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Endocannabinoid regulation of β -cell functions: implications for glycaemic control and diabetes

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Abstract

Visceral obesity is a major risk factor for the development of insulin resistance which can progress to overt type 2 diabetes (T2D) with loss of β -cell function and, ultimately, loss of β -cells. Insulin secretion by β -cells of the pancreatic islets is tightly coupled to blood glucose concentration and modulated by a large number of blood-borne or locally released mediators, including endocannabinoids. Obesity and its complications, including T2D, are associated with increased activity of the endocannabinoid/CB₁ receptor (CB₁R) system, as indicated by the therapeutic effects of CB₁R antagonists. Similar beneficial effects of CB₁R antagonists with limited brain penetrance indicate the important role of CB₁R in peripheral tissues, including the endocrine pancreas. Pancreatic β -cells express all of the components of the endocannabinoid system, and endocannabinoids modulate their function via both autocrine and paracrine mechanisms, which influence basal and glucose-induced insulin secretion and also affect β -cell proliferation and survival. The present brief review will survey available information on the modulation of these processes by endocannabinoids and their receptors, with an attempt to assess the contribution of such effects to glycaemic control in T2D and insulin resistance.

Keywords

diabetes; endocannabinoids; inflammation; pancreas

Introduction

Type 2 diabetes (T2D) or non-insulin-dependent diabetes is a chronic disease characterized by the body's impaired ability to metabolize glucose, which commonly develops in obese/overweight people. Obesity is a risk factor for developing insulin resistance, which is defined as the inability of cells in insulin-sensitive tissues to respond normally to insulin produced and released by β -cells of pancreatic islets in response to an increase in blood glucose concentration. A commonly held view is that in a subset of obese, insulin-resistant individuals, β -cell dysfunction ensues, leading to decreased insulin production, poor blood glucose regulation, and ultimately T2D [1]. It is also possible that β -cell dysfunction arises

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before or in parallel with insulin resistance, as in some cases it can be detected well before the onset of T2D [2].

The β -cells of the pancreatic islets are exquisitely sensitive to changes in blood glucose concentration. The type 2 glucose transporter protein (GLUT-2) mediates the entry of glucose into β -cells, thus allowing intracellular and extracellular glucose to equilibrate across the cell membrane. Glucose metabolism via glycolysis generates ATP, and a higher ATP/ADP ratio triggers the closure of the ATP-dependent potassium (K_{ATP}) channels responsible for maintaining the resting membrane potential, thus preventing potassium ions from being shunted across the cell membrane. The ensuing rise in positive charge inside the cell leads to depolarization of the cell membrane, resulting in the opening of voltage-gated calcium channels and a rise in intracellular calcium concentration [3]. The glucose-induced Ca^{2+}_i signal is organized in a synchronous and homogeneous oscillatory pattern [4], resulting in pulsatile insulin secretion [5]. The ultimate result is the export of insulin from β -cells into the systemic circulation.

The endocannabinoid system (ECS) comprises G-protein coupled cannabinoid receptors [CB_1 receptor (CB_1R) and CB_2 receptor (CB_2R)], their endogenous lipid ligands or endocannabinoids, such as arachidonylethanolamide (AEA) or anandamide and 2-arachidonoylglycerol (2-AG), and their biosynthetic and degrading enzymes, as detailed below. The discovery of the ECS has triggered an avalanche of experimental studies exploring its diverse biological functions [6]. Evidence from both preclinical and human studies indicates that overactivity of the CB_1R system contributes to the development of insulin resistance and both type 1 diabetes (T1D) and T2D [7,8], and that CB_1R blockade has beneficial effects in mitigating obesity/metabolic syndrome and its complications, including T2D [9–11]. This implies that components of the ECS are present and functional in tissues prominently involved in glycaemic control, including the endocrine pancreas, although a consensus has not yet emerged as to the cellular location and exact function of specific ECS components. In the present paper, we will provide a brief overview of current knowledge about the role of the ECS in β -cell function and survival, as it relates to its involvement in T2D.

Marijuana, Cannabinoids and the Endocannabinoid System

Marijuana has been used for millennia, both for its psychoactive and medicinal properties. The resolution of the chemical structure of Δ^9 -tetrahydrocannabinol (Δ^9 -THC) in 1964 [12] rekindled interest in cannabinoid biology. The ensuing discovery of G-protein-coupled cannabinoid receptors, first by radioligand binding [13] and then by molecular cloning [14,15] triggered a search for endogenous ligands. This culminated in the discovery of two endogenous lipids, AEA or anandamide and 2-AG, which bind to the same receptors – CB_1R and CB_2R – that recognize Δ^9 -THC, with a different affinity to each receptor [16–18]. Although these two endocannabinoids have been a major focus of research over the last two decades, there is some evidence for the existence of additional, endogenous peptide ligands for cannabinoid receptors [19,20], and there are also additional G-protein-coupled receptors (GPCRs), such as GPR-55, that recognize certain cannabinoid ligands [21]. Of the two main cannabinoid receptors, CB_1R is highly conserved across mammalian species [22–24] and is

by far the most abundant of all known class A GPCRs in the mammalian central nervous system. CB₁R is also expressed in peripheral tissues at low yet fully functional levels, where they directly influence metabolic, cardiovascular, reproductive and other biological functions; for review, see also Mazier et al. [8], Pacher and Kunos [25] and Maccarrone et al. [26]. CB₂Rs, conversely, are mainly expressed in immune and hematopoietic cells and once activated, they can modulate immune cell migration and cytokine release [21]. Both CB₁R and CB₂R signal through G_{i/o} proteins to inhibit adenylyl cyclase and regulate ion channels, but can also activate G-protein-independent tyrosine kinase pathways [27]. A unique mechanism of CB₁R action first reported in islet β -cells is the ability of CB₁R to form a heterotrimeric complex with the insulin receptor and G_{i α} , which results in inhibition of insulin signalling by inducing autophosphorylation of the insulin receptor [28]. Additionally, endocannabinoids acting via CB₁R were found to tonically inhibit both the secretion and the insulin secretagogue action of the incretin, glucagon-like peptide-1 [29].

Both AEA and 2-AG are generated on demand from membrane phospholipid precursors via multiple, parallel biosynthetic pathways [30]. AEA is generated from its immediate precursor *N*-arachidonoyl phosphatidylethanolamine (NAPE), mainly via cleavage by aNAPE-specific phospholipase D (NAPE-PLD), whereas 2-AG is derived from diacylglycerol (DAG) via cleavage by DAG-lipases (DAGL α or DAGL β). As a result of their lipophilicity and rapid degradation after release, endocannabinoids act locally as autocrine or paracrine mediators [31]. This is particularly relevant for the endocrine pancreas, where an endocannabinoid produced by one type of islet cell may act on its receptor located either on the same cell or on an adjacent cell of a different type [32]. Endocannabinoid action is terminated by metabolic degradation of the ligand, AEA being degraded by fatty acid amide hydrolase (FAAH) [33], whereas 2-AG is preferentially metabolized by monoglyceride lipase (MAGL) [34]. Potentiation of a biological response in the presence of a FAAH or MAGL inhibitor has been used to implicate AEA or 2-AG in that response, respectively, and such inhibitors are being evaluated for their therapeutic potential in pathological conditions where an increase in endocannabinoid tone is desirable, such as pain, anxiety or post-traumatic stress.

Association of Cannabinoids and the Endocannabinoid System with Obesity, Insulin Resistance and Type 2 Diabetes

Marijuana, Obesity and Diabetes

The term ‘cannabinoid’ represents Δ^9 -THC, its endogenous counterparts, and all of their structural analogues [21]. Based on this definition, numerous non-psychoactive and psychoactive compounds are part of this superfamily such as the non-psychoactive compounds found in *Cannabis sativa* L. as well as their synthetic analogues, non-psychoactive acylethanolamides, non-psychoactive lipoamino acids and other psychoactive cannabinoid receptor ligands. These cannabinoids have been implicated in regulating or modulating the functions of the endocrine pancreas.

Well before the discovery of cannabinoid receptors and their endogenous ligands, Δ^9 -THC, the psychoactive ingredient of marijuana and now known to be a CB₁R/CB₂R agonist, was

reported to induce glucose intolerance when administered acutely to normal human volunteers [35,36]. Glucose intolerance was also induced in mice by chronic administration of a synthetic 9 -THC analogue with more potent CB₁R agonist activity [37], whereas an opposite effect, i.e. improved glucose tolerance, was reported after chronic CB₁R blockade in overweight, insulin-resistant human subjects [10,11] or obese mice [38–40]. Together, these findings point to a deleterious role of CB₁R activation, either by plant-derived or endogenous cannabinoids, on glycaemic control, as discussed later. These findings stand in contrast, however, with the results of epidemiological studies in marijuana users, which indicate that regular chronic use of marijuana is associated with a lower prevalence of obesity [41], diabetes mellitus [42], lower fasting insulin and homeostatic model assessment of insulin resistance values and lower waist circumference [43], as well as absence of hepatic steatosis, and normal insulin sensitivity and β -cell function [44]. A possible explanation of this paradox, first suggested in 1974 [35], is that chronic heavy marijuana use leads to rapid loss and desensitization of CB₁R, so the associated phenotype reflects decreased rather than increased CB₁R activity. Indeed, chronic 9 -THC administration to mice, resulting in plasma 9 -THC levels regularly found in heavy marijuana smokers, was found to cause the rapid loss of CB₁R in axon terminals of γ -aminobutyric acid- (GABA)ergic neurons in the brain that took several weeks to recover, as documented recently through the use of super-resolution imaging [45].

Similarly, downregulation rather than activation of CB₁R may explain the protective effect of chronic 9 -THC treatment on β -cell function and survival, although cannabinoid receptor-independent mechanisms may also play a role. In streptozotocin-induced diabetic rats, 7-day treatment with 3 mg/kg/day 9 -THC significantly increased pancreatic glutathione levels, catalase and superoxide dismutase activity, which should protect against oxidative damage in the diabetic pancreas [46]. In a mouse model of autoimmune diabetes, 9 -THC transiently reduced blood glucose and strongly reduced inflammatory markers such as interferon- γ , tumour necrosis factor- α and interleukin (IL)-12 [47]. Finally, a cannabinoid receptor-independent mechanism probably accounts for the effects of cannabidiol, a non-psychoactive marijuana constituent with no significant CB₁R or CB₂R activity, in reducing inflammatory changes and arresting the onset of diabetes in non-obese diabetic mice [48], a model of T1D.

Increased Endocannabinoid System Activity Linked to Insulin Resistance and Type 2 Diabetes

In preclinical studies using mouse models of diet-induced obesity/metabolic syndrome, pharmacological or genetic deletion of CB₁R showed the pathogenic role of an overactive endocannabinoid/CB₁R system in metabolic obesity and the associated insulin resistance [49–51]. The beneficial effect of CB₁R blockade was also documented in a rat model of T2D linked to progressive β -cell loss [52,53], more directly indicating a role of the ECS in the control of β -cell functions. The role of peripheral as opposed to central CB₁R is further indicated by the improved glycaemic control achieved in such studies by treatment with non-brain-penetrant CB₁R antagonists [39,40,53] or antisense oligonucleotides [54]. Similar effects observed in clinical trials with the first in class CB₁R antagonist rimonabant supported these conclusions and suggested that the effects of rimonabant were mediated, at

least in part, via its interaction with peripheral CB₁R. In overweight individuals with metabolic syndrome, rimonabant significantly reduced fasting blood glucose and glycated haemoglobin levels relative to placebo [11,21]. In drug-naïve patients with T2D, rimonabant treatment resulted in significant improvements in glycaemic control, body weight and metabolic profile [55] and, in insulin-treated patients with T2D, CB₁R blockade caused further improvements in glycaemic control and cardiometabolic risk factors [56]. The pathogenic role of the ECS in T2D was further suggested by the elevated plasma levels of both AEA and 2-AG compared with healthy subjects [57,58].

Cellular Localization and Function of the Endocannabinoid System in the Endocrine Pancreas

Cannabinoid Receptors

The ability of ⁹-THC to modulate insulin release from isolated pancreatic islets was first documented in 1986 [59]. The subsequent discovery of the ECS and studies showing that CB₁R blockade improves glycaemic control in preclinical models of metabolic syndrome [38] and in diabetic human subjects [11] prompted inquiries into the presence and function of the ECS in the endocrine pancreas, which were the subject of a recent detailed review [60]. The first such study indicated that mouse islets express CB₁R mRNA and protein predominantly in α -cells, while CB₂R was detectable in both α - and β -cells [61], and similar findings were reported by others [62]. In contrast, another group reported that mouse isolated islets express CB₁R but not CB₂R, and CB₁Rs were localized mostly in β -cell rich areas [63]. The unique co-localization of CB₁R with β -cells has also been documented in fetal mouse islets [32] as well as in mouse pancreatic sections, from which β -cells were micro-dissected by laser capture microscopy and the presence of CB₁R mRNA confirmed by quantitative real-time PCR [64]. Both CB₁R and CB₂R were found to be expressed in the MIN6 mouse insulinoma cell line, which also expressed DAGL α and DAGL β [65]. Transient receptor potential vanilloid-1 (TRPV1) receptors, a putative secondary target of AEA, were identified in both α - and β -cells of mouse islets, and their genetic disruption or pharmacological blockade resulted in increased islet size because of an increased ratio of β - to α -cells, implying a developmental role of tonic TRPV1 activation in the mouse islet [32].

In the rat, β -cells were found to express both CB₁R and CB₂R [66], with selective activation of CB₂R by the synthetic CB₂R-selective agonist JWH133 resulting in improved glucose tolerance [66] and selective activation of CB₁R causing glucose intolerance [67]. Additionally, CB₁R and CB₂R as well as the enzymes involved in the biosynthesis and degradation of AEA and 2-AG have been identified in insulinoma cell lines RIN-m5F [57] and INS-1E [68], the latter also expressing vanilloid TRPV1 receptors, a potential secondary target of AEA [68]. In contrast, another group using normal rat islets reported that CB₁R and CB₂R are expressed exclusively in glucagon-producing α -cells, as documented using immunohistochemistry and confocal microscopy [69], whereas yet another group detected CB₁R expression uniquely in somatostatin-producing δ -cells, and neither in α - or β -cells, or in islets from Zucker fatty rats, mice or humans [70]. Finally, the putative cannabinoid receptor GPR-55 has been identified in the rat pancreas, with both GPR-55 mRNA and protein colocalized with β -cells [71]. Incubation of rat- or mouse-isolated islets with the

putative GPR-55 agonist O-1602 increased calcium transients and glucose-stimulated insulin secretion (GSIS), and these effects were absent in islets from GPR-55 knockout mice [71].

Cannabinoid receptor expression was also analysed in human isolated islets. In one study, CB₁R was highly expressed in glucagon-producing α -cells and less so in β -cells, with CB₂R expression being restricted to somatostatin-producing δ cells [72], and similar findings were reported by others [64]. A different picture emerges from a more recent study, in which CB₁R was uniquely localized in β -cells but not α -cells, and the reverse was true for DAGL α and MAGL in both human and fetal mouse islets, suggesting that α -cell-derived 2-AG may be involved in the developmental priming and recruitment of β -cells via CB₁R activation [32].

The apparent lack of consensus about the cell-type-specific expression of CB₁R and CB₂R protein in the endocrine pancreas may be related, at least in part, to the uncertain specificity and sensitivity of the respective antibodies, as discussed recently [73,74]. The level of CB₁R expression in islets may also depend on metabolic state, with food restriction resulting in upregulation, whereas high glucose caused downregulation of CB₁R expression in rat islets [75]. This highlights the need to combine morphological studies aimed at analysing cannabinoid receptor expression in specific islet cell types, most notably β -cells, with functional studies of the effects of selective CB₁R and CB₂R ligands on β -cell functions, such as basal and glucose-stimulated insulin release and β -cell proliferation/survival, using both *in vitro* and *in vivo* paradigms.

Endocannabinoid Biosynthetic and Metabolizing Enzymes

The presence in the pancreatic islets of the enzymatic machinery involved in the biosynthesis and degradation of endocannabinoids has also been documented. Bermudez-Silva et al. reported the presence of DAGL α , DAGL β , MAGL and FAAH in isolated human islets, with minimal to no expression of NAPE-PLD, although the localization of the enzymes to β -cells has only been determined for FAAH [72]. RIN-m5F rat insulinoma cells were reported to express all of the key enzymes involved in the biosynthesis and degradation of AEA and 2-AG [57]. Similarly, in mouse and human isolated islets, NAPE-PLD, DAGL α and FAAH were detected predominantly in β -cells [64], whereas MAGL was present both in α -cells and β -cells [64,65]. The amount of endocannabinoids produced by RIN-m5F cells was increased by high glucose concentrations and this increase was inhibited by insulin [57], which may be one of the mechanisms underlying the increased endocannabinoid ‘tone’ in metabolic syndrome and its complications, including diabetes [11,58]. The ability of glucose to increase endocannabinoid production was also documented in mouse and human isolated islets [64]. MAGL is also expressed in MIN6 mouse insulinoma cells where its pharmacological blockade, aimed to increase endogenous 2-AG levels, resulted in increased Ca²⁺_i and insulin secretion [65], and similar effects could be elicited by exogenous CB₁R and CB₂R agonists [76]. MAGL inhibition also resulted in increased insulin and glucagon secretion from human isolated islets *in vitro*, suggesting an important role of endogenous 2-AG in both β -cell and α -cell function [65]. Overall, these studies have revealed the presence of a functional ECS in pancreatic islets, however, the effects of endocannabinoids and

cannabinoid receptor activation on downstream signalling and on insulin release still remain unclear.

Endocannabinoid Modulation of Insulin Secretion from Pancreatic β Cells

In mouse cultured islets, the endocannabinoid 2-AG was found to inhibit calcium oscillations implicated in pulsatile insulin release from β -cells, via pertussis toxin-sensitive CB₂R, as deduced from the effects of selective CB₁R and CB₂ R agonists and antagonists [61]. Similar effects of AEA and its non-hydrolysable analogue R(+)-methanandamide suggested that CB₁R also contributes to these effects, with the dominant effect of endocannabinoids being inhibition of insulin release [61]. The inhibitory role of CB₁R is more directly indicated by another study, in which AEA or the CB₁R-selective agonist arachidonylcyclopropylamide inhibited GSIS and Ca²⁺ oscillations in mouse islets, which were found to express CB₁R but not CB₂R [63]. These findings are compatible with the role of the protein kinase A/cAMP system in sensitizing insulin release [77], and also with *in vivo* observations of restoration of GSIS by CB₁R antagonist treatment of Zucker diabetic fatty (ZDF) rats [53]. In contrast to these findings, several other studies using isolated islets [72] and insulinoma cell lines [57,65,68,76] have documented cannabinoid stimulation of both basal and glucose-stimulated insulin release mediated by CB₁R. The inability of CB₁R or CB₂ R antagonists to inhibit such effects in other studies suggested cannabinoid receptor-independent mechanisms [69,78], such as direct inhibition of K_{ATP} channels [79]. GSIS is known to involve cytoskeletal and focal adhesion remodelling at β -cells, which allows docking and fusion of insulin granules to the cell membrane for exocytosis. Recent findings indicate that glucose-induced adhesion remodelling involves increased phosphorylation of focal adhesion kinase (FAK), paxillin and ERK1/2, and also requires the interaction of β integrin [80]. CB₁R activation also leads to FAK phosphorylation in INS-1E insulinoma cells [68].

Although the reasons for these discrepant findings are not clear, differences between normal intact islets and insulinoma cells, signalling via G-protein-dependent versus G-protein-independent pathways, using freshly isolated or chronically cultured preparations, or activation versus downregulation of CB₁R depending on the concentration of agonists or the length of agonist exposure may be factors. Indeed, AEA inhibited insulin secretion in freshly isolated rat islets but stimulated it in overnight cultured islets via CB₁R/CB₂R-independent mechanisms [81], whereas, in another study, 48 h exposure of mouse islets to a potent CB₁R agonist blunted its subsequent acute insulin secretory effect at low but not at high glucose [82]. Furthermore, acute exposure of INS-1E insulinoma cells to a maximally effective concentration of 0.1 μ M AEA caused a twofold increase in insulin secretion in 2.5mM glucose, whereas a 24-h exposure to a much higher effective concentration of 10 μ M AEA caused only a 1.4-fold increase under the same conditions (Figure 3A vs Figure 5A in [68]). This discrepancy may be related, in part, to the differential affinity of AEA for CB₁R versus CB₂R. One should also keep in mind that different mechanisms may underlie effects on basal insulin release, which is increased in diabetic islets and reversed by CB₁R blockade [83], or on GSIS, which is reduced or absent in overt T2D. Similarly, chronic rimonabant treatment of hyperinsulinaemic ZDF rats normalized their plasma insulin levels and also resulted in reduced basal insulin secretion by islets isolated from such animals without

affecting GSIS, thus resulting in an improved stimulation index [84]. An analogous finding is the increased GSIS in isolated islets from $CB_1R^{-/-}$ mice compared with wild-type controls [32]. Although some of the above *in vitro* findings on cannabinoid modulation of insulin release are in good agreement with *in vivo* data, it is likely that the pro-diabetic function of the endocannabinoid/ CB_1R system indicated by *in vivo* studies is more closely linked to parallel effects on the survival and proliferation of β -cells, as discussed below.

Endocannabinoid Regulation of Pancreatic β -Cell Survival and Proliferation

Insulin is known to act as an autocrine factor promoting β -cell proliferation, and this mechanism is required for the maintenance of β -cell mass. T2D is associated with reduced total β -cell mass, and a recent study showed that GPCRs, signalling via $G_{\alpha_{i/o}}$ proteins (G_i -GPCRs), regulate β -cell mass by inhibiting β -cell proliferation, and in particular the developmental expansion of β -cell mass during the perinatal period [85]. Indeed, β -cell-specific transgenic overexpression of different G_i -GPCRs in mice resulted in reduced β -cell mass and impaired glucose homeostasis in adult animals, whereas β -cell-specific transgenic expression of the gene encoding the $G_{i/o}$ inhibitor pertussis toxin, selectively induced during the perinatal period, resulted in increased β -cell mass and improved glucose homeostasis [85]. In line with the above findings, an attractive hypothesis has recently been put forward to provide a mechanistic basis for the effects of the ECS on β -cell function and glucose homeostasis [28,64]. Accordingly, agonist-activated CB_1R in mouse β -cells was found to form a heterotrimeric complex with $G_{i\alpha}$ and the insulin receptor, and $G_{i\alpha}$ inhibited insulin receptor autophosphorylation by binding to the activation loop in its tyrosine kinase domain. This caused inhibition of the phosphorylation of the apoptotic protein BAD, resulting in its increased apoptotic activity and β -cell death [28]. In turn, genetic deletion or pharmacological blockade of CB_1R in diabetic (*db/db*) mice resulted in increased insulin receptor signalling via insulin receptor substrate 2 (IRS2)/AKT in β -cells, reduced blood glucose, and increased β -cell mass [64]. In another study, however, the size of immature (developing) islets and their β -cell content remained unchanged in $CB_1R^{-/-}$ compared with $CB_1R^{+/+}$ mice [32].

It is now recognized that T2D is an inflammatory condition, with earlier studies focusing on adipose tissue inflammation and its contribution to insulin resistance. More recent evidence indicates that inflammatory cell infiltration into pancreatic islets leads to cytokine-driven insulinitis, which may contribute to β -cell loss and the consequent progression of insulin resistance to overt T2D [86–88]. This hypothesis is supported by the documented therapeutic efficacy in T2D of the IL-1 receptor antagonist anakinra [89] or a monoclonal anti-IL-1 β antibody [90,91]. Although anti-IL-1 treatment has not yet gained regulatory approval, the concept of anti-inflammatory therapy for T2D continues to be evaluated by ongoing clinical trials with anakinra in T2D (NCT02310009; NCT00928876, listed at clinicaltrials.gov). Additional observations indicate that glucotoxicity-induced endoplasmic reticulum (ER) stress in β -cells activates the thioredoxin interacting protein TXNIP and its downstream targets, the Nlrp3 inflammasome and IL-1 β , leading to β -cell death [92–94]. As high glucose levels can induce endocannabinoid synthesis in β -cells [57,64] and activation of CB_1R on β -cells can cause their apoptosis [28], these findings could suggest an autocrine

mechanism through which β -cell-derived endocannabinoids acting on CB₁R on the same cells cause apoptosis via activation of a proinflammatory cascade.

An alternative paracrine mechanism may be more important in the diabetic islet *in vivo*, as indicated by the results of a recent study using ZDF rats, a model of T2D with progressive β -cell loss [53]. In that study, increased expression of CB₁R and the Nlrp3/Asc inflammasome in islets of ZDF compared with non-diabetic control rats co-localized with infiltrating M1 macrophages rather than β -cells, and *in vivo* treatment with a peripherally restricted CB₁R antagonist, depletion of macrophages, or macrophage-specific *in vivo* knockdown of CB₁R prevented the progressive β -cell loss and the associated hyperglycaemia, and restored GSIS. Incubation of isolated human or rodent macrophages with AEA elicited increased expression of Nlrp3/ASC and the macrophage chemotactic protein (MCP)-1, as well as the proteolytic activation and release of active IL-1 β and IL-18 [53]. Interestingly, IL-1 β induced a robust downregulation of CB₁R gene expression in MIN6 insulinoma cells that would further attenuate a direct effect of endocannabinoids on β -cells in the diabetic islet. Conversely, CB₁R expression by macrophages was 'auto-induced' by endocannabinoids [53], which might represent a feed-forward mechanism whereby the initial CB₁R-induced inflammatory signalling is amplified through upregulation of CB₁R. Another interesting aspect of this study was that chronic CB₁R blockade prevented β -cell loss and the development of hyperglycaemia and restored GSIS in ZDF rats, but did not reduce basal hyperinsulinaemia and insulin resistance, providing another indication that different mechanisms may be involved in basal insulin hypersecretion and GSIS [53]. Together, these findings support a model whereby endocannabinoid activation of CB₁R on pro-inflammatory macrophages promotes their transmigration into islets where they induce the apoptosis of neighbouring β -cells via the release of cytotoxic IL-1 β and IL-18, as illustrated schematically in Figure 1. Although the applicability of this model to human T2D remains to be tested, a recent report of increased expression of the NLRP3 inflammasome in monocytic cells isolated from the peripheral blood of untreated patients with diabetes compared with control subjects without diabetes is compatible with this hypothesis [95]. These findings also highlight the therapeutic potential of peripherally restricted CB₁R antagonist in T2D.

Glycaemic Regulation via Extrapancreatic CB₁R

Although the focus of the present paper is β -cell function, there is evidence of modulation of insulin resistance by endocannabinoids via CB₁R outside the pancreas. Mice with hepatocyte-specific deletion of CB₁R are protected from high-fat diet-induced glucose intolerance and insulin resistance [96], and the diabetic phenotype can be rescued by transgenic re-expression of CB₁R in hepatocytes of mice with a global CB₁R^{-/-} background [97]. These findings implicate hepatocyte CB₁R in the hepatic insulin resistance that accompanies diet-induced obesity, in which the increased hepatic glucose production may result from a CB₁R-mediated increase in glycogenolysis [97] and/or gluconeogenesis [98]. Furthermore, CB₁R activation on skeletal muscle cells inhibits glucose uptake by suppressing GLUT4 expression [99], which contributes to peripheral insulin resistance. The dominant role of peripheral CB₁R in the insulin resistance of obese animals is indicated by its reversal by peripherally restricted CB₁R antagonists [39,40], although hypothalamic

CB₁R may also be involved under certain conditions, such as following short-term overfeeding [100].

Future Directions

Current evidence for the involvement of endocannabinoids and their receptors in the control of glycaemic functions has been based largely on studies using rodent models of diabetes and obesity. Although early clinical studies with the CB₁R antagonist rimonabant supported the role of CB₁R in insulin resistance and T2D in obese/overweight individuals, the withdrawal of rimonabant from the market because of neuropsychiatric side effects has hampered further exploration of the underlying mechanisms in humans. Recent evidence in rodent models of T2D and obesity indicate that peripherally restricted CB₁R antagonists retain the metabolic benefits, including the β -cell protecting effect, of globally acting compounds without the neuropsychiatric liability of the latter may revive the therapeutic potential of CB₁R blockade in diabetes. One such compound, JD-5037, is currently undergoing IND-enabling toxicology screening.

Conclusions

Mounting evidence indicates that an overactive ECS is involved in the development of diabetes and insulin resistance, with the prominent involvement of CB₁R in multiple peripheral organs. Pancreatic β -cells, the major source of insulin in the body, have a fully functional ECS, and there is both *in vitro* and *in vivo* evidence of endocannabinoid modulation of basal insulin secretion and GSIS as well as the survival and proliferation of β -cells. Studies using isolated pancreatic islets have often provided conflicting information as to the cell-type specific localization CB₁R in the islet and the direction of change – increase or decrease – elicited by CB₁R activation in basal insulin secretion and GSIS. In contrast, there is unambiguous evidence that chronic CB₁R blockade attenuates diabetes and insulin resistance and protects against β -cell loss in both human and experimental diabetes and obesity/metabolic syndrome. These latter findings are consistent with the documented role of endocannabinoids in promoting the apoptosis and inhibiting the proliferation of β -cells. The results of *in vitro* studies are compatible with an autocrine model whereby β -cell-derived endocannabinoids promote apoptosis via CB₁R-mediated induction of apoptotic and inflammatory gene expression. Evidence from *in vivo* studies supports an alternative paracrine model whereby activation of CB₁R on proinflammatory macrophages promotes their expression of the Nlrp3 inflammasome and their infiltration into diabetic islets, where they release cytotoxic IL-1 β and IL-18 that cause β -cell death. Prevention of this process by peripherally restricted CB₁R antagonists highlights the therapeutic potential of this class of compounds.

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Figure 1. Schematic illustration of the mechanism of CB₁ receptor (CB₁R)-mediated β -cell loss in the diabetic islet. High circulating glucose and/or palmitic acid induces arachidonylethanolamide (AEA) synthesis in infiltrating macrophages. The released AEA acts in an autocrine fashion to activate macrophage CB₁R (inhibited by the CB₁R antagonist JD5037) to induce the Nlrp3 inflammasome. This results in the release of active IL-1 β , which then acts on interleukin (IL)-1 receptors on neighboring β -cells to cause their apoptosis. IL-1 β also stimulates macrophage chemotactic protein (MCP)-1 production by β -cells, and the released MCP-1 acts on the chemokine receptor CCR2 to promote the transmigration of additional macrophages into the islet. This figure was prepared using a template on the Servier medical art website (<http://www.servier.fr/servier-medical-art>) and reproduced with permission from Jourdan et al. [53].