghrelin, produced both by the GI tract and CNS, increased 2-AG levels in mouse hypothalamus (Kola et al., 2008). The expression of CB1 receptors in rat vagal fibres is negatively regulated by another regulatory peptide, CCK, which is also produced by the GI system (Burdyga et al., 2006). In addition, CB1 receptor agonists also inhibit gastric acid secretion in rats (Adami et al., 2002) and intestinal motility in humans (Esfandyari et al., 2007). CB2 receptors, which are expressed by activated macrophages in the intestine, are believed to be largely associated with the regulation of immune responses (Wright et al., 2005), although the exact role of CB2 receptor activity in the GI tract remains to be clarified. Not surprisingly, both endocannabinoids, AEA and 2-AG, are also found to be locally produced by the intestinal tissue (Liu et al., 2005). The level of AEA is negatively coupled to feeding

behaviour with elevations observed in rat small intestine but not the brain during food deprivation and reductions after re-feeding (Gomez et al., 2002; Petersen et al., 2006). The level of 2-AG in the gut was unchanged by starvation or re-feeding (Petersen et al., 2006), so although the local concentration of AEA in the intestine is more than 200-fold lower than that of 2-AG (Izzo & Camilleri, 2008), AEA is likely to be the major functional endocannabinoid in the gut.

5.2. Adipose tissue

Components of the ECS are also expressed by adipocytes (Bluher et al., 2006), and activity of this system is up-regulated in both diet-induced and genetically obese mouse models (Matias et al., 2006). In particular, activity of the CB1 receptor has been highlighted by numerous studies for its regulatory function in adipocyte differentiation, lipogenesis and adipokines production (Osei-Hyiaman et al., 2005; Matias et al., 2006). Administration of the CB1 receptor antagonist rimonabant in diet-induced obesity significantly enhanced lipolysis, which was reported to be directly responsible for the reduction in adipose mass observed (Vettor & Pagano, 2009). A recent study has provided more information on how rimonabant stimulates lipolysis, with evidence to suggest that it acts by increasing sympathoadrenal activity rather exerting direct lipolytic action on adipocytes (Mølhøj et al., 2010).

The endocannabinoids 2-AG and AEA have also been isolated from mature adipocytes (Gonthier et al., 2007), and 2-AG is elevated in the serum and visceral fat of obese individuals (Bluher et al., 2006). Moreover, circulating concentrations of 2-AG correlate directly with lipid mass (Matias et al., 2006), and increased expression of the insulin-dependent glucose transporter GLUT-4 has been reported in adipocytes of rimonabant-treated animals (Pagano et al., 2007), all of which suggest a significant role for the ECS in modulating energy storage and metabolism in adipose tissue. Indeed, CB1 receptors have been reported to regulate the production of adiponectin, an adipocyte-derived peptide hormone that improves peripheral insulin sensitivity (Iqbal, 2007; Bellocchio et al., 2008). Circulating adiponectin levels are reduced in obese and insulin-resistant individuals (Iqbal, 2007), and long term administration of a synthetic cannabinoid resulted in a reduction in adiponectin mRNA expression in mouse adipocytes (Matias et al., 2006). Consistent with this, chronic blockade of CB1 receptors with rimonabant induced a 4-fold increase in adiponectin mRNA in adipocytes in culture and in obese rats in vivo (Bensaid et al., 2003; Matias et al., 2006), and rimonabant also increased adiponectin release from cultured mouse
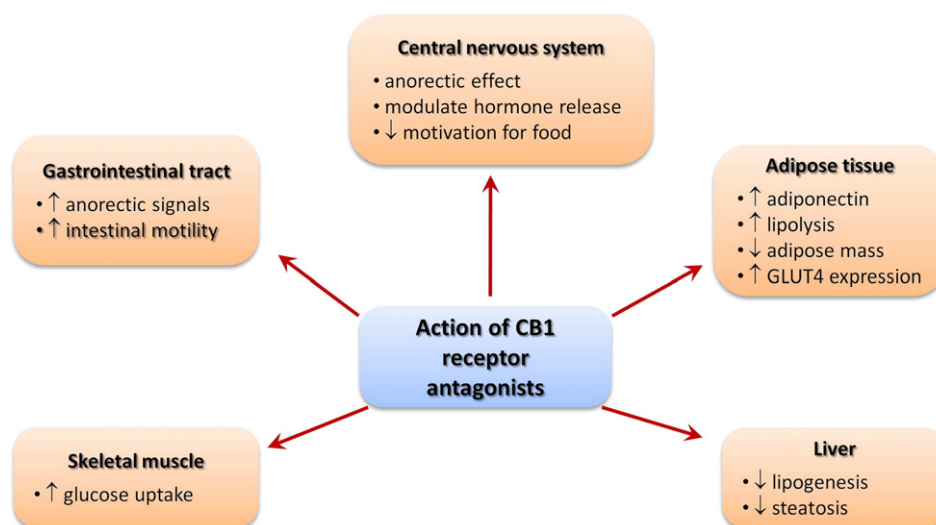
Fig. 3. Systemic effects of CB1 receptor antagonism. CB1 receptor antagonism reduces food intake by acting centrally on the hypothalamus and limbic forebrain, which results in anorectic effects. CB1 receptor antagonists also exert peripheral effects on the gastrointestinal tract to stimulate secretion of anorectic signals; adipose tissue to increase lipolysis and glucose uptake; skeletal muscles to stimulate glucose uptake and liver where de novo lipogenesis is inhibited.

adipocytes (Gary-Bobo et al., 2006). Thus, the improvements in lipid and glucose metabolism observed upon CB1 receptor antagonism may be attributable, at least in part, to elevations in adiponectin secretion. This is supported by observations that rimonabant improved insulin sensitivity in wild type mice fed on a high fat diet, but not in adiponectin-deficient mice fed on the same high fat diet (Migrenne et al., 2009).

5.3. Skeletal muscle

Expression of CB1 receptors has been reported in human and mouse soleus muscles (Liu et al., 2005; Cavuoto et al., 2007a,b). Genetically obese mice exhibited increased expression of CB1 receptors compared to the wild type animals, and the same study also demonstrated significant up-regulation of glucose uptake into the soleus muscle of these obese mice after a seven day-treatment with rimonabant (Liu et al., 2005). Consistent with this, CB1 receptor activation is reported to contribute to skeletal muscle insulin resistance in human obesity (Eckardt et al., 2009).

5.4. Liver

Despite being expressed at a relatively low level, CB1 receptors have been identified in the liver, as have the endocannabinoids 2-AG and AEA (Hanus et al., 2003). Indeed, a role for this system in regulating hepatic lipogenesis has been reported in experiments where 2-AG stimulated de novo fatty acid synthesis (Osei-Hyiaman et al., 2005) and application of endocannabinoids to mice promotes lipogenesis in the liver and glucose intolerance (de Kloet & Woods, 2009). Results obtained from CB1 receptor-deficient mice further confirmed the lipogenic function of CB1 receptors in hepatocytes, and it was suggested that CB1 receptor antagonism may also protect the liver from high fat diet-induced phenomena such as hepatic steatosis (Izzo & Camilleri, 2008).

6. Expression and function of the endocannabinoid system in islets of Langerhans

The hypothesis that the ECS is overactive in obesity and this increases the risks of other metabolic syndromes has been proposed (Engeli et al., 2005), although a detailed physiological impact of the ECS has not been delineated yet. Importantly, reduced fasting plasma glucose and improved insulin sensitivity have been reported following administration of rimonabant both in rodents and humans, independent from its central effects (Scheen, 2007). Given the vital roles of the pancreas in maintaining glucose homeostasis, recent studies have investigated the potential regulatory functions of the ECS in the endocrine islets.

6.1. Islets of Langerhans

Islets of Langerhans are clusters of endocrine cells, ~100–500 μm in diameter, which are distributed throughout the pancreas. Insulin-secreting β-cells are the most abundant cell type within islets, constituting ~60% of the islet mass in humans and 80–90% in rodents. The other major islet cell types are glucagon-secreting α-cells, somatostatin-secreting δ-cells, and pancreatic polypeptide-secreting PP cells (Jones & Persaud, 2010). There are paracrine interactions between different islet endocrine cells, with insulin secretion being stimulated by glucagon and inhibited by somatostatin, while insulin inhibits glucagon release and stimulates somatostatin secretion. Elevations in circulating blood glucose levels result in increased insulin output, which increases glucose, fatty acid and amino acid storage as glycogen, triacylglycerol and protein in liver, fat and muscle. Hyperglycaemia also inhibits glucagon secretion, resulting in decreased hepatic glycogenolysis and gluconeogenesis, thus complementing the anabolic effects of insulin.

In addition to the regulation of insulin secretion by glucagon and somatostatin, β-cells are also equipped with numerous cell surface receptors that modulate the exocytotic release of insulin (Jones & Persaud, 2010), and there is evidence of local signalling systems where islets synthesise ligands that activate particular β-cell receptors. For example, the hypothalamic peptide kisspeptin is expressed by islets and exogenous kisspeptin binds to GPR54 receptors on β-cells to stimulate insulin secretion (Hauge-Evans et al., 2006; Bowe et al., 2009). Similarly, islets express a renin–angiotensin signalling system by which locally generated angiotensin II can bind to its receptors on mouse and human β-cells to stimulate insulin secretion (Ramracheya et al., 2006). In this way locally produced ligands can play important roles in co-ordinating insulin secretory responses, and studies have been carried out to identify the presence of elements of the ECS in islets, to determine whether cannabinoids regulate islet function.

6.2. Expression of endocannabinoid system components by islets of Langerhans

Although there have been reports of cannabinoid receptor expression by the islets of Langerhans, no consensus has yet been reached regarding which cannabinoid receptor subtypes are present or localisation of the receptors to particular endocrine cells. Expression profiles of CB1 and CB2 receptors have been investigated in mouse (Juan-Pico et al., 2006; Matias et al., 2006; Bermudez-Silva et al., 2007; Nakata & Yada, 2008; Tharp et al., 2008), rat (Bermudez-Silva et al., 2007; Tharp et al., 2008) and human (Bermudez-Silva et al., 2008; Tharp et al., 2008) islets.

It was demonstrated in a rat cell line (RINm5F) that insulin-secreting cells express CB1 and CB2 receptor mRNAs (Matias et al., 2006) and in the same year another study detected the presence of both CB1 and CB2 receptor mRNAs in mouse islets (Juan-Pico et al., 2006). In addition, this latter study reported that CB1 receptors were found in non-β-cells that exhibited α-cell morphologies, and CB2 receptors were present in both β- and non-β-cells (Juan-Pico et al., 2006). The same group later reported co-expression of CB1 and CB2 receptors in both insulin positive and non-insulin-expressing rat dissociated islet cells (Bermudez-Silva et al., 2007), suggesting a degree of species-specificity in islet cell types expressing cannabinoid receptors. These findings were supported by an immunohistochemistry study of mouse and rat pancreas sections (Starowicz et al., 2008) and peripheral localisation of CB1 receptors in rat islets has also been reported in a recent study (Vilches-Flores et al., 2010). Thus, two groups have proposed that in islets CB1 receptors localise strictly to mouse α-cells, and mainly to rat α-cells, with some expression also detectable in small selective populations of rat β-cells, while CB2 receptors are proposed to be present in both α- and β-cells in mouse and rat.

In direct contrast, another study described expression of CB1, but not CB2 receptor, mRNA in mouse islets and a lack of CB2 receptors in mouse pancreas was demonstrated by immunohistochemistry (Nakata & Yada, 2008). A separate study also reported that CB2 receptors were not expressed by rat or human islets and, to add further to the discrepancies between published studies, the same report used immunofluorescence microscopy to demonstrate exclusive co-expression of CB1 receptors with somatostatin-producing δ-cells in mouse, rat and human (Tharp et al., 2008). We have also investigated cannabinoid receptor expression by mouse islets and the mouse MIN6 β-cell line, and have detected mRNAs coding for CB1 and CB2 receptors (Li et al., 2010a,b), and for the novel cannabinoid receptor GPR55 (Fig. 4). Immunohistochemistry also revealed that both CB1 and CB2 receptors are expressed by mouse islet β-cells, and co-staining of pancreas sections with glucagon antibodies failed to support CB1 or CB2 receptor expression by mouse α-cells (Li et al., 2010a) (Fig. 5). We detected some CB1 receptor-positive, insulin- and glucagon-negative mouse islet cells (Fig. 5, upper panel), and these

Fig. 4. Detection of GPR55 mRNA in MIN6 β-cells, mouse and human islets. A product of the correct size coding for GPR55 (60 bp) was amplified from MIN6 β-cell, mouse islet and human islet cDNAs. No products were amplified when water was used in place of the cDNA template.

may be the CB1 receptor-expressing δ-cells that were identified in another study (Tharp et al., 2008). Neither CB1 nor CB2 receptors were detected in mouse exocrine pancreas by immunohistochemistry (Fig. 5) (Starowicz et al., 2008; Li et al., 2010a), suggesting specific roles for these receptors in pancreatic endocrine cell function.

In addition to the expression studies in rodents described earlier, one report in human islets using quantitative RT-PCR, western blotting and immunohistochemistry showed that while the mRNAs and proteins of both cannabinoid receptors could be detected in whole islet cDNA and protein extracts, CB1 receptors were mainly localised to α-cells with certain populations of β-cells also expressing this receptor at a low level. On the other hand, the CB2 receptor, whose mRNA level was ~100-fold lower than that of the CB1 receptor, was strictly expressed by islet δ-cells and, unexpectedly, abundantly by the pancreatic exocrine tissue (Bermudez-Silva et al., 2008). We have also identified CB1 and CB2 mRNAs in human islets by RT-PCR, and our fluorescence immunohistochemistry studies have indicated that both receptor subtypes are expressed by β-cells, but not α-cells (Li, Jones & Persaud unpublished), consistent with our observations in mouse islets (Li et al., 2010a). Contrary to the report by Bermudez-Silva et al. (2008), we detected very little extra-islet expression of CB1 or CB2 receptors in human pancreas sections.

Expression profiles of the other ECS components have also been examined in some of these studies. Our own unpublished data have indicated that mRNAs coding for DAG-lipase, responsible for synthesis of 2-AG, and the endocannabinoid-degrading enzymes FAAH (for AEA) and MGL (for 2-AG) are expressed by human islets. These observations are consistent with the identification of DAG-lipases α and β, FAAH and MGL in human islet protein extracts, (Bermudez-Silva et al., 2008) and the same study indicated that NAPE-PLD, the AEA-generating lipase was not present in human islets. Cellular localisation within islets of some ECS enzymes has also been obtained. Thus, an immunohistochemical study reported co-expression of DAG-lipase and NAPE-PLD in mouse α-cells, while FAAH and MGL were found in the β-cells (Starowicz et al., 2008). Another report using

immunofluorescence confocal microscopy revealed co-localisation of MGL in rat δ-cells and FAAH was mainly detectable in rat and human α-cells, with weak expression in β-cells (Tharp et al., 2008). A summary of the observations made by different groups on ECS expression profiles in mouse, rat and human islets is shown in Table 1.

6.3. Effects of cannabinoid receptors on islet Ca^{2+} and cyclic adenosine monophosphate levels

Although there are some discrepancies among current studies on ECS expression and localisation in islets (Table 1), the possibility of a paracrine regulatory function of this system has nonetheless been strongly implicated. However, similar to the localisation profile reports, measurements of downstream signalling upon cannabinoid receptor activation with endogenous or pharmacological cannabinoids has also led to conflicting results (Table 2). A stimulatory effect of CB1 receptor activation on $[Ca^{2+}]_i$ was initially suggested in RINm5F insulin-secreting cells (Matias et al., 2006), but Ca^{2+} microfluorimetry in dispersed single mouse islet cells demonstrated profound inhibitory effects of ACPA, a pharmacological CB1 receptor-specific agonist, on glucose-induced elevation in $[Ca^{2+}]_i$ (Nakata & Yada, 2008). In addition, the endocannabinoid 2-AG was reported to reduce the amplitude of glucose-stimulated $[Ca^{2+}]_i$ oscillations in mouse islets via activation of CB2 receptors, and similar results were obtained following direct stimulation of mouse islets with a CB2 receptor agonist, suggesting a potential CB2 receptor-mediated inhibitory mechanism of endocannabinoids in mouse β-cells (Juan-Pico et al., 2006). In the same experiments a CB1 receptor agonist did not decrease glucose-stimulated Ca^{2+} oscillations in mouse islet cells, in contrast to the complete cessation of oscillations following exposure to ACPA that was observed in the study of Nakata and Yada (2008).

We have also investigated whether CB1 and CB2 receptor agonists can regulate $[Ca^{2+}]_i$ at a substimulatory glucose concentration (2 mM) in Fura 2-loaded MIN6 β-cells and dispersed mouse islet cells, and found that activation of both receptor subtypes stimulated reversible increases in $[Ca^{2+}]_i$ (Li et al., 2010a,b). Furthermore, we demonstrated for the first time that islet CB1 and CB2 receptors are coupled to decreases in cyclic AMP levels (Li et al., 2010a,b), suggesting that a $G_{i/o}$-coupled inhibition of adenylate cyclase is downstream of cannabinoid receptor activation in β-cells, as it is in other cell types (summarised in Fig. 2). The capacity of cannabinoids to reduce cyclic AMP generation indicates that the elevations in $[Ca^{2+}]_i$ that we observed in β-cells are not secondary to the PKA-mediated phosphorylation of VGCC that was reported in neuronal cells (Rubovitch et al., 2002), but it is possible that β-cell cannabinoid receptors may elevate Ca^{2+} through G_q-mediated PIP_2 hydrolysis, as
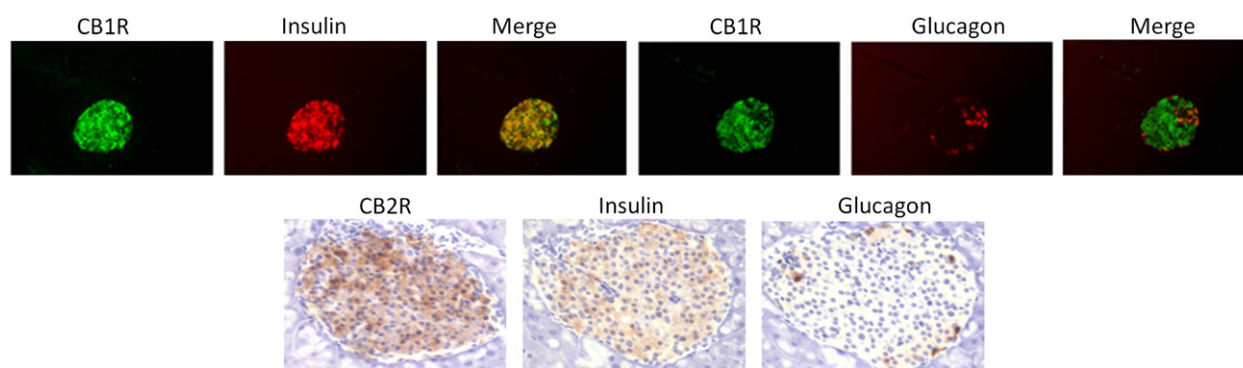
Fig. 5. Cannabinoid receptor expression by mouse islets. Mouse pancreas sections were immunoprobed with antibodies directed against CB1 receptors (upper panel) or CB2 receptors (lower panel) and the immunoreactive cells were detected using a FITC-conjugated secondary antibody (green, CB1R, upper) or a DAB-reactive horseradish peroxidase-conjugated secondary antibody (brown, CB2R, lower). The sections were also probed with anti-insulin and anti-glucagon primary antibodies and a Texas Red-conjugated secondary antibody (red, upper) and a DAB-reactive horseradish peroxidase-conjugated secondary antibody (brown, lower). Merged fluorescent images (yellow, upper) and DAB staining (lower) are consistent with CB1 and CB2 receptor co-localisation to insulin-expressing β-cells, but not to glucagon-expressing α-cells.

Table 1
Summary of ECS element expression profiles in islets.

Mouse	Juan-Pico et, al. 2006 Bermudez-Silva et al., 2007	Nakata & Yada, 2008	Juan-Pico et al., 2009	Li et al., 2010a (and unpublished)	Starowicz et al., 2008
CB1 receptor	Non-β-cells	α- and β-cells	α- and δ-cells	β-cells	α-cells
CB2 receptor	Non-β-cells and β-cells	No expression	α- and δ-cells	β-cells	α- and β-cells
DAG-lipase	Not done	Not done	Not done	Islet cell type not defined	α-cells
NAPE-PLD	Not done	Not done	Not done	Not done	α-cells
MGL	Not done	Not done	Not done	No expression	β-cells
FAAH	Not done	Not done	Not done	Islet cell type not defined	β-cells

Rat	Bermudez-Silva et al., 2007	Tharp et al., 2008	Starowicz et al., 2008
CB1 receptor	Non-β-cells and β-cells	δ-cells	Mainly non-β-cells, and β-cells
CB2 receptor	Non-β-cells and β-cells	Non-endocrine pancreas	Not done
DAG-lipase α	Not done	Not done	Not done
NAPE-PLD	Not done	Not done	Not done
MGL	Not done	δ-cells	Not done
FAAH	Not done	Mainly α-cells, and β-cells	Not done

Human	Bermudez-Silva et al., 2008	Tharp et al., 2008	Li et al., unpublished
CB1 receptor	Mainly α-cells, and β-, δ-cells	δ-cells	β-cells
CB2 receptor	δ-cells and exocrine pancreas	No expression	β-cells
DAG-lipase	Islet cell type not defined	Not done	Islet cell type not defined
NAPE-PLD	No expression	Not done	Not done
MGL	Islet cell type not defined	Unreliable staining	Selectively expressed by some β-cells and α-cells
FAAH	Islet cell type not defined	Mainly α-cells, and β-cells	Islet cell type not defined

Components of the ECS, including CB1 and CB2 cannabinoid receptors, upstream enzymes for endocannabinoid biosynthesis, DAG-lipase (for 2-AG) and NAPE-PLD (for AEA) and downstream enzymes for 2-AG and AEA degradation, MGL and FAAH respectively, have been identified in mouse, rat and human pancreas. Several reports have been published over the years suggesting expression of the ECS by the main islet cell types α, β, and δ, the details of which are listed here. 'Not done' indicates that the study did not investigate expression of this component of the ECS.

outlined in Fig. 2. The few reports published to date therefore demonstrate that signalling cascades downstream of islet cannabinoid receptor activation in β-cells may be as complex as those identified in other cell types and the net effects on Ca^{2+} levels are likely to depend on a number of variables such as the glucose concentration, agonist used, administration duration, etc.

Table 2
Summary of effects of CB1 and CB2 receptor agonists on islet hormone secretion. Several groups have investigated the effects of cannabinoid receptor stimulation on $[Ca^{2+}]_i$, cyclic AMP and islet hormone secretion using insulin-secreting cell lines and isolated mouse, rat and human islets. 'Not done' indicates that the study did not assess the effects of cannabinoid agonists on this particular aspect.

Insulin-secreting cell lines

	RINm5F insulin-secreting cells Matias et al., 2006	MIN6 β-cells and pseudoislets Li et al., 2010b
$[Ca^{2+}]_i$	Not done	Increase, CB1 and CB2 receptors
Cyclic AMP	Not done	Decrease, CB1 and CB2 receptors
Insulin	Increase, CB1 receptors	Increase, CB1 and CB2 receptors

Mouse islets

	Juan-Pico et al., 2006	Nakata & Yada, 2008	Li et al., 2010a
$[Ca^{2+}]_i$	No effect (3 mM glucose), CB1 and CB2 receptors Decrease (11 mM glucose), CB2 receptors	Decrease (8.3 mM glucose), CB1 receptors	Increase (2 mM glucose), CB1 and CB2 receptors
Cyclic AMP	Not done	Not done	Decrease, CB1 and CB2 receptors
Insulin	No effect (3 mM glucose), CB1 and CB2 receptors Decrease (11 mM glucose), CB2 receptors	Decrease (8.3 mM glucose), CB1 receptors	Increase (2 mM glucose), CB1 and CB2 receptors No effect (20 mM glucose), CB1 receptors Increase (20 mM glucose), CB2 receptors

Rat islets

	Vilches-Flores et al., 2010
Insulin	Increase (3 mM and 16 mM glucose), CB1 receptors

Human islets

	Bermudez-Silva et al., 2008	Li et al., unpublished
Insulin	Increase (3 mM glucose and 11 mM glucose), CB1 receptors Decrease (11 mM glucose), CB2 receptors	Increase (2 mM and 20 mM glucose), CB2 receptors Increase (2 mM glucose), CB1 receptors No effect (20 mM glucose), CB1 receptors
Glucagon	Increase (3 mM glucose), CB1 receptors No effect (3 mM glucose), CB2 receptors	Increase (2 mM glucose), CB2 receptors
Somatostatin	Increase (11 mM glucose), CB1 receptors No effect (11 mM glucose), CB2 receptors	Not done

Several groups have investigated the effects of cannabinoid receptor stimulation on $[Ca^{2+}]_i$, cyclic AMP and islet hormone secretion using insulin-secreting cell lines and isolated mouse, rat and human islets. 'Not done' indicates that the study did not assess the effects of cannabinoid agonists on this particular aspect.

6.4. Effects of cannabinoid receptors on insulin secretion

Elevations in both $[Ca^{2+}]_i$ and cyclic AMP are known to play important roles in the biphasic insulin secretory responses of β-cells (reviewed in Jones & Persaud, 1998; Prentki & Matschinsky, 1987; Shibasaki et al., 2004) and the observations that islet cannabinoid receptors regulate β-cell cyclic AMP generation and Ca^{2+} levels, as described in Section 6.3, suggest that these changes may be coupled to modified insulin output. The opposing effects of cannabinoid receptor activation on the levels of these two second messengers make it difficult to predict islet functional responses to cannabinoids, particularly modulation of insulin secretion, and it is perhaps not surprising that there have been reports that cannabinoid receptor activation either stimulates or inhibits insulin secretion.

In addition there may be differences in islet secretory responses to CB1 and CB2 receptor agonists. Thus, there are several reports indicating that CB1 receptor activation is coupled to increased insulin secretion, possibly via its stimulatory effect on β-cell $[Ca^{2+}]_i$, (Matias et al., 2006; Bermudez-Silva et al., 2008), and this is consistent with observations that the CB1 receptor antagonist rimonabant inhibits glucose-stimulated insulin secretion (Getty-Kaushik et al., 2009). However, there is one report that the CB1-selective agonist ACEA inhibits glucose-stimulated insulin secretion from mouse islets in static incubations (Nakata & Yada, 2008) and inhibitory effects have also been reported for CB2 receptor activation on glucose-stimulated insulin secretion from isolated mouse (Juan-Pico et al., 2006) and human (Bermudez-Silva et al., 2008) islets.

It is worth pointing out that the studies to date in which cannabinoids inhibited insulin secretion have been performed using static incubation studies, where insulin secreted from β-cells into the supernatant was determined over a fixed time-course (30 min–2 h). These static incubation protocols do not provide any dynamic information about the insulin secretory responses and somatostatin and GABA, which might be released from islets during the experimental protocols, can accumulate to exert paracrine inhibitory effects on insulin output. In studies where a dynamic perifusion system has been used to deliver agonists of interest to islets and rapidly remove secreted products, the CB1- and CB2-selective receptor agonists ACEA and JWH015 reversibly stimulated insulin secretion from mouse islets

(Li et al., 2010a) and MIN6 β-cells (Li et al., 2010b), and ACEA also stimulated insulin release from human islets (Fig. 6). Potentiation of glucose-induced insulin release from human islets by the endocannabinoids AEA and 2-AG has also been reported (Bermudez-Silva et al., 2008). In the same experiments the CB1 receptor agonist ACEA induced insulin release at a sub-stimulatory glucose concentration (3 mM), consistent with the data shown in Fig. 6. This was abolished by pharmacological blockade of the CB1 receptors with AM251, confirming a CB1 receptor-dependent stimulation of insulin secretion from human islets. Furthermore, the local concentration of 2-AG in human islets, which was undetectable at 3 mM glucose, was markedly elevated by an increase in the glucose concentration to 11.1 mM (Bermudez-Silva et al., 2008), suggesting a feedback mechanism for the local islet ECS that would lead to a regulatory effect on plasma glucose in vivo.

Secretion of glucagon and somatostatin has also been assessed following cannabinoid receptor stimulation and inhibition in isolated human islets (Bermudez-Silva et al., 2008). In these experiments application of ACEA stimulated both glucagon and somatostatin secretion, and the stimulatory effects were blocked in the presence of a CB1 receptor antagonist. No effect on glucagon or somatostatin secretion was identified upon CB2 receptor activation, suggesting that only CB1 receptors are coupled to regulation of hormone secretion from human islet α- and δ-cells.

6.5. Contribution of islet cannabinoid receptors to energy homeostasis

Despite the lack of consensus on expression and function of cannabinoid receptors in islets, there are some consistent observations in the studies published to date. Thus, it is beyond dispute that islets express cannabinoid receptors that are coupled to regulation of islet cell second messenger formation and changes in hormone output. Although the key question of whether cannabinoids exert inhibitory or stimulatory effects on insulin secretion has not been resolved, the only studies performed using the most clinically relevant research model, isolated human islets of Langerhans, indicate that exogenous CB1 receptor agonists stimulate insulin release (Bermudez-Silva et al., 2008; Fig. 6). What then, is the endogenous trigger for islet CB1 receptor activation? As outlined in Section 6.2, islets express

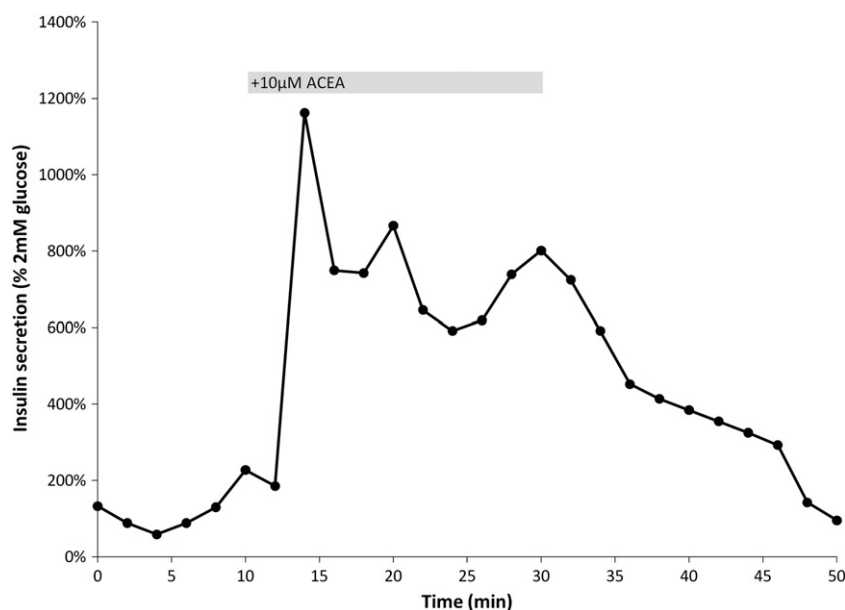**Fig. 6.** Stimulation of insulin secretion from isolated human islets by ACEA. Isolated human islets were perifused (37 °C, 0.5 ml/min) with a physiological buffer containing 2 mM glucose and supplemented with 10 μM ACEA for 20 min as shown. ACEA stimulated insulin secretion, an effect that was reversible upon removal of the agonist. Data are presented as a percentage of insulin secretion at 2 mM glucose.

elements of the ECS such as DAG-lipase and MGL that will allow local production and subsequent degradation of the endocannabinoid 2-AG, and glucose stimulates 2-AG accumulation in human islets (Bermudez-Silva et al., 2008). This indicates that there is a local cannabinoid signalling system within islets through which 2-AG may be generated from DAG to increase β-cell insulin secretory responses. In addition to this locally produced 2-AG, cannabinoid receptors on β-cells may also be activated under pathophysiological conditions of obesity and type 2 diabetes, where circulating 2-AG levels are elevated (Matias et al., 2006). Moreover, 2-AG levels are higher in the visceral fat of obese humans (Matias et al., 2006) so under conditions of obesity the normal islet ECS generation of 2-AG might be overwhelmed by adipocyte-generated 2-AG leading to hyperstimulation of insulin secretion and consequent exacerbation of the adipocyte hypertrophy and elevated lipid levels.

The CB1 receptor antagonist rimonabant can reverse diet-induced obesity in mice (Poirier et al., 2005) and it has been reported to improved lipid profiles and reduce insulin resistance, independently of central effects to reduce food intake (Scheen & Paquot, 2009), confirming the importance of peripheral cannabinoid receptors in dysregulation of energy homeostasis in obesity. Similar effects have been observed in CB1 receptor knockout mice, which were resistant to diet-induced obesity, did not develop insulin resistance and had reductions in plasma insulin levels (Ravinet Trillou et al., 2004). Moreover, rimonabant directly inhibited the basal insulin hypersecretion that is observed in islets isolated from obese rats (Duvivier et al., 2009; Getty-Kaushik et al., 2009), and this reduced overactivity of islets in obesity may contribute to the improvements in insulin sensitivity that are observed with rimonabant use in vivo (Despres et al., 2005).

7. Multiple sites of action of cannabinoids

Another layer of complexity arises when interpreting data generated using pharmacological cannabinoids since experiments on mice lacking CB1 or CB2 receptors have suggested CB1/2 receptor-independent sites of action for cannabinoids in the periphery as well as in the CNS (Begg et al., 2005; Mackie & Stella, 2006). Thus, in mesenteric arteries AEA, but not synthetic cannabinoid receptor agonists, caused vasodilation (Begg et al., 2005). This was blocked by the CB1 receptor antagonist, rimonabant, but not by a chemically similar CB1 receptor blocker, AM251. Furthermore, a cannabidiol analogue, with negligible activities towards CB1 and CB2 receptors, was reported to induce vasodilation (Jarai et al., 1999), suggesting CB1/2 receptor-independent mechanisms. Another study has demonstrated that WIN55,212-2, a pharmacological CB1/2 receptor agonist, and another cannabinoid, CP55940, inhibited glutaminergic excitatory postsynaptic currents in CB1 receptor-depleted mouse hippocampal pyramidal cells that do not express CB2 receptors (Hajos et al., 2001), further confirming the presence of non-CB1/2 receptor activities. Moreover, similar cannabinoid-induced CB1/2 receptor-independent effects were also reported in the heart (Begg et al., 2005), smooth muscle cells (O'Sullivan et al., 2005a) and T-cells (Rao & Kaminski, 2006).

In addition to the recently identified cannabinoid receptor GPR55, another GPCR, GPR119, has also been demonstrated to possess cannabinoid receptor characteristics (Godlewski et al., 2009). Furthermore, anandamide has been shown to act via the transient receptor potential vanilloid type 1 receptor (TRPV1) (Di Marzo et al., 2002; Ross, 2003; Starowicz et al., 2008), and direct activity towards other potential targets such as the peroxisome proliferator activated family of receptors (PPARs) has also been demonstrated in studies using CB receptor agonists (Burstein, 2005; O'Sullivan et al., 2005b). The following sections summarise information available about CB1/CB2 receptor-independent effects of cannabinoids and it is possible

that more cannabinoid receptors and other molecular targets that can be stimulated by cannabinoid agonists will be identified in the future.

7.1. GPR55

GPR55 was initially discovered as an orphan receptor of the purinergic GPCR superfamily, closely related to GPR35, GPR23 and the purinoceptor $P2Y_5$ (Sawzdargo et al., 1999). The relationship between GPR55 and cannabinoids was first established and patented by GlaxoSmithKline and AstraZeneca, in which the CB1 receptor antagonists AM251 and rimonabant exhibited significant affinity and activity towards recombinant yeast GPR55. Furthermore, radioligand binding studies by AstraZeneca indicated that binding sites for the synthetic cannabinoid CP55940, but not WIN55,212-2, were found in membrane extracts of GPR55-transfected HEK293 cells, implying selective cannabinoid activities towards GPR55. In addition, the endocannabinoid 2-AG shows nearly 200-fold greater potency for GPR55 than it does for CB1 or CB2 receptors (Ryberg et al., 2007). Thus, GPR55 activity may contribute to the effects of some cannabinoids that were previously thought to act exclusively at CB1/2 receptors. It has been proposed that GPR55 stimulation leads to the activation of G_{13} (Baker et al., 2006), which is known to initiate the Ras/Raf/MAPK pathway in the regulation of cell proliferation (Kratz et al., 2007). Furthermore, a G_q-PLC-mediated signalling mechanism downstream of GPR55 activation has also be reported (Lauckner et al., 2008), which leads to increased $[Ca^{2+}]_i$ and decreased M-type K^+ current in transfected human embryonic kidney (HEK293) cells. However, the detailed intracellular pathways that are coupled to GPR55 activation are still presently unknown and the effects of G_{13}-linked GPR55 activation in vivo and its physiological significance also remain to be unravelled. In addition, discrepancies in receptor–ligand interactions between recombinant and intrinsic GPR55 have been reported (Brown, 2007), suggesting the involvement of other G-protein-dependent and independent downstream cascades.

7.2. GPR119

Another G-protein orphan receptor, GPR119, was first classified as a member of the rhodopsin GPCR superfamily (Fredriksson et al., 2003). Subsequent studies indicated its expression in the gastrointestinal tract (Soga et al., 2005) and islet β-cells (Chu et al., 2007). The receptor was later proposed to possess certain structural similarity with the cannabinoid receptors (Brown, 2007). Indeed, GPR119 can be activated by certain ethanolamine derivatives (common precursors of anandamide), as well as some lysophospholipid species (Morgan & Chan, 2001). Activation of GPR119 by lysophosphatidylcholine (LPC) was reported to potentiate glucose-stimulated insulin secretion from rat pancreas and mouse NIT-1 β-cells via G_s-enhanced cyclic AMP generation (Soga et al., 2005). However, as commonly occurs in agonist/antagonist-based receptor studies, receptor-independent effects of LPC on insulin secretion were also observed in studies using gpr119 silenced RINm5F and MIN6 insulin-secreting cells (Ning et al., 2008) and GPR119 knockout mice (Lan et al., 2009). In fact, LPC has repeatedly been mis-assigned as an endogenous ligand of different orphan GPCRs due to its pleiotropic effects in vitro. As a result, a selection of small molecule agonists of GPR119 has been developed for the investigation of receptor function: studies in rats reported significant reductions in cumulative food intake 24 h after administration of PSN632408, a GPR119-selective agonist, and decreased diet-induced weight gain after a 14-day oral treatment with the same compound (Overton et al., 2006). Another GPR119-activating agent, AR-231453, is reported to improve metabolic profiles by enhancing glucose-dependent insulin secretion in mice. Better glucose tolerance was also observed in both healthy and diabetic mice after AR-231453 treatment, and the effect was lost in GPR119 knockout mice, confirming the involvement of the receptor

(Jones, 2006). GPR119 therefore has been strongly implicated in the regulation of energy homeostasis. However, despite some interaction with cannabinoid precursors and/or derivatives, the nature of the endogenous GPR119 agonist and its physiological relevance remain uncertain.

7.3. TRPV1

The TRPV1 receptor belongs to the six-transmembrane transient receptor potential (TRP) family, a collection of non-selective cation channels. Formerly known as a vanilloid receptor (VR_1), TRPV1 is the only member characterised so far of a possible family of capsaicin-activated, resinoferatoxin (RTX)-sensitive nociceptors (Caterina et al., 1997). Initially believed to mainly localise to the sensory C fibres and act as a ligand-, proton- and heat-activated molecular sensor of nociceptive stimuli, TRPV1 receptors are now known to be widely distributed in the CNS (Mezey et al., 2000) and various peripheral tissues including gut, liver, and pancreas in both rodents and humans (Cortright et al., 2001). Activation of TRPV1 receptors by the endocannabinoid AEA was first reported in the arteries of rat and guinea pig (Zygmunt et al., 1999), the application of which resulted in vasodilation. Interaction between AEA and TRPV1 receptors was later confirmed in human VR_1-transfected HEK293 cells (Smart & Jerman, 2000), rat mesenteric arteries (Vanheel & Van de Voorde, 2001) and in mouse trigeminal neurones (Roberts et al., 2008). The downstream consequences of AEA-induced TRPV1 receptor activation by AEA are elevations in $[Ca^{2+}]_i$, (Smart et al., 2002) and, in certain tissues at least, AEA is considered to be an endovanilloid, an endogenous activator of TRPV1 (Di Marzo et al., 2002).

7.4. PPARγ

Peroxisome proliferator activated receptor γ (PPARγ) is a member of the nuclear receptor superfamily and is most abundantly expressed in adipose tissue, where it exerts important roles in regulation of lipid metabolism. The improvements in glucose homeostasis following PPARγ activation (Lemberger et al., 1996; Corton et al., 2000) made it an attractive target in the development of therapies for diabetes and thiazolidinediones (TZDs), which are PPARγ ligands, are used clinically to improve insulin sensitivity in T2DM. Despite some disagreement regarding the impact of PPARγ activation on the regulation of insulin secretion at sub-stimulatory glucose levels, a positive effect on glucose-induced insulin secretion has been observed consistently in islets treated with PPARγ agonists (Santini et al., 2004; Shimomura et al., 2004). Increasing evidence has indicated a correlation between the activities of CB1 and PPARγ receptors (Burstein, 2005; O'Sullivan et al., 2005b). Up-regulation of CB1 receptor expression and endocannabinoid levels has been observed following mouse and human adipocyte differentiation, accompanied by an increase in PPARγ mRNA (Idris et al., 2009; Karaliota et al., 2009). Direct activation of PPARγ by the cannabinoid THC has been observed in HEK293 cells, and THC also stimulated adipocyte differentiation, which is an established mode of action of PPARγ agonists (O'Sullivan et al., 2005b). It is therefore possible that as key regulators in metabolism and lipogenesis, both CB1 and PPARγ receptors interact with each other to maintain glucose and energy homeostasis. In contrast to the increased lipid storage that occurs as a consequence of cannabinoid receptor activation of PPARγ receptors, another cannabinoid, N-oleoylethanolamide (OEA), stimulates lipolysis and this occurs via activation of PPARα receptors (Guzman et al., 2004). Thus, cannabinoid activation of PPARγ (and CB1) receptors leads to triacylglycerol synthesis, while PPARα is coupled to anorexigenic pathways that decrease food intake and body weight (Fu et al., 2003). This suggests that the pathways downstream of PPAR activation depend on the receptor subtype activated, and this may vary from tissue to tissue depending on receptor density,

endocannabinoid species and receptor affinity for that particular ligand.

7.5. CB1 and CB2 receptor-independent effects of cannabinoids in islets

There is currently little published data available on the effects of cannabinoids on islets independent of activation of CB1 and/or CB2 receptors, but there are several lines of evidence to suggest that at least some signalling occurs via CB1 and/or CB2-receptor-independent mechanisms. First, our preliminary experiments using islets isolated from CB1 receptor-deficient mice indicate that ACEA elevates $[Ca^{2+}]_i$, (Li, Bowe, Baker, Jones & Persaud, unpublished), as it does in normal mouse islets (Li et al., 2010b), indicating that ACEA-induced increases in Ca^{2+} in islet cells can occur by a non-CB1 receptor pathway. Second, we have found that antagonism of CB1 and CB2 receptors by AM251 and JTE907 does not inhibit ACEA and JWH015-stimulated insulin release from human islets and that AM251 and JTE907 alone exert reversible, stimulatory effects on insulin secretion (Li, Jones & Persaud unpublished). The direct stimulation of insulin release by AM251 may occur via activation of GPR55, which is expressed by islets (Fig. 4), and activated by AM251 in HEK293 cells (Henstridge et al., 2009; Kapur et al., 2009). It is not currently clear by what mechanism JTE907 increases insulin secretion, but it is an inverse agonist rather than a receptor antagonist and this may play a role in its functional effects in islets. Third, since it is established that AEA can activate TRPV1 receptors (Di Marzo et al., 2002), it is possible that the effects of AEA in islets, such as elevation in $[Ca^{2+}]_i$, may occur via vanilloid receptors, rather than conventional CB1/2 receptors. Alternatively, the endocannabinoid OEA is reported to decrease β-cell $[Ca^{2+}]_i$ through activation of PPARα receptors (Ropero et al., 2009), and this might explain the inhibitory effects of cannabinoids on $[Ca^{2+}]_i$ that have been reported previously (Juan-Pico et al., 2006; Nakata & Yada, 2008).

8. Conclusions and future directions

It has become apparent in recent years that receptors for cannabinoids are expressed not only in the central nervous system (CB1) or immune cells (CB2), but also by a number of tissues that participate in the regulation of energy metabolism. CB1 receptors are most commonly reported to be responsible for cannabinoid-induced increased food intake and lipogenesis that contribute to weight gain and reduced insulin sensitivity, since antagonism of these receptors by rimonabant is effective in normalising abdominal adiposity and metabolic dysfunction. However, CB2 receptors are also expressed by some tissues involved in energy balance, as is the novel cannabinoid receptor GPR55. In addition, there is a variety of other receptors through which cannabinoids signal, and further work is required to define their roles, if any, in energy homeostasis.

The endocrine pancreas plays vital roles in controlling fuel storage and utilisation through the secretion of insulin and glucagon, two hormones that maintain blood glucose levels within the appropriate range. The identification of ECS elements in islet endocrine cells, and the coupling of cannabinoid receptor activation to increased insulin output place the islets as a key target tissue for cannabinoids. Thus, while activation of cannabinoid receptors in adipocytes, skeletal muscle and liver promotes reduced sensitivity to insulin, glucose intolerance and lipogenesis, the insulin resistance and hyperglycaemia will be ameliorated by cannabinoid-induced insulin secretion from islet β-cells. This suggests that under normal circumstances cannabinoid-induced insulin secretion is a beneficial stimulus–response coupling mechanism. The problems arise when the ECS is overactive and excessive secretion of insulin will compound the lipogenesis and contribute to the weight gain promoted by CB1 receptor activation.

There are still unanswered questions about cannabinoid signalling in islets, mainly whether effects on insulin secretion are mediated by

the classic CB1 and/or CB2 receptors, or if other receptors are involved. Given the capacity of "endocannabinoids" and so-called selective cannabinoid receptor agonists and antagonists to exert off-target effects, new models are required for further research in this area. In particular, it is likely that islets isolated from mice in which CB1, CB2 and GPR55 receptors have been knocked out will be important in further delineating the roles of these particular receptors in regulating islet function.

Another interesting area that requires further investigation is the unexpected stimulation of insulin secretion following antagonism of human islet CB1 receptors with AM251 in vitro. If this also occurs in vivo therapeutics that have dual actions to antagonise the obesogenic effects of cannabinoids while directly stimulating insulin secretion could be developed. Obviously this would require modifications in drug design to minimise the incidence of serious psychiatric problems that were associated with rimonabant use, and which led to its withdrawal. A novel peripherally restricted CB1 receptor antagonist, AM6545, which improves glucose homeostasis without affecting CB1 receptor-regulated behavioural responses (Tam et al., 2010), may be a useful starting point for clinical drug development.

Acknowledgments

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