REVIEW

Inhibitory G proteins and their receptors: emerging therapeutic targets for obesity and diabetes

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The worldwide prevalence of obesity is steadily increasing, nearly doubling between 1980 and 2008. Obesity is often associated with insulin resistance, a major risk factor for type 2 diabetes mellitus (T2DM): a costly chronic disease and serious public health problem. The underlying cause of T2DM is a failure of the beta cells of the pancreas to continue to produce enough insulin to counteract insulin resistance. Most current T2DM therapeutics do not prevent continued loss of insulin secretion capacity, and those that do have the potential to preserve beta cell mass and function are not effective in all patients. Therefore, developing new methods for preventing and treating obesity and T2DM is very timely and of great significance. There is now considerable literature demonstrating a link between inhibitory guanine nucleotide-binding protein (G protein) and G protein-coupled receptor (GPCR) signaling in insulin-responsive tissues and the pathogenesis of obesity and T2DM. These studies are suggesting new and emerging therapeutic targets for these conditions. In this review, we will discuss inhibitory G proteins and GPCRs that have primary actions in the beta cell and other peripheral sites as therapeutic targets for obesity and T2DM, improving satiety, insulin resistance and/or beta cell biology.

INTRODUCTION

Insulin resistance is a classic risk factor for the development of type 2 diabetes mellitus (T2DM).[1,2] A significant predisposition to insulin resistance is observed with obesity,[3] classified as a body mass index greater than 30. Obesity and its associated comorbidities are reaching never-seen-before levels worldwide.[4,5] With sufficient increases in basal and nutrient-stimulated insulin levels, insulin resistance may not progress to T2DM.[6] Systemic insulin levels can be increased by one of two general mechanisms: increased insulin secretion from individual beta cells (that is, beta cell function) and increased total numbers of beta cells (that is, beta cell mass).[7]

Reduced beta cell function in T2DM is clearly an early and important aspect underlying the pathophysiology of T2DM;[8–10] hence, addressing this dysfunction is a valid therapeutic strategy for T2DM. (Little is known of the effects of the therapeutics discussed in this review on alpha cell function. As dysregulated glucagon secretion has also been noted as a key issue in T2DM pathophysiology, these are clearly important questions to address, but are outside of the scope of this review.) The first T2DM therapeutics to target the beta cells were the sulfonylurea compounds, which act directly on the ATP-sensitive potassium channels of the beta cell to enhance membrane depolarization, stimulating exocytosis of insulin granules.[11] While still used clinically and excellent drugs in some circumstances, sulfonylureas act in a glucose-independent manner, increasing the potential risk of hypoglycemia.[12] Furthermore, sulfonylureas and other long-established therapeutics, including the insulin-sensitizer metformin, do not prevent the continuing loss of beta cell function observed in T2DM.[10] However, weight loss from metformin may indirectly benefit beta cell function and limit beta cell loss in T2DM.[13,14]

A different aspect of beta cell dysfunction is being addressed by recent T2DM therapeutics: the lack of potentiation of insulin secretion in response to an oral glucose/nutrient challenge.[15] This potentiation occurs because of peptide hormones termed incretins that are secreted from the digestive tract and act on the pancreas and other peripheral

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tissues to elicit biological effects, including augmenting nutrient-stimulated insulin secretion. This effect of incretins is lost or blunted in T2DM.[16]

The incretin receptors belong to a superfamily of transmembrane proteins termed as G protein-coupled receptors (GPCRs) that promote intracellular signal transduction pathways mediated by guanine nucleotide-binding proteins (G proteins). There are four major classes of G proteins: G_s, G_i, G_q, and G_{12}.[17] These proteins are classified based on the sequence identity of their alpha subunit, which confers similar downstream signaling mechanisms among subfamily members. G_s is the sole member of its subfamily not restricted to olfactory/taste sensory cells, while similar members of the G_i subfamily are G_{i1}, G_{i2}, G_{i3}, G_{o1}, G_{o2} and G_z. The Gs and Gi subfamilies also include specialized members: G_{olf} is a G_s subfamily member restricted to olfactory cells, while G_t and G_{gust} are G_i subfamily members restricted to the retina and taste cells, respectively. While taste and olfactory cell signaling is an interesting focus of current obesity and diabetes research (for example, see work from Dotson et al.[18]), only the more ubiquitously expressed G proteins will be discussed in this review.

The G_s and G_i subfamily members all signal by modulating the levels of the intracellular second-messenger cyclic AMP (cAMP), either stimulating (G_s) or inhibiting (G_i) the production of cAMP by adenylate cyclase. The impact of these proteins on cAMP production mirrors their effect on insulin secretion. For instance, most of the incretin receptors signal through G_s.[19] cAMP has also been shown to regulate beta cell proliferation and survival, potentially linking these receptors to regulation of beta cell mass.[20,21] The G_q class of G proteins is also stimulatory toward insulin secretion, but acts through separate second-messenger pathways: inositol trisphosphate and diacylglycerol, which are formed when G_q-activated phospholipase C-β cleaves phosphatidylinositol bisphosphate.[22] A smaller number of incretin receptors have been linked with G_q.[23] Not as much is known regarding the role of G_{12} on beta cell function, but its actions through the small G protein, Rho, appear to be important for promoting the exocytosis process itself.[24]

Drugs targeting the glucagon-like peptide 1 (GLP-1) receptor, a G_s-coupled incretin receptor, are some of the newer T2DM therapeutics, having been used clinically for nearly a decade.[25] The stable GLP-1 analogs, such as exenatide (also known as Byetta, Amylin Pharmaceuticals, San Diego, CA, USA), are resistant to inactivating cleavage by dipeptidyl peptidase 4, which rapidly degrades endogenous GLP-1. In addition, endogenous GLP-1 can be stabilized by treatment with dipeptidyl peptidase inhibitors, such as sitagliptin (also known as Januvia, Merck, Whitehouse Station, NJ, USA). Several other G_q- and G_s-coupled incretin receptors are being explored as T2DM therapeutic targets and have been reviewed elsewhere.[21,26,27] Even with these successes, incretin-based therapeutics do not work in all individuals and safety concerns, including pancreatitis and gastrointestinal distress, in addition to possible side effects such as weight changes lead to non-compliance.[28] These concerns raise the possibility of targeting potential dysfunction in other components of beta cell signaling pathways.

Endogenous inhibitors of beta cell function have long been known, even before the identity of their receptors and associated G proteins were established. For example, alpha adrenergic receptor (α-AR) agonists such as epinephrine and norepinephrine are released from the sympathetic nervous system as part of the fight-or-flight response, one aspect of which is to maintain higher glucose levels in the bloodstream.[29] α-ARs are expressed in beta cells, and agonists of the α2-AR subfamily, which are G_i-coupled receptors, rapidly reduce glucose-stimulated insulin secretion.[30] Another long-established inhibitor of beta cell function, prostaglandin E2 (PGE2), is produced by cyclooxygenase (COX)-catalyzed metabolism of arachidonic acid and acts in an autocrine and/or paracrine fashion to modulate beta cell growth and insulin secretion.[31] Interestingly, receptors for adrenergic agonists and PGE2, as well as their associated G proteins, have all been linked with diabetic beta cell pathophysiology.[32–35]

Insulin-responsive tissues besides the pancreas also play critical roles in the pathophysiology of T2DM. The pancreatic circulation empties directly into the portal vein, meaning that the liver is the first stop for hormones secreted from the pancreas, including insulin and glucagon. The liver is critical for the maintenance of whole-body glucose homeostasis. Insulin acts on the liver to signal the fed state of the body, promoting glucose storage into glycogen and blocking gluconeogenesis and lipolysis, blocking hepatic glucose and free fatty acid production. It is well accepted that hepatic fuel production is dysfunctional in T2DM, as insulin-resistant alpha cells secrete glucagon in an unregulated manner, further exacerbating the phenotype of liver insulin resistance.[36,37] In addition, muscle and adipose tissue are stimulated by insulin to increase glucose uptake for use as fuel and storage as glycogen and triglycerides, respectively. Inefficient muscle and adipose glucose uptake is also observed in T2DM and contributes to the pathology of hyperglycemia.[38,39] There is much debate surrounding the impact of enhanced beta cell function and mass to the development of insulin resistance in T2DM. This discussion is beyond the scope of this review, but has been well described elsewhere.[40] Specific GPCRs, including melatonin receptor isoform 2 (MT2), have been shown to have important roles in regulating insulin sensitivity.[41]

Metabolic syndrome is often linked to obesity and visceral fat accumulation known to perturb blood glucose regulation and increase insulin resistance; however, obesity by itself is not a definitive predictor of metabolic syndrome as some obese individuals do not present with typical etiologies.[42] A diagnosis of metabolic syndrome is a significant risk factor, though, for the development of T2DM, cardiovascular disease, neurodegeneration, polycystic ovarian syndrome, cancer and non-alcoholic fatty liver disease, among others.[43] Therefore, developing anti-obesity therapeutics as a preventative for these significant, chronic diseases is a major focus of current research. Several effective medical treatments for obesity have been removed from the market or are strictly

regulated due to unacceptable side effects. Fenfluramine/ phentermine (also known as phen-fen) was pulled from the US market in 1997. Sibutramine (also known as Reductil, Meridia and Sibutrex, Abbott Labs, Abbott Park, IL, USA) is a schedule IV-controlled substance and was pulled from the US market in 2010. Dextromethamphetamine (also known as Desoxyn, Abbot Labs) is a methamphetamine derivative and a schedule II-controlled substance. Furthermore, the few widely available medical treatments such as orlistat (also known as Xenical, Roche, Basel, Switzerland; and Alli, GlaxoSmithKline, Research Triangle Park, NC, USA) have only modest effects at best.[44] Surgical techniques such as gastric bypass and sleeve gastrectomy are often quite effective, but are also major procedures that have continuously decreasing, yet still not insignificant, peri- and post-operative morbidity and mortality risks.[45] A safe, effective medical treatment to augment healthy lifestyle changes in the efforts to combat obesity and metabolic syndrome is sorely needed. One inhibitory GPCR in particular, cannabinoid receptor type 1 (CB1) has been demonstrated as a potentially effective anti-obesity pharmacological target.

In this review, we will discuss G_i subfamily members that have been linked to beta cell dysfunction, insulin resistance or both. Next, we will discuss a select number of inhibitory GPCRs that have been shown to have significant pre-clinical or clinical evidence of efficacy as anti-T2DM and/or anti-obesity targets. These include GPCRs whose major effect is in the beta cell, as potential targets to maintain beta cell function and/or mass; and GPCRs with primary effects in other insulin-responsive tissues, as targets to improve insulin resistance and/or obesity. It is our hope that this review will stimulate further research into these relatively under-studied proteins as potentially useful therapeutic targets for the obesity and T2DM that is becoming a worldwide public health problem.

G_i SUBFAMILY PROTEINS AS TARGETS FOR BETA CELL DYSFUNCTION IN T2DM

Beta cell dysfunction and insulin resistance are early and necessary events in the development of T2DM,[8] and G_i signaling has been shown to be an important regulator of both these events. The G_i family inhibitor, pertussis toxin (PTX), was originally named islet-activating protein due to its ability to promote glucose- and hormone-stimulated insulin secretion long before its mechanism of action was known.[46] Even so, no insulin secretion phenotypes had been reported in mouse lines deficient in individual G_i subfamily members, until those lacking only the PTX-insensitive G_i subfamily alpha subunit, $G\alpha_z$, were studied (Since then, other G_i subfamily members that are sensitive to PTX have been linked with regulating beta cell function *in vivo*, explaining the effect of PTX on insulin secretion: these will be discussed in more detail below). By sequence and functional similarity, G_z is clearly a member of the G_i subfamily, but it is unique in that its alpha subunit ($G\alpha_z$) lacks the C-terminal cysteine residue that is the substrate for PTX-mediated ADP ribosylation and inactivation.[47] $G\alpha_z$-null mice exhibit improved insulin secretion response and glucose clearance after glucose

challenge, correlating with constitutively increased cAMP levels and improved glucose-stimulated insulin secretion from their isolated islets.[48] These effects were observed even though no GPCR had been specifically targeted. The slow $G\alpha_z$ GTP hydrolysis and deactivation rate were posited as a potential explanation for constitutive effects,[47,48] although endogenous beta cell GPCRs have been linked specifically with G_z. Agonists activating the prostaglandin E receptor 3 (EP3) isoform of the PGE2 receptor block glucose-stimulated insulin secretion from insulinoma cells and isolated mouse islets in a PTX-insensitive manner, suggesting coupling of the EP3 receptor specifically to G_z and not other G_i proteins.[33,49] These results were confirmed with specific inhibitors of $G\alpha_z$ itself.[49] In addition, the modulation of endocytosis following exocytosis (a required event in the maintenance of insulin secretion) by norepinephrine is also PTX-insensitive. A synthetic peptide mimicking the C-terminus of the $G\alpha_z$ blocked this norepinephrine effect.[50]

There appears to be no effect of $G\alpha_z$ on insulin sensitivity. This is not unexpected, as $G\alpha_z$ has little-to-no expression in the liver, adipose tissue or skeletal muscle,[47,51,52] and although expressed in the brain, does not seem to regulate appetite or energy metabolism.[33] Even so, $G\alpha_z$-null mice are completely protected from the development of glucose intolerance upon high-fat diet feeding, primarily through a significant augmentation of beta cell mass and maintenance of beta cell function.[33] These results suggest $G\alpha_z$ as an emerging target to improve both defects of the diabetic islet: reduced beta cell mass and decreased beta cell function.

Other G_i subfamily members, including both G_i and G_o isoforms, have been implicated in directly inhibiting exocytosis from beta cells,[53] which, together with their subcellular localization, indicates a role for G_i proteins in the regulation of a distal step in the stimulated secretion pathway. In studies in an insulinoma cell line, norepinephrine inhibited the refilling of the readily releasable pool of secretory granules, an effect that was abolished by a dual $G\alpha_{i1/2}$-blocking peptide.[54] Perplexingly, mice deficient in $G\alpha_{i2}$ expression did not have noticeably improved insulin secretion, but instead had reduced insulin sensitivity.[55] Furthermore, expression of constitutively active $G\alpha_{i2}$ in mouse liver and adipose tissue enhanced glucose tolerance.[56] Among other effects, a direct effect of $G\alpha_{i2}$ signaling on GLUT4 membrane translocation through a phosphoinositide 3-kinase-dependent mechanism may be responsible for these phenotypes.[57]

Early studies with $G\alpha_o$-null mice indicated changes in the nervous system structure and aberrant heart calcium channel signaling, but no effect on blood glucose levels.[58] In more recent work, one of the two splice variants of $G\alpha_o$, $G\alpha_{o2}$, was revealed as a key regulator of insulin secretion. Mice lacking $G\alpha_{o2}$, but not those lacking $G\alpha_{o1}$ or any $G\alpha_i$ isoforms have augmented glucose-stimulated insulin secretion and improved glucose tolerance,[59] just as observed with $G\alpha_z$-null mice. Moreover, these results help support earlier findings that targeting PTX-sensitive proteins, such as $G\alpha_{o2}$, can potentiate insulin secretion but in a different manner than

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$G\alpha_z.$[46,49] These results are important, as they suggest that the G_i subfamily members in the beta cell are not redundant, as has been suggested in other systems[60] and that specific isoforms and/or their associated receptors might be useful T2DM therapeutic targets. Of note, no small-molecule pharmaceutical agents in use clinically target a GPCR/G protein pathway directly at the G protein level (although some have been proposed hypothetically and/or tested *in vitro*[61–63]). In addition, biased GPCR ligands can preferentially effect one versus another G protein-dependent or -independent downstream signaling pathway, but these are still receptor-based drugs and not G protein-based.[64] Thus, understanding more about the endogenous GPCRs that these inhibitory G proteins couple to will likely be very important in pharmaceutically targeting their activation.

THE EP3 RECEPTOR: TARGETING DYSFUNCTIONAL AGONIST PRODUCTION AND RECEPTOR SIGNALING IN T2DM

The pathophysiology of T2DM is increasingly being linked with inflammation.[65–67] Many metabolites of the ω-6 polyunsaturated fatty acid (PUFA) arachidonic acid have been linked directly with inflammation.[68,69] E-series prostaglandins, comprising a major family of arachidonate metabolites, were posited as playing a role in T2D over 30 years ago.[70,71] PGE2 in particular has long been known as a physiological inhibitor of insulin secretion, acting via autocrine or paracrine mechanisms.[72] This means that islet cells themselves, including beta cells, express the enzymes required for synthesis of PGE2, including phospholipase A2, which cleaves arachidonate from plasma membrane phospholipids; COX, which convert the fatty acid into the intermediate PGH2; and PGE synthases (Ptges), which convert PGH2 to PGE2.

The ω-3 PUFAs compete with ω-6 PUFAs for some of the same metabolic enzymes. Many groups (including the American Diabetes Association) have recommended a high ω-3 PUFA diet as a means to prevent disease.[73] Yet, there still exists some controversy as to the benefits of ω-3 PUFAs on chronic disease[74,75] as well as the impact of high ω-3 PUFAs in the context of a diet that remains high in ω-6 PUFAs. Furthermore, few studies have explored these interventions specifically in the beta cell. Notable exceptions include two groups that generated transgenic mice expressing a *Caenorhabditis elegans* desaturase that converts ω-6 PUFAs to the ω-3 form. These mice robustly increased ω-3 PUFA concentrations (and reduced ω-6 PUFAs to near zero) in all tissues, with improved insulin secretion from isolated islets.[76,77]

Inhibition of COX (which, in beta cells, is primarily COX-2[31,78]) can completely block PGE2 production along with that of numerous other metabolites downstream of PGH2 and the other COX intermediates. The upregulation of COX-2 expression and/or activity has been clearly linked with the diabetic state.[66,79–82] The potential utility of COX-2 inhibitors in T2DM is overshadowed by concerns of significant

cardiovascular risks, especially in a population at greater intrinsic risk of cardiovascular disease.[83,84] Interestingly, after nearly a half century, there has been a recent resurgence in studies on the utility of salicylate, which acts directly at the site of inflammation to reduce COX-1 and COX-2 expression, as a T2D therapeutic.[85,86]

Recently, a specific PGE2 receptor isoform, EP3, has been suggested as an emerging target for T2DM therapeutics. EP3 receptor expression and activity was significantly upregulated in diabetic mouse islets, as was the production of PGE2.[32] Targeting the EP3 receptor with a specific antagonist, L798,106, significantly improved the insulin secretory response of diabetic mouse and human islets.[32] Interestingly, EP3 signaling was found to oppose the action of the GLP-1 receptor, discussed above as a significant potentiator of glucose-stimulated insulin secretion. Specifically, the maximal effect of GLP-1 on insulin secretion was blunted by EP3 activation, and a combination of an EP3 antagonist and GLP-1 agonist additively promoted insulin secretion from diabetic mouse islets.[32] The mechanisms mediating dysfunctional EP3 signaling in diabetic beta cells and potential ways to target EP3 in T2DM therapy are shown on the left half of Figures 1.

Although the EP3 receptor plays critical roles in islet biology, extrapancreatic function may be equally important in T2DM pathophysiology; in particular, in mediating T2DM complications. The migratory response of vascular smooth muscle cells is required for the dysfunctional vascular remodeling observed with cardiovascular disease. Genetic deletion or pharmaceutical inhibition of EP3 results altered polarity and directional migration of vascular smooth muscle cells, suggesting that blockade of EP3 might protect from dysfunctional vascular remodeling.[87] In addition, mice lacking EP3 have reduced baseline and stimulated mean arterial pressures, and pharmacological inhibition of EP3 blocks angiotensin 2-mediated vasoconstriction.[88] Thus, it appears that PGE2 signaling through EP3 may play a significant role in the progression of T2DM and its associated comorbidities.

Mice lacking EP3 develop obesity, insulin resistance, glucose intolerance and eat considerably more than wild-type controls.[89] Part of this phenotype can be explained by increased light cycle eating, most likely due to unstable sleep patterns, as PGE2 (acting through hypothalamic EP3) may act as a somnogen.[90] Furthermore, high levels of PGE2 and increased signaling through EP3 both augment nitric oxide synthase expression, an enzyme critical in the brain development of newborns.[91] This signaling cascade is suggested to play a significant role in connecting brain circulation and synaptic activity in perinatal development. If EP3 antagonists are pursued as therapeutics for T2DM and/or metabolic disease, a peripherally restricted EP3 antagonist might be necessary. Interestingly, an EP3 antagonist, DG-041, completed a European phase II clinical trial by Decode Genetics testing its effect in treating peripheral vascular disease, which is typically caused and/or complicated by hypertension. The results of this trial were never published, so questions remain as to whether this drug was effective as an anti-
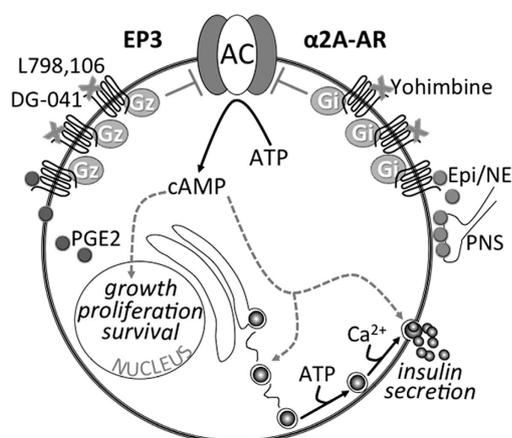
Figure 1 Summary of some of the proposed mechanisms of dysfunctional E prostanoid receptor 3 (EP3) and α2A-adrenergic receptor (α2A-AR) signaling in diabetic beta cells and how these receptors might be targeted in type 2 diabetes mellitus (T2DM) therapy. In a schematic of a diabetic beta cell, both expression and/or activity of both EP3 (left) and α2A-AR (right) have been shown to be dysfunctionally upregulated. Increased EP3 expression/activity is exacerbated by increased prostaglandin E2 (PGE2) production, acting in an autocrine/paracrine manner to further reduce cyclic AMP (cAMP) production by G_i subfamily member, G_z, signaling to adenylate cyclase (AC). In contrast, the release of α2A-AR agonists epinephrine and norepinephrine (Epi/NE) after stimulation by the parasympathetic nervous system (PNS) is not necessarily dysfunctionally upregulated in T2DM; rather, a specific *ADRA2A* single nucleotide polymorphism (SNP) confers increased stability of α2A-AR at the plasma membrane, allowing Epi/NE to tonically signal through α2A-AR and associated G_i subfamily proteins to reduce cAMP production by AC. Of note, cAMP is one of the only signaling pathways shown to positively impact on both beta cell mass (that is, growth, proliferation and survival) and beta cell function (that is, insulin secretion). Gray arrows and text indicate confirmed or potential downregulation of these effects with dysfunctional EP3 or α2A-AR signaling. The EP3 antagonist, L798,106, has been shown to reverse diabetic beta cell dysfunction in isolated islets *in vitro*, while DG-041 has gone through clinical trials for a different indication, indicating potential *in vivo* utility. Also suggested in this figure is the potential to target EP3 signaling by reducing PGE2 production with nutritional or pharmacological interventions. With regards to α2A-AR, a specific antagonist, yohimbine, improves insulin secretion from islets isolated from individuals with the specific *ADRA2A* SNP conferring α2A-AR stability. A clinical trial to confirm this finding *in vivo* has been initiated. Again, both EP3 and α2A-AR antagonists have the potential to improve beta cell mass, but this has yet to be confirmed.

hypertensive agent. Recently, DG-041 treatment in mice was found to limit atherothrombosis without altering hemostasis, suggesting that DG-041 can decrease clot formation without increasing bleeding risk, critical in the prevention of cardiovascular disease.[92]

THE α2A AR: TARGETING GENETIC SUSCEPTIBILITY TO T2DM

Dysfunctional α-AR signaling was noted in T2DM at least 40 years ago.[34,93] In an early clinical study of six diabetic individuals, α-AR blockade with phentolamine restored the weak diabetic insulin secretion response to glucose.[34] α-AR antagonists were never much pursued as anti-diabetic agents, though, perhaps due to the systemic effects of α-AR signaling, including smooth muscle contraction, inhibition of neurotransmitter release, transient hypertension followed by sustained hypotension, and constriction of certain veins and arteries, including those leading to and from the heart, among others.

This possible thinking that α-AR signaling was too pleiotropic to be targeted therapeutically in T2DM changed in 2010, when a single nucleotide polymorphism in the α2A-AR gene, *ADRA2A*, was discovered as a susceptibility factor for T2D: rs553668.[35] In two different studies, including over 5000 individuals, Rosengren *et al.*[35,94] showed that individuals with a specific rs553668 genotype trended toward elevated fasting plasma glucose levels and reduced serum insulin levels. Furthermore, these individuals were significantly more prone to a hypoinsulinemic response during glucose tolerance testing, indicating a defect in the insulin secretion process itself.[35,94] When human pancreatic islet preparations were genotyped, those with the specific rs553668 variant had reduced insulin granule docking and secreted less insulin in response to glucose due to increased expression of α2A-AR at the membrane.[35,94] Treatment of isolated islets with an α2A-AR antagonist, yohimbine, corrected the insulin secretory dysfunction in these islets.[35] A clinical trial initiated in the US is currently enrolling patients with this specific genotypic risk variant in a randomized, controlled trial of yohimbine as a T2D therapeutic.

A more recent meta-analysis of four prospective studies confirmed the linkage of rs553668 with fasting glucose and T2DM risk, and found another variant of *ADRA2A*, rs17186196, associated with fasting glucose levels, fasting insulin levels and whole-body insulin sensitivity.[95] Furthermore, rs491589 was associated with systolic and diastolic blood pressure, while rs36022820 correlated with body mass index.[95] Additional *ADRA2A* variants have been linked with hypertension, a significant risk factor for cardiovascular disease, a common complication of T2DM.[96,97] In a study of 70 hypertensive patients, 35 with and 35 without T2DM, individuals with a genotype encoding for a small deletion in *ADRA2A* were significantly more likely to have hypertension, regardless of T2DM status.[98] A potential physiological mechanism for this association was the significantly lower high-density lipoprotein and higher low-density lipoprotein in individuals homozygous for the deletion phenotype.[98] Overall, these results confirm the utility of targeting the α2A-AR to improve beta cell function in T2DM in a specific population, and also indicate its possible utility as a target for insulin resistance, obesity and/or T2DM complications. The mechanisms mediating dysfunctional α2A-AR activity in a specific subset of diabetic beta cells and potential ways to target α2A-AR in T2DM therapy are shown on the right half of Figure 1.

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THE MT2 RECEPTOR: TARGETING DYSREGULATED INSULIN SECRETION AND PERIPHERAL INSULIN RESISTANCE

It has been demonstrated in humans that disruption of the circadian rhythm promotes obesity and diabetes, and leads to perturbations in plasma glucose and insulin concentrations.[99] For instance, melatonin (N-acetyl-5-methoxytryptamine) is secreted from the pineal gland and has a major role in the regulation of circadian rhythms. It has also been shown to affect carbohydrate metabolism and insulin secretion.[100] Melatonin acts through two receptors, MT1 (gene name: MTNR1A) and MT2 (gene name: MTNR1B). Activation of MT1 or MT2 receptors in the beta cell inhibit insulin secretion.[100] However, it is not melatonin antagonists that have been proposed as obesity/T2DM therapeutics, but rather melatonin agonists. This is because MT1/MT2 receptor signaling also reduces dyslipidemia, reduces hepatic glucose output, decreases adiposity and improves metabolic function.[41,101,102] Low melatonin levels have also been independently associated with T2DM risk.[103] Thus, stimulating melatonin signaling has been proposed as a treatment for metabolic syndrome.[41]

Altered melatonin receptor expression or loss-of-function mutations and altered melatonin secretion itself have all been previously linked with T2DM or glucose intolerance.[103] Melatonin was shown to function by reducing hepatic gluconeogenesis and phosphorylation and activation of Akt in the hypothalamus.[101] Most of the recent research into the links between the melatonin receptors and diabetes is focused on MT2. Independent genome-wide association studies in European populations identified single nucleotide polymorphisms near MTNR1B that were associated with fasting plasma glucose, reduced beta cell function, and significantly increased T2DM risk: rs1387153 and rs10830963.[104,105] Expression of these two single nucleotide polymorphisms is also significantly associated with the development of gestational diabetes,[106,107] as well as elevated fasting glucose and reduced insulin secretion after parturition.[106] A diagnosis of gestational diabetes is one of the highest predictors for the later development of T2DM.[106] A clinical trial was registered in late 2012 to test the effects of 3 months of melatonin treatment on glucose tolerance in individuals with different MTNR1B variants.

THE CB1 RECEPTOR: TARGETING APPETITE AND SATIETY AS AN ANTI-OBESITY THERAPEUTIC

CB1 is a G_i-coupled receptor located in the central and peripheral nervous system as well as other insulin-responsive tissues. Endogenous CB1 ligands include anandamide (which is responsible for pleasurable effects, such as that from eating chocolate) and 2-arachidonoyl glyceride.[108] Natural and synthetic cannabinoids such as tetrahydrocannabinol, the main active compound of the psychoactive drug cannabis, and its synthetic analog, dronabinol, are potent CB1 agonists. In the brain, CB1 is highly expressed in the hypothalamus, which regulates appetite, and the medulla, which contains the

chemoreceptor trigger zone, regulating nausea and vomiting, and where it is involved in the regulation of normal brain aging.[108] This explains why tetrahydrocannabinol is such an effective drug at combatting the nausea and loss of appetite associated with chemotherapy. Despite potential side effects such as nausea, the appetite-suppressive effects of central CB1 stimulated research into antagonists of the receptor as potential anti-obesity therapeutics.[44]

Several trials of the systemic CB1 antagonist, rimonabant, as a weight-loss agent were initiated in both Europe and North America. Overall, rimonabant was superior to both sibutramine and orlistat, with a nearly 5-kg average weight loss over a 1-year period (vs 4.5 kg for sibutramine and 2.5 kg for orlistat).[44] Also, T2DM patients treated with rimonabant in addition to metformin or sulfonylurea had significantly lower HbA1c levels than those treated with metformin or sulfonylurea alone.[109] This effect was at least partially independent of changes in body mass index. Not surprisingly, nausea was a common adverse effect in the rimonabant group (13%, as compared with 4% with placebo), as well as were a number of severe psychological alterations. Specifically, 15% percent of rimonabant-treated patients withdrew from the trials, and nearly half of these dropouts were attributed to mood alterations such as depression and increased suicide ideation.[109] These outcomes halted any ongoing rimonabant trials, and the CB1 antagonist field made little progress over the next few years.

Perhaps due to the importance of CB1 in mediating appetite in the central nervous system, alternative CB1 antagonists had not been tested until recently. These include the neutral CB1 antagonists, which have a weaker affinity for receptor, but also peripherally restricted CB1 antagonists, which cannot cross the blood-brain barrier and thus have no direct central nervous system effects. Insulin-responsive tissues such as the liver, pancreas, adipose and muscle also express CB1, and antagonism of these receptors promotes insulin signaling, blocks lipogenesis, promotes fatty acid oxidation and stimulates energy-requiring processes such as mitochondrial biogenesis and cell growth and differentiation.[110] CB1 receptors are also located on afferent and efferent nerve fibers; thus, the insulin-responsive state of the body is transmitted to the brain, where it can send signals to decrease food intake.[110] Overall, a requirement for direct central nervous system activation of CB1 receptors does not seem to exist in order to elicit positive metabolic changes, and perhaps, weight loss. Several recent publications show strong pre-clinical efficacy of these peripherally restricted CB1 antagonists.[111–113]

SUMMARY

In recent years, we have learned much about the role of inhibitory G proteins and GPCRs in mediating dysfunctional signaling pathways in metabolic diseases such as T2DM and confirmed their efficacy in pre-clinical models and select human studies. A number of other inhibitory GPCRs not discussed above may also have therapeutic potential (for example, serotonin 2A, ghrelin, NPY and GPR41, among

others; reviewed by Ahren[26]). Yet, we still have a long way to go before demonstrating that any of these proteins are suitable targets for T2DM and obesity in the general population. Even so, the current results are quite promising.

CONFLICT OF INTEREST
The authors declare no conflict of interest

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