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Review

Treatment

G protein-coupled receptor signaling: Implications for the treatment of diabetes and its complications

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Abstract

Introduction

G protein coupled receptors (GPCRs) represent the most abundant receptor family encoded by the human genome, and are the targets of a large percentage of drugs currently prescribed in the United States. Recently, several GPCRs have been identified as potential therapeutic targets for the treatment of diabetes and diabetes-associated complications, including retinopathy, nephropathy, and neuropathy.

Conclusion

In this review, we will discuss GPCR structure and signalling cascades, as well as highlight GPCR-based therapeutics that are currently prescribed or are in clinical trials to combat diabetes and its complications, a disease which now affects over 347 million patients worldwide.

Introduction

Diabetes is a multi-factorial disease process with a complex aetiology involving insulin resistance, aberrations in glucose homeostasis, lipid metabolism, and mitochondrial function, as well as inflammation and pancreatic beta cell toxicity[1,2,3] Both type 1 and type 2 diabetes are associated with the development of multiple microvascular complications including retinopathies, nephropathies, and neuropathies, which can lead to blindness, kidney disease and failure, and loss of feeling or function of the lower extremities, sometimes requiring amputation of one or more limbs[4]. The underlying causes of diabetes-associated microvascular dysfunction are not fully understood, but likely involve aberrations in multiple cellular processes, including nutrient metabolism and mitochondrial function, as well as intracellular signalling mechanisms and enhanced inflammation. Although dysfunctional insulin signalling often is implicated in the aetiology of diabetes-associated complications, many of these cellular activities may be modulated by G-protein coupled receptor (GPCR) signalling as well. Consequently, GPCRs may prove to be effective targets for the treatment of diabetes and its complications. The aim of this review was to discuss the Implications for the treatment of diabetes and its complications of G protein-coupled receptor signalling.

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Discussion

The authors have referenced some of their own studies in this review. The protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed.

GPCR Structure and Signalling

GPCRs are the most abundant receptor family encoded by the human genome, and, accordingly, are the targets of a large percentage (~30%) of pharmaceuticals currently prescribed in the United States[5]. All GPCRs share basic structural features including seven transmembrane domains, three extracellular and three intracellular loops, and conserved cysteine residues in the second extracellular loop which may play a role in the formation of the ligand binding pocket[6,7]. With a few notable exceptions, the N terminal region of GPCRs are extracellular and may function in ligand specificity, while the C terminal cytoplasmic tail may play a role in GPCR signalling, including association with G protein Receptor Kinases (GRKs)[8].

GPCRs are categorized based on homology into six classes; however, most small peptide hormones that signal via a GPCR, such as glucagon and GLP-1, interact with either Class A (Rhodopsin-Like) or Class B (Secretin-Like) GPCRs. The "classical" signalling pathways initiated by activation of these Class A and B receptors have been very well described and include a complex series of steps, including dissociation of G proteins, GTP hydrolysis, and re-association of the G protein trimer[6,7]. This traditional GPCR signalling often involves stimulation or inhibition of adenylate cyclase by Gas or Gai, respectively (Figure 1). Adenylate cyclase activation triggers cAMP formation, cAMP binds to the regulatory subunits of PKA (PKAr) and a consequent allosteric change leads to release of the PKA catalytic subunits (PKAc)[9]. PKAc then phosphorylates downstream targets, and is a key player in the regulation of metabolic enzymes and activation of transcription factors.

In the last several years, multiple reports have been published on the possibility of cAMP-independent activation of PKAc (Figure 1). In 1997, Zhong et al. (10) were the first to characterize a novel signalling pathway with cAMP-independent activation of PKAc in rabbit lung cytosol extract as well as mouse and human lymphocytes. They showed that while they were able to measure increased PKAc phosphorylation as a result of LPS treatment, they were unable to detect an elevation in cAMP. An eloquent set of pharmacological experiments demonstrated that a portion of intracellular PKAc (but not PKAr) was bound to the NFkB-IkB complex and that

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degradation of IkB with NFkB inducers led to the release and activation of PKAc. These same NFkB-IkB-PKAc isolated complexes were insensitive to increases in cAMP, and increased cAMP levels did not lead to increased PKAc activation. Since then, there have been several reports in the literature linking GPCRs with cAMP-independent PKAc activation. Dulin et al.[11] reported that two separate vasoactive peptides, endothelin-1 (ET1) and angiotensin II, upon GPCR binding, induced cAMP-independent PKAc activation. Their data complemented Zhong et al.[10] by demonstrating the NFkB-IkB complex bound to PKAc, rendering it inactive by blocking its ATP binding site. Ubiquitination and subsequent proteosomal degradation of IkB resulted in release of both PKAc and NFkB, followed by immediate phosphorylation and activation of NFkB by PKAc. Additionally, Vinciguerra et al.[12] reported that principal cells of the cortical collecting duct in the mammalian kidney, when stimulated, showed an increase in ouabain binding sites and an increase in PKAc that was prevented by PKA inhibitors. No increase in cAMP was observed, and PKAc activation was prevented by inhibiting the proteasome. Gambaryan et al.[13] observed a similar PKA-dependent, cAMP-independent phenomenon in human platelets, which serves as a novel inhibitory feedback signalling mechanism for preventing undesired platelet activation. Thrombin, signalling through a non-traditional GPCR[14], activated PKAc associated with an NFkB-IkB complex without measurable increases in cAMP.

Chemokines and classic chemoattractants are examples of other known ligands that signal through seven-transmembrane receptors, triggering degradation of IkB and activation of NFkB-dependent transcription[15]. For example, the chemokine fractalkine binds the GPCR CXCR3 and signals through the heterotrimeric G protein Gai, phosphoinositide 3-kinase (PI3-kinase), phosphoinositide-dependent kinase 1 (PDK1), Akt, NIK, IKK and finally NF-kB[16]. Phophorylation of Akt through the phosphorylation of IKK, can result in degradation of IkB by the proteosome and release of NFkB[17]. Therefore any ligand that signals through a GPCR to stimulate PI3K and subsequently Akt could potentially intersect with the NFkB-IkB-PKAc complex.

Clearly GPCR-mediated signalling is more complex than originally realized, and multiple non-traditional GPCR signalling pathways exist. For example, another possible mechanism for cAMP-independent protein kinase A activation is through the heterotrimeric G protein Ga13. Niu et al.,18 demonstrated that Ga13, AKAP110, PKAr, and PKAc can form a complex, and Ga13-induced release of PKAc from the AKAP110-PKAr complex results in cAMP-independent PKA activation. Additional alternative signalling mechanisms likely exist, and represent potential targets that can be manipulated for the treatment of diabetes and its complications.

GPCRs as Therapeutic Targets in Diabetes

The pharmaceutical therapeutic options for diabetes have traditionally included both injectable insulin regimens as well as oral agents. Insulin therapy has been the mainstay treatment of type 1 diabetes. Exogenous insulin treatment lowers blood glucose levels but must be administered to approximate physiologic insulin delivery requiring careful dosing, attention to pharmacokinetics, and current state of health. Though many formulations exist (regular, intermediate, and long-acting insulin) from either human or animal species with a variety of absorption rates and concentrations, the daily use of these products can be difficult requiring substantial empirical adjustment. Significant side effects can include hypoglycaemia, insulin allergy, antibody-mediated insulin resistance, and local lipodystrophy at injection sites[19].

Oral agents are typically first line therapy in type 2 diabetics and commonly used drugs include sulfonylureas (Glipizide, Gliburide) and biguanides (e.g. Metformin). Sulfonylureas stimulate insulin secretion from the beta cells of the pancreas through an ATP-dependent potassium channel and therefore require the function of these cells. A major complication of this approach is the possibility of developing hypoglycaemia due to increased insulin release. Biguanides increase insulin sensitivity and glucose uptake and decrease gluconeogenesis. Because they do not affect the output of insulin, do not require functional pancreatic beta cells and therefore are useful in type 2 and type 1 diabetics where they can be used in combination with insulin therapy. A side benefit in some patients desiring weight loss is anorexia, but side effects can include abdominal pain, nausea, and diarrhoea[19].

In addition to insulin itself, which signals via a tyrosine kinase receptor, several new therapies for diabetes and diabetic complications target GPCRs (Table 1). In many cases, therapies targeting GPCRs require once or twice daily dosage regimens. While in some cases clinical trials have indicated utility as monotherapy, addition of these agents to sulfonylureas or metformin offer advantages. These include GLP-1 based agonists, the related DPP-4 antagonists[20], bromocriptine, serotonin agonists, and, more recently, GPR40 agonists and selective endothelin-A receptor antagonists. The GLP-1 agonists and DPP4 inhibitors currently represent the most well-developed of therapies based on GPCRs for diabetes. GLP-1 (glucagon like peptide 1) is an incretin hormone that is released from the gut after a meal. GLP-1 stimulates insulin secretion, inhibits glucagon secretion, and may stimulate islet proliferation and cytoprotection through an interaction with its receptor, GLP1R[21,22]. While its effect on the pancreatic beta cell is direct, its effect on alpha cells and glucagon secretion may be either direct or indirect [23,24,25].

In 1992 it was suggested that GLP-1 might be a therapeutic target in type 2 diabetes[26] and the first GLP-1-based pharmaceutical came to market in 2005. Several strategies aimed at the GLP-1 receptor have been used to circumvent the otherwise short half-life of GLP-1 (usually 1-2 minutes) that is the result of inactivation by the DPP4 enzyme[27]. One of these strategies was to use a mimetic from the Gila monster called exendin-4 that shows 53% homology to GLP-1 and binds the receptor with the same affinity as the

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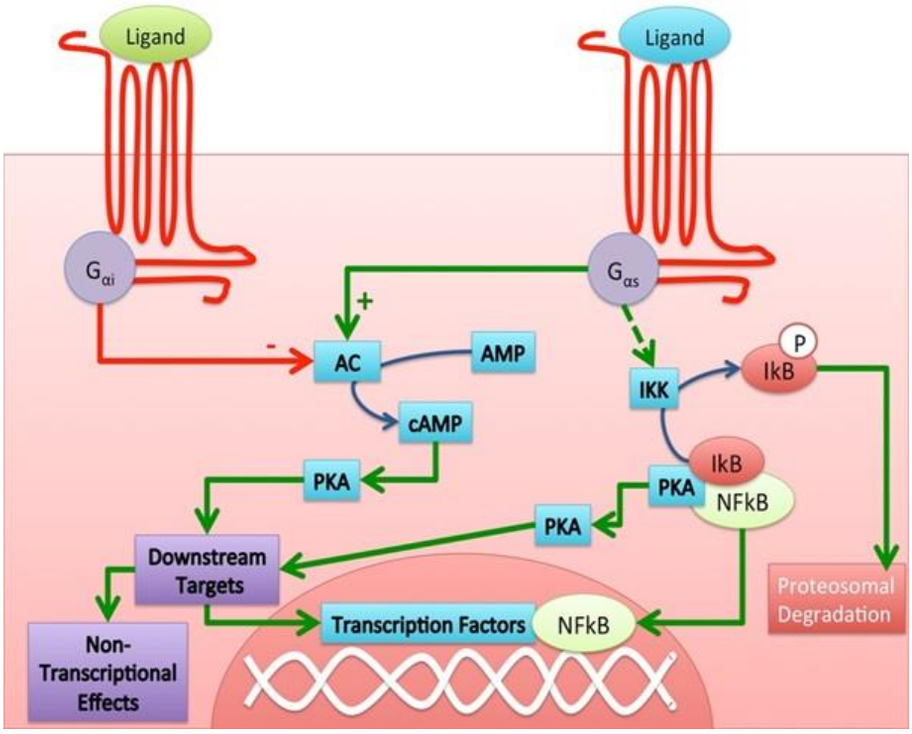

Figure 1: Classical and Non-Traditional GPCR-Mediated Signaling Cascades. Activation of GPCRs coupled to Gαi leads to an inhibition of adenyl cyclase (AC) activity, resulting in reduced intracellular levels of cyclic AMP (cAMP). Activation of GPCRs coupled to Gαs leads to stimulation of AC activity, resulting in increased protein kinase A (PKA) activity, and downstream signaling events, including alterations in gene transcription. Alternatively, dissociation of Gαs can lead to activation of IKK (IkB kinase) through an unknown mechanism (dotted green arrow). IKK in turn phosphorylates IkB (inhibitor of kappa B), leading to the proteosomal degradation of IkB and dissociation of the IkB-NFkB-PKA complex. NFkB (nuclear factor kappa-light-chain-enhancer of activated B cells) then translocates to the nucleus to act as a transcription factor, while PKA has additional downstream effects.

human homologue[28,29]. It is resistant to DPP4 and has a half-life of 30 minutes (intravenous administration) or 2-3 hours (sub-cutaneous administration). A synthetic form called exenatide or Byetta (Amylin/Eli Lilly) was shown to reduce body weight and HbA1c levels when administered twice daily subcutaneously with metformin, a sulphonylurea, or both[30,31,32]. The use of exenatide is approved by the FDA as both a monotherapy and in combination with other hypoglycemic agents. Though well tolerated overall, the predominate side effects include nausea during the beginning of treatment (~40% of patients) and hypoglycaemia when used with sulfonylureas (30% of patients). When combined with metformin, hypoglycaemia occurs with a similar frequency as when metformin is given alone[30,33]. Antibodies can be formed against exenatide in as many as 38% of treated patients. However in the vast majority these are low-titer with no clinical consequences[34]. The half-life of exenatide also has been modified using extension of the carboxy-terminus, conjugation to albumin, or containment within polymer microspheres, which have given rise to once daily and once weekly forms[20].

Efforts to extend the half-life of GLP-1 agonists also have included modifications of the native GLP-1 structure itself for resistance to DPP4 activity. Liraglutide (Novo Nordisk) was developed to include an attached C16 fatty and a Lys28Arg substitution allowing the molecule to bind albumin protecting it from DPP4 and renal clearance. It also has a slower absorption rate with a half-life of 12 hours after subcutaneous administration, resulting in once daily dosing (35). HbA1c levels and body weight were reduced in clinical trials. The most common side effect is nausea (~10%)[36,37,38,39]. Several other GLP-1 agonists have had varying success and have entered phase 3 clinical trials (albiglutide, dulaglutide, semaglutide, lixisenatide). In addition, combination therapies utilizing GLP-1 agonists and insulin are also currently in phase 3 clinical trials [iDegLira (combined insulin degludec and liraglutide); LixiLan (combined lixisenatide and insulin glargine)].

DPP4 inhibitors prolong endogenous GLP-1 concentrations that are typically observed after a meal and increase fasting levels of GLP-1. This results in the same type of augmentation of insulin and inhibition of glucagon secretion observed with GLP-1 agonists[20,40]. Several DPP4 inhibitors have been approved by the FDA as adjuvant treatment for diabetes, including alogliptin (Xanthine based compound), saxagliptin (substrate like inhibitor), and sitagliptin (non-substrate like inhibitor)[41,42,43]. However, DPP4 also is involved in suppressing tissue invasion/metastasis in certain cancers, including non-small cell carcinoma in the lung and colon cancer[44,45]. Both DPP4 and GLP-1-based therapies have been at the center of

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Table 1: Examples of GPCR-Based Therapeutics for the Treatment of Diabetes and its Complications.

Compound Name	Trade Name(s)	GPCR Targeted	Clinical Utility	Status	References
Exenatide	Byetta, Bydureon	GLP1R	Incretin mimetic; enhances insulin release	Currently Prescribed	28-34
Liraglutide	Victoza	GLP1R	Incretin mimetic; enhances insulin release	Currently Prescribed	29; 36-39
Alogliptin	Nesina	GLP1R (indirect)	DPP4 inhibitor; enhances endogenous GLP-1 levels	Currently Prescribed	40, 43, 45
Bromocriptine	Cycloset	D2R	Dopamine agonist; reduces HbA1C and serum lipids	Currently Prescribed	72-77
C-peptide	Ersatta	CpepR	May reverse diabetic neuropathy	Phase 2b Clinical Trials	78-82

an academic debate [46,47,48,49] over the risk of pancreatitis and pancreatic cancer associated with incretin-based therapies. Reviews of current therapies by the European Medicines Agency and the Food and Drug Administration have not altered recommendations at this time[50,51]. There have been recent calls for efforts to investigate the possible risks by the American Diabetes Association and The Endocrine Society as of mid-2013[51,53].

Free fatty acids stimulate insulin secretion, a process that operates not only through the formation of long-chain CoA esters[54] that modulate beta cell ion channels and exocytosis, but also through the activation of islet GPCR signalling by lipids themselves[55,56,57]. One such receptor, GPR40 (free fatty acid receptor 1 or FFAR1), is expressed in pancreatic beta cells as well as in brain, omental adipocytes, and endocrine cells of GI mucosa. Activation by medium- or long-chained free fatty acids leads to insulin secretion in a glucose-dependent manner[20,58]. A loss of function mutation in the GPR40 gene is found in 0.75% of healthy subjects and is associated with obesity and impaired insulin secretion[59]. GPR40 is not likely to be involved in beta-cell lipotoxicity under high fat diet conditions but may be involved in the adaption of islets to insulin resistance[60,61,62]. Because of its intestinal expression and because of a lack of incretin responses in GPR40 knockout mice, GPR40 has been linked to incretin responses to free fatty acids[63]. A large number of GPR40 agonists are being developed. TAK-875 (fasiglifam, Takeda Pharmaceutical Company) was developed as an oral agent and was shown in rats to enhance glucose dependent insulin secretion and improve postprandial and fasting hyperglycemia [64]. In these studies there was a low risk of hypoglycaemia and no evidence of beta cell toxicity. In a human phase 2 clinical trial, TAK-875 monotherapy reduced HbA1c significantly compared to placebo without an increase in hypoglycaemic events, substantiating the utility in targeting FFAR1 for the treatment of type 2 diabetes [65]. However, a phase 3 clinical trial of TAK-875 was voluntarily terminated in December 2013 due to concerns related to liver safety [66].

The D2 dopamine receptor is also a GPCR. Bromocriptine (a sympatholytic) targets this receptor and was approved recently for the treatment of type 2 diabetes when given once daily within 2 hours of awakening [67]. It is thought that low hypothalamic dopamine levels and excessive sympathetic tone in the CNS alter the metabolic state so that it resembles the changes that occur in mammalian species that undergo hibernation. As in type 2 diabetics, these animals switch to a state of insulin resistance in muscle and liver along with accelerated hepatic glucose production and gluconeogenesis, hyperglycaemia, adipocyte insulin resistance, increased lipolysis, enhanced fat oxidation with increased plasma free fatty acids and triglycerides along with obesity [68,69,70,71]. Endogenous dopaminergic and serotonergic rhythms in the SCN and VMH are involved in the transition to this state and an insulin sensitive state [68]. A modified, oral form of bromocriptine (bromocriptine mesylate, Cycloset) is rapidly dissolved and absorbed within 30 min. Hepatic first pass metabolism is high (leaving 5-10% of ingested dose) with a biliary elimination half-life of 6 hours [72,73,74,75]. Phase 2 and phase 3 clinical trials have shown improvement in HbA1c, plasma triglycerides, and free fatty acid concentrations when given both as monotherapy and in combination with oral hypoglycemic agents [72, 77,78].There is evidence from cardiovascular safety trials that Cycloset may improve major adverse cardiovascular outcomes (myocardial infarction, stroke, death) in type 2 diabetics by 40% [77].

A promising new therapeutic for the treatment of diabetes-associated microvascular complications currently in clinical trials is synthetic C-peptide or C-peptide conjugates [78]. C-peptide, or Connecting Peptide, is part of the insulin prohormone, and for many years was thought to be biologically inert and to have little physiological significance beyond its role in the proper folding of insulin. It is now known that C-peptide plays a protective role in the microvasculature [79, 80], likely through an interaction with the previously orphaned GPCR, GPR146 [81]. In type 1 diabetic patients, higher levels of endogenous C-peptide correlated with a significant reduction in the incidence of retinopathy, neuropathy, and nephropathy [82]. Importantly, administration of exogenous C-peptide was shown to at least partially reverse microvascular dysfunction in diabetic humans with mild to undetectable side effects [80]. Cebix recently completed a phase 1/2 clinical trial

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evaluating Ersatta, a long-acting C-peptide agonist, in type 1 diabetic patients. The results of this study were favourable in terms of safety and improvements in neuropathy, and a phase 2b trial currently is underway [78].

Conclusion

GPCRs are abundantly expressed in the pancreatic islets and may play an important role in normal glucose homeostasis and microvascular function. Although many of the current therapies for diabetes and its complications target tyrosine kinase receptors (insulin) or ion channels (sulfonylureas), several GPCRs, including the GLP-1 receptor, D2 receptor, and the C-peptide receptor (GPR146), may provide novel targets for the treatment of this disease. At least 293 different GPCRs are expressed by pancreatic islets[83], and thus future studies may reveal even more targets for the development of GPCR-based therapeutics.

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