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G-PROTEIN COUPLED RECEPTORS- A POTENTIAL NEW DRUG TARGET TO COMBAT DIABETIC SYNDROME: AN OVERVIEW

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ABSTRACT

G protein-coupled receptors (GPCRs), also known as seven-transmembrane domain receptors, 7TM receptors, heptahelical receptors, serpentine receptor, and G protein-linked receptors (GPLR), comprise a large protein family of transmembrane receptors that sense molecules outside the cell and activate inside signal transduction pathways and, ultimately, cellular responses. GPCRs are involved in a wide variety of important physiological processes like visual sense, sense of smell, behavioral and mood regulation, regulation of immune system activity and inflammation, autonomic nervous system transmission and cell density sensing. There are two principal signal transduction pathways involving the G protein-linked receptors, cAMP signal pathway and Phosphatidylinositol signal pathway. Diabetes mellitus, often simply referred to as diabetes, is a group of metabolic diseases in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced. One of the reasons for the growing public health concern over the rapidly increasing prevalence of obesity in society is its association with the growing incidence of type II diabetes, a combination of conditions often accompanied by increased cardiovascular risk factors. Much effort among health-care providers and pharmaceutical companies is now focused on the discovery of new treatments that alleviate this medical problem. As part of this effort, the G-protein-coupled receptors have recently attracted a lot of attention. GPR119 particularly is of importance because of evidence from in vitro systems and animal models that its modulation may produce favorable effects on glucose homoeostasis, food intake/body weight gain and possibly also β-cell preservation. Many modulators of GPCRs like GPR119, GPR40, GPR41, GPR43 and GPR120 can be used with a great added advantage of improvement in glucose handling and homeostasis in treating diabetes. Relatively high 'druggability' of G-protein coupled receptors as compared with many other molecular target classes may provide an insight into the treatment of diabetes. Hence, G protein coupled receptors show a great potential in drug targeting in the process of drug discovery and development. Provided that being clinically well tolerated, these GPCR effectors will prove a boon to the patients suffering from apathy of metabolic syndrome of diabetes.

INTRODUCTION: G protein-coupled receptors (GPCRs), also known as seven-transmembrane domain receptors, 7TM receptors, heptahelical receptors, serpentine receptor, and G protein-linked receptors (GPLR), comprise a large protein family of transmembrane receptors that sense molecules outside the cell and activate inside signal transduction pathways and, ultimately, cellular responses.

G protein-coupled receptors are involved in many diseases, and are also the target of approximately 30% of all modern medicinal drugs [1-2]. When a ligand binds to the GPCR it causes a conformational change in the GPCR, which allows it to act as a guanine nucleotide exchange factor (GEF). Guanine nucleotide exchange factors are components of intracellular signalling networks that stimulate the release of guanosine diphosphate (GDP) which is replaced by guanosine triphosphate (GTP). Guanine nucleotide exchange factors stimulate the exchange of GDP for GTP to generate the activated form, which is then capable of recognizing downstream targets, or effector proteins. GTPase activating proteins (GAPs) accelerate the intrinsic GTPase activity of Rho family members to inactivate the switch [3].

They function as activators of small GTPases [4]. G proteins function as molecular switches, where the resting (inactive) state they are bound to GDP and their activation requires the dissociation of GDP and binding of GTP, which exists at an approximate 10-fold higher concentration in the cell cytoplasm. The GPCR can then activate an associated G-protein by exchanging its bound GDP for a GTP. The G-protein's α subunit, together with the bound GTP, can then dissociate from the β and γ subunits to further affect intracellular signaling proteins or target functional proteins directly depending on the α subunit type (Gαs, Gαi/o, Gαq/11, Gα12/13) [5].

In all, GPCRs can be grouped into 6 classes based on sequence homology and functional similarity as class A (Rhodopsin-like), class B (Secretin receptor family), class C (Metabotropic glutamate/pheromone), class D (Fungal mating pheromone receptors), class E (Cyclic AMP receptors) and class F (Frizzled/Smoothened) [6]. The very large rhodopsin A group has been further subdivided into 19 subgroups (A1-A19) [7]. More recently, an alternative classification system called GRAFS (Glutamate, Rhodopsin, Adhesion, Frizzled, Secretin) has been proposed [8]. The human genome encodes thousands of G protein-coupled receptors [9], about 350 of which detect hormones, growth factors and other endogenous ligands. Approximately 150 of the GPCRs found in the human genome have unknown functions.

Pepducins, for example, are novel cell-penetrating peptides that act as intracellular inhibitors of signal transference from receptors to G proteins [10]. A CXCR4 agonist pepducin has been found to mobilize bone marrow hematopoietic cells [11]. On the same grounds, many modulators of GPCRs like GPR119, GPR40, GPR41, GPR43 and GPR120 can be used with an advantage of improvement in glucose handling and homeostasis in treating diabetes. Provided that being clinically well tolerated, these GPCR effectors will be of great use to the patients suffering from apathy of metabolic syndrome of diabetes.

Structure of G-Protein Coupled Receptors: GPCRs are integral membrane proteins that possess seven membrane-spanning domains or transmembrane helices (**figure 1**). The extracellular parts of the receptor can be glycosylated. These extracellular loops also contain two highly-conserved cysteine residues that form disulfide bonds to stabilize the receptor structure. Some seven-transmembrane helix proteins (channelrhodopsin) that resemble GPCRs may contain ion channels, within their protein. Early structural models for GPCRs were based on their weak analogy to bacteriorhodopsin, for which a structure had been determined by both electron diffraction [12] and X ray-based crystallography. In 2000, the first crystal structure of a mammalian GPCR, that of bovine rhodopsin, was solved. While the main feature, the seven transmembrane helices, is conserved, the relative orientation of the helices differs significantly from that of bacteriorhodopsin.

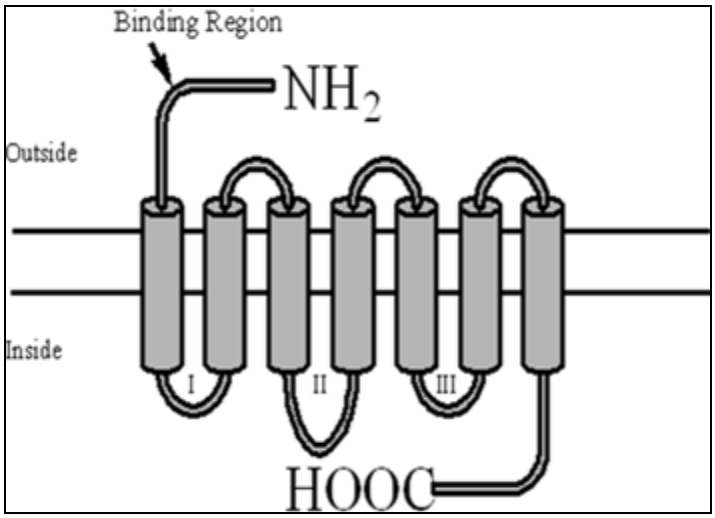

FIG. 1: THE SEVEN-TRANSMEMBRANE α-HELIX STRUCTURE OF A G-PROTEIN-COUPLED RECEPTOR

In 2007, the first structure of a human GPCR was solved. This was followed immediately by a higher resolution structure of the same receptor [13]. Structurally GPCRs are characterized by an extracellular N-terminus, followed by seven transmembrane (7-TM) α-helices (TM-1 to TM-7) connected by three intracellular (IL-1 to IL-3) and three extracellular loops (EL-1 to EL-3), and finally an intracellular C-terminus. The GPCR arranges itself into a tertiary structure resembling a barrel, with the seven transmembrane helices forming a cavity within the plasma membrane which serves a ligand-binding domain that is often covered by EL-2.

Mechanism of Action of GPCRs: GPCRs that act as receptors for stimuli that have not yet been identified are known as orphan receptors. Whereas, in other types of receptors that have been studied, wherein ligands bind externally to the membrane, the ligands of GPCRs typically bind within the transmembrane domain (**figure 2**). However, protease-activated receptorsare activated by cleavage of part of their extracellular domain [14]. Ligand binding disrupts an ionic lock between TM-3 and acidic residues of TM-6. As a result the GPCR reorganizes to allow activation of G-alpha proteins [15].

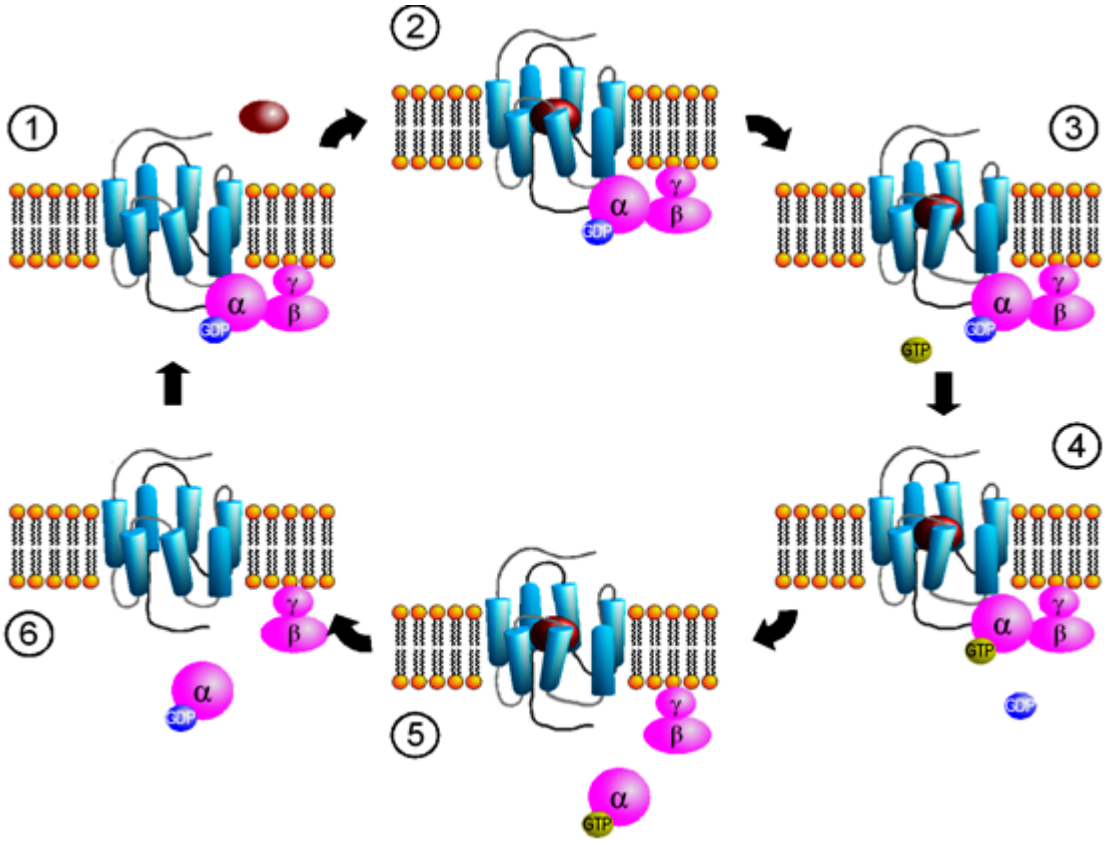

FIG. 2: G-PROTEIN-COUPLED RECEPTOR MECHANISM

The inactive G protein is bound to the receptor in its inactive state. Once the ligand is recognized, the receptor shifts conformation and, thus, mechanically activates the G protein, which detaches from the receptor. The receptor can now either activate another G protein or switch back to its inactive state. It is believed that a receptor molecule exists in a conformational equilibrium between active and inactive biophysical states [16]. The binding of ligands to the receptor may shift the equilibrium toward the active receptor states. Three types of ligands exist: agonists are ligands that shift the equilibrium in favour of active states; inverse agonists are ligands that shift the equilibrium in favour of inactive states; and neutral antagonists are ligands that do not affect the equilibrium. G proteins function as molecular switches. When they bind guanosine triphosphate (GTP), they are 'on', and when they bind guanosine diphosphate (GDP), they are 'off'. G proteins belong to the larger group of enzymes called GTPases. GTPases are a large family of hydrolase enzymes that can bind and hydrolyze guanosine triphosphate (GTP). Receptor-activated G proteins are bound to the inside surface of the cell membrane.

They consist of the G_α and the tightly associated $G_{\beta\gamma}$ subunits. There are many classes of G_α subunits: $G_s\alpha$ (G stimulatory), $G_i\alpha$ (G inhibitory), $G_o\alpha$ (G other), $G_{q/11}\alpha$, and $G_{12/13}\alpha$ are some examples. They behave differently in the recognition of the effector, but share a similar mechanism of activation. $G_{\alpha s}$ activates the cAMP-dependent pathway by stimulating the production of cAMP from ATP. This is accomplished by direct stimulation of the membrane-associated enzyme adenylate cyclase. cAMP acts as a second messenger that goes on to interact with and activate protein kinase A (PKA). PKA can then phosphorylate myriad downstream targets.

Gαi inhibits the production of cAMP from ATP. Gαq/11 stimulates membrane-bound phospholipase C beta, which then cleaves PIP2 (a minor membrane phosphoinositol) into two second messengers, IP3 and diacylglycerol (DAG). Gα12/13 are involved in Rho family GTPase signaling (through RhoGEF superfamily) and control cell cytoskeleton remodeling, thus regulating cell migration. Gβγ sometimes also have active functions, e.g., coupling to and activating G protein-coupled inwardly-rectifying potassium channels. When the receptor is inactive, the GEF domain may be bound to an also inactive α-subunit of a heterotrimeric G-protein.

These “G-proteins” are a trimer of α, β, and γ subunits (known as Gα, Gβ, and Gγ, respectively) which is rendered inactive when reversibly bound to guanosine diphosphate (GDP), but active when bound to guanosine triphosphate (GTP). Upon receptor activation, the GEF domain, in turn, allosterically activates the G-protein by facilitating the exchange of a molecule of GDP for GTP at the G-protein's α-subunit. The cell maintains a 10:1 ratio of cytosolic GTP:GDP so exchange for GTP is ensured. At this point, the subunits of the G-protein dissociate from the receptor, as well as each other, to yield a Gα-GTP monomer and a tightly interacting Gβγ dimer, which are now free to modulate the activity of other intracellular proteins.

The extent to which they may diffuse, however, is limited due to the palmitoylation of Gα and the presence of a molecule of Glycosylphosphatidylinositol (GPI) that has been covalently added to the C-termini of Gγ. The phosphatidylinositol moiety of the GPI-linkage contains two hydrophobic acyl groups that anchor any GPI-linked proteins (e.g. Gβγ) to the plasma membrane, and also, to some extent, to the local lipid raft. Because Gα also has slow GTP→GDP hydrolysis capability, the inactive form of the α-subunit (Gα-GDP) is eventually regenerated, thus allowing reassociation with a Gβγ dimer to form the “resting” G-protein which can again bind to a GPCR and await activation. The rate of GTP hydrolysis is often accelerated due to the actions of another family of allosteric modulating proteins called Regulators of G-protein Signaling, or RGS proteins, which are a type of GTPase-Activating Protein, or GAP.

In fact, many of the primary effector proteins (e.g. adenylate cyclases) that become activated/inactivated upon interaction with Gα-GTP also have GAP activity. Thus, even at this early stage in the process, GPCR-initiated signaling has the capacity for self-termination. The ERK2 mitogen-activated protein kinase, a key signal transduction mediator downstream of receptor activation in many pathways, has been shown to be activated in response to cAMP-mediated receptor activation in the slime mold D. discoideum despite the absence of the associated G protein α- and β-subunits [17].

In mammalian cells, the much-studied β2-adrenoceptor has been demonstrated to activate the ERK2 pathway after arrestin-mediated uncoupling of G-protein-mediated signaling. Therefore it seems likely that some mechanisms previously believed to be purely related to receptor desensitisation are actually examples of receptors switching their signaling pathway rather than simply being switched off. In kidney cells, the bradykinin receptor β2 has been shown to interact directly with a protein tyrosine phosphatase. The presence of a tyrosine-phosphorylated ITIM (immunoreceptor tyrosine-based inhibitory motif) sequence in the β2 receptor is necessary to mediate this interaction and subsequently the antiproliferative effect of bradykinin.18.

GPCR Signal Transduction: There are two principal signal transduction pathways involving theG protein-linked receptors: cAMP signal pathway and Phosphatidylinositol signal pathway.

cAMP Signal Pathway: The cAMP signal transduction contains 5 main characters: stimulative hormone

receptor (Rs) or inhibitory hormone receptor (Ri); Stimulative regulative G-protein (Gs) or inhibitory regulative G-protein (Gi); Adenylyl cyclase; Protein Kinase A (PKA); and cAMP phosphodiesterase. Stimulative hormone receptor (Rs) is a receptor that can bind with stimulative signal molecules, while inhibitory hormone (Ri) is a receptor that can bind with inhibitory signal molecules. Stimulative regulative G-protein is a G protein-linked to stimulative hormone receptor (Rs) and its α subunit upon activation could stimulate the activity of an enzyme or other intracellular metabolism.

On the contrary, inhibitory regulative G-protein is linked to an inhibitory hormone receptor and its α subunit upon activation could inhibit the activity of an enzyme or other intracellular metabolism. The Adenylyl cyclase is a 12-transmembrane glucoprotein that catalyzes ATP to form cAMP with the help of cofactor Mg2+ or Mn2+. The cAMP produced is a second messenger in cellular metabolism and is an allosteric activator to Protein kinase A. Protein kinase A is an important enzyme in cell metabolism due to its ability to regulate cell metabolism by phosphorylating specific committed enzymes in the metabolic pathway.

It can also regulate specific gene expression, cellular secretion, and membrane permeability. The protein enzyme contains two catalytic subunits and two regulatory subunits. When there is no cAMP, the complex is inactive. When cAMP binds to the regulatory subunits, their conformation is altered, causing the dissociation of the regulatory subunits, which activates protein kinase A and allows further biological effects. cAMP phosphodiesterase is an enzyme that can degrade cAMP to 5'-AMP, which will terminate the signal.

Phosphatidylinositol Signal Pathway: In the phosphatidylinositol signal pathway, the extracellular signal molecule binds with the G-protein receptor (Gq) on the cell surface and activates phospholipase C, which is located on the plasma membrane. The lipase hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP2) into two second messengers: Inositol 1, 4, 5-trisphosphate (IP3) and Diacylglycerol (DAG). IP3 binds with the receptor in the membrane of the smooth endoplasmic reticulum and mitochondria, helps open the Ca^{2}+ channel.

DAG will help activate Protein Kinase C (PKC), which phosphorylates many other proteins, changing their catalytic activities, leading to cellular responses. The effects of Ca^{2+} is also remarkable: it cooperates with DAG in activating PKC and can activate CaM kinase pathway, in which calcium modulated protein calmodulin (CaM) binds Ca^{2+}, undergoes a change in conformation, and activates CaM kinase II, which has unique ability to increase its binding affinity to CaM by autophosphorylation, making CaM unavailable for the activation of other enzymes. The kinase then phosphorylates target enzymes, regulating their activities. The two signal pathways are connected together by Ca^{2+}-CaM, which is also a regulatory subunit of adenylyl cyclase and phosphodiesterase in cAMP signal pathway.

G-Protein Coupled Receptor Regulation: GPCRs become desensitized when exposed to their ligand for a prolonged period of time. There are two recognized forms of desensitization:

1) homologous desensitization, in which the activated GPCR is downregulated; and

2) heterologous desensitization, wherein the activated GPCR causes downregulation of a different GPCR. The key reaction of this downregulation is the phosphorylation of the intracellular (or cytoplasmic) receptor domain by protein kinases.

Phosphorylation by cAMP-Dependent Protein Kinases: Cyclic AMP-dependent protein kinases (protein kinase A) are activated by the signal chain coming from the G protein (that was activated by the receptor) via adenylate cyclase and cyclic AMP (cAMP). In a feedback mechanism, these activated kinases phosphorylate the receptor. The longer the receptor remains active, the more kinases are activated, the more receptors are phosphorylated. In β2-adrenoceptors, this phosphorylation results in the switching of the coupling from the Gs class of G-protein to the Gi class [19].

Phosphorylation by G protein-Coupled Receptor Kinases: The G protein-coupled receptor kinases (GRKs) are protein kinases that phosphorylate only active GPCRs. G protein-coupled receptor kinases (GRKs, GPCRKs) are a family of protein kinases which regulate the activity of G protein-coupled receptors

(GPCRs) by phosphorylating their intracellular domains after their associated G proteins have been released and activated. Phosphorylation of the receptor can have two consequences, first being translocation and the second is arrestin linking [20-22].

GPCR Signal Termination: G-proteins may terminate their own activation due to their intrinsic GTP→GDP hydrolysis capability. However, this reaction proceeds at a slow rate (≈.02 times/sec) and thus it would take around 50 seconds for any single G-protein to deactivate if other factors did not come into play. Indeed, there are around 30 isoforms of RGS proteins (Regulators of G protein signaling) that, when bound to Gα through their GAP domain (GTPase-Activating Proteins), accelerate the hydrolysis rate to ≈30 times/sec. In addition, the GPCR may be desensitized itself by other processes as well.

GPCR Downregulation: Receptor desensitization is mediated through a combination phosphorylation, β-arr binding, and endocytosis. Downregulation occurs when endocytosed receptor is embedded in an endosome that is trafficked to merge with an organelle called a lysosome. Because lysosomal membranes are rich in proton pumps, their interiors have low pH which acts to denature the GPCRs. Additionally, lysosomes contain many degradative enzymes, including proteases, which can only function at such low pH, and so the peptide bonds joining the residues of the GPCR together may be cleaved.

It is generally accepted that G-protein-coupled receptors can form heteromers such as homo- and heterodimers as well as more complex oligomeric structures, and indeed heterodimerization has been shown to be essential for the function of receptors such as the metabotropic GABA(B) receptors. Present biochemical and physical techniques lack the resolution to differentiate between distinct homodimers assembled into an oligomer or true 1:1 heterodimers. The best-studied example of receptor oligomerisation is the metabotropic $GABA_B$ receptors. These receptors are formed by heterodimerization of $GABA_BR1$ and $GABA_BR2$ subunits. It has been shown that GABABR2 binding to GABABR1 causes masking of a retention signal of functional receptors [23]. A novel GPCR containing a lipid kinase domain has recently been identified in Dictyostelium discoideum that regulates cell density sensing [24]. Examples of orphan receptors are found in the G protein-coupled receptor (GPCR) [25]. GPCR orphan receptors are usually given the name "GPR" followed by a number, for example GPR1. G protein-coupled receptor 1, also known as GPR1, is a protein that in humans is encoded by the GPR1 gene. GPR1 is a member of the G protein-coupled receptor family of transmembrane receptors. It functions as a receptor for chemerin [26].

GPCRs as Potential Target for Antidiabetic Drugs

- **Diabetes Mellitus:** Diabetes mellitus, often simply referred to as diabetes, is a group of metabolic diseases in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced. This high blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger). There are three main types of diabetes:
  - Type 1 diabetes: results from the body's failure to produce insulin, and presently requires the person to inject insulin.
  - Type 2 diabetes: results from insulin resistance, a condition in which cells fail to use insulin properly, sometimes combined with an absolute insulin deficiency.
  - Gestational diabetes: is when pregnant women, who have never had diabetes before, have a high blood glucose level during pregnancy. It may precede development of type 2 DM.

Management of Diabetes Mellitus: Management concentrates on keeping blood sugar levels as close to normal (euglycemia) as possible without presenting undue patient danger. This can usually be done with close dietary management, exercise, and use of appropriate medications, insulin only in the case of type 1 diabetes mellitus. Oral medications like sulfonylureas, biguanides, thiazolidinediones, alpha-glucosidase inhibitors etc. may be used in the case of type 2 diabetes, as well as insulin. Patient education, understanding, and participation is vital since the complications of diabetes are far less common and less severe in people who have well-managed blood sugar

levels [27-28]. Wider health problems may accelerate the deleterious effects of diabetes. These include smoking, elevated cholesterol levels, obesity, high blood pressure, and lack of regular exercise. There are roles for patient education, dietetic support, sensible exercise, with the goal of keeping both short-term and long-term blood glucose levels within acceptable bounds. In addition, given the associated higher risks of cardiovascular disease, lifestyle modifications are recommended to control blood pressure [29].

Role of Insulin: Insulin is a natural peptide hormone composed of 51 amino acids which is produced in the islets of langerhans of the pancreas. In β-cells, insulin is synthesized from the proinsulin precursor molecule by the action of proteolytic enzymes, known as prohormone convertases (PC1 and PC2), as well as the exoprotease carboxypeptidase E [30]. The actions of insulin on the global human metabolism level include control of cellular intake of certain substances, most prominently glucose in muscle and adipose tissue, increase of DNA replication and protein synthesis via control of amino acid uptake and modification of the activity of numerous enzymes. An insulin molecule produced endogenously by the pancreatic beta cells is estimated to be degraded within about one hour after its initial release into circulation (insulin half-life~ 4-6 minutes) [31].

Role of GPCRs in Diabetes Mellitus: One of the reasons for the growing public health concern over the rapidly increasing prevalence of obesity in society is its association with the growing incidence of type II diabetes, a combination of conditions often accompanied by increased cardiovascular risk factors. Much effort among health-care providers and pharmaceutical companies is now focused on the discovery of new treatments that alleviate this medical problem. As part of this effort, the G-protein-coupled receptors have recently attracted a lot of attention [32-36]. GPR119 particularly is of importance because of evidence from in vitro systems and animal models that its modulation may produce favourable effects on glucose homoeostasis, food intake/body weight gain and possibly also β-cell preservation. In other words, GPR119 modulators may influence parameters related to diabetes [37-39].

GPR119: GPR119 is a class 1 (rhodopsin-type) orphan G-protein-coupled receptor having no close primary sequence relative in the human genome. GPR119 has various synonyms including SNORF25, RUP3, GPCR2, 19AJ, OSGPR116 and glucose-dependent insulinotropic receptor.

- **GPR119 Agonists with Antidiabetic Property:** The first molecule to be described as a potential endogenous ligand of GPR119 was all-trans retinoic acid. Treatment of GPR119-transfected Cos-7 cells with all-trans retinoic acid was reported to produce a dose-dependent increase in intracellular cAMP. However, it was unable to detect any activity for all-trans retinoic acid using human embryonic kidney cells transiently expressing GPR119. A number of phospholipid molecules have been reported to act as GPR119 agonists. The action of oleoyl LPC as a GPR119 agonist has been confirmed in a yeast-based reporter assay.

  Moreover, LPC has been found to stimulate insulin release from neonatal rat islet cells and a possible role for GPR119 in this effect is suggested by its β-cell localization. Because of the remote sequence relationship between GPR119 and the cannabinoid receptors, for which the fatty acid arachidonyl ethanolamide is an endogenous ligand, a series of related fatty acid amides have been found to exhibit agonist activity.

  The identification of OEA as a potential endogenous ligand for GPR119 is of particular interest, since this compound produces a number of pharmacological effects in rodent studies, including reduction of food intake and body weight gain, modifying feeding behaviour and motor activity and increasing fatty acid uptake by adipocytes. It has been suggested that the reported effects of OEA on feeding may be mediated in part by GPR119 in view of the gastrointestinal localization of this receptor. The endovanilloid compounds N-oleoyldopamine and olvanil have recently been described as GPR119 agonists with in vitro potencies similar to that of OEA. It is of course possible that many further potential endogenous ligands remain to be identified.

- **Effect of GPR119 Agonists on Glucose Homoeostasis:** The expression of GPR119 in pancreatic islet β-cells led to the hypothesis that this receptor could play a role in the modulation of insulin secretion. In principle, a molecule acting via GPR119 to raise intracellular cAMP concentrations in pancreatic β-cells would be expected to potentiate glucose-stimulated insulin secretion (GSIS) in a manner analogous to that of the incretin hormones glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide/glucosedependent insulinotropic peptide (GIP), which also act via Gas-coupled, β-cell receptors (**figure 3**).

The insulinotropic actions of GPR119 agonists have been revealed in a number of in vitro systems. The insulinotropic effects of oleoyl LPC are attenuated in the presence of either an adenylate cyclase inhibitor or a GPR119-selective siRNA. The presence of GPR119 in the enteroendocrine cell line suggests that intestinally expressed receptor may also be involved in glucose homoeostasis via the modulation of incretin hormone release. GLUTag cell lines are known to secrete the incretin hormone GLP-1, and they respond to the signals controlling GLP-1 release in a similar manner to primary intestinal L cells.

Small-molecule GPR119 agonists have been shown to elevate cAMP levels and stimulate GLP-1 secretion from GLUTag cells. Moreover, they increase plasma GLP-1 levels acutely when administered to rodents. GPR119 may be expressed in GIP-secreting K cells, but this has not yet been demonstrated directly. Hence, GPR119 agonists might exert a twofold effect in lowering blood glucose, acting directly at the pancreatic β-cell to promote glucose-stimulated insulin secretion and, indirectly, via the enteroendocrine cells, by stimulating the release of the incretin hormones GLP-1 and GIP, which are powerful antihyperglycaemic agents [40].

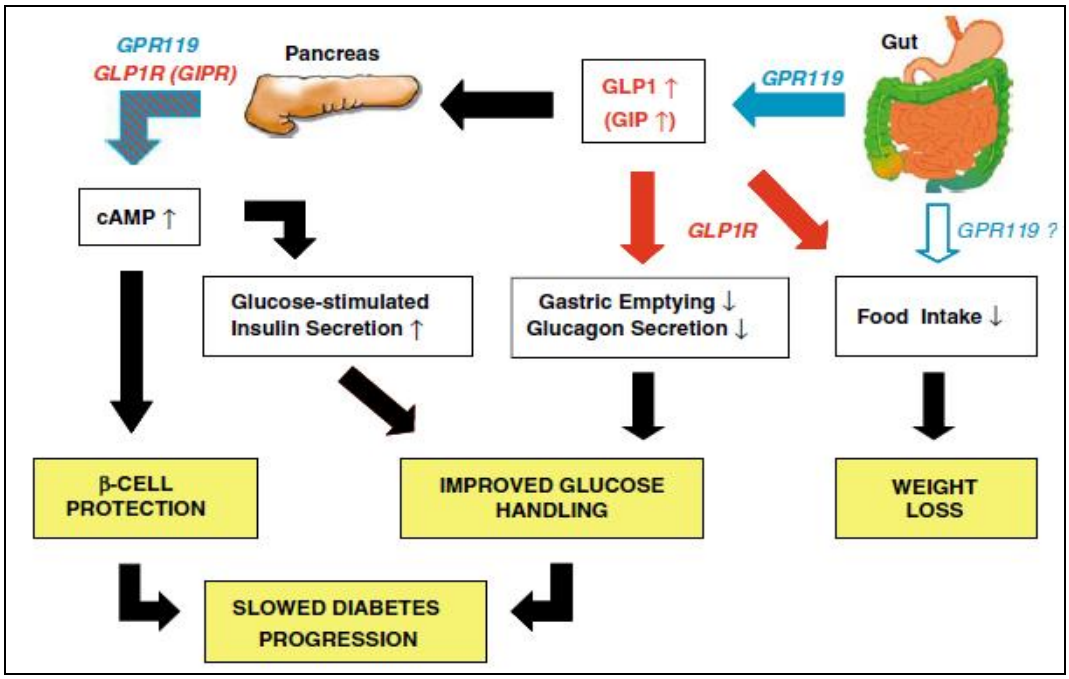

FIG. 3: MECHANISM OF GPR119 AGONIST ACTION

- **GPR119 and Drug Discovery:** Current publicly available data on the effects of GPR119 agonists in animal models indicate that they could prove valuable agents for the treatment of type 2 diabetes and obesity by improving glucose homoeostasis while concurrently limiting food intake and body weight gain [41-43]. It appears that their ability to stimulate GLP-1 secretion from intestinal L-cells may be important for these observed effects. Using a GPR119 agonist to stimulate secretion of endogenous GLP-1 may provide a great approach, giving improved glycaemic control and associated weight loss through an oral dosing regime. The ability of GPR119 agonists to elicit GIP secretion could be of limited therapeutic value during the initial stages of

treatment, since type 2 diabetic patients are generally GIP resistant. However, β-cell responsiveness to GIP is enhanced with improving blood glucose control, so one could envisage GIP secretion gaining more pharmacological importance as chronic treatment with a GPR119 agonist progresses. As GPR119 agonists raise cAMP levels in the β-cell, there is potential for these agents to exert a beneficial effect on disease progression beyond what could be achieved by improving glucose homoeostasis alone. The concomitant increase in circulating GLP-1, itself capable of raising β-cell cAMP levels, might contribute to such an effect. The value of GPR119 agonists as a new class of therapeutics for type II diabetes and associated obesity is therefore likely to be determined within the next few years.

GPR40: Fatty acids serve vital functions as sources of energy, building materials for cellular structures, modulators of physiological responses, and in some cases, biomarkers for disease. Recent reports have identified fatty acids as cognate ligands for a small family of G-protein-coupled receptors, thus providing evidence that these receptors function as nutrient sensors [44-46]. Fatty acid carbon chain length has been shown to confer activity and specificity, with short chain fatty acids (SCFAs) activating GPR41 and GPR43, and long chain fatty acids (LCFAs) activating GPR40. Activation of a fourth fatty acid receptor, GPR120, which bears little homology to the GPR40 family, stimulates *in vivo* and *in vitro* secretion of the insulinotropic incretin GLP-1 (glucagon-like peptide 1).

Within the last decade, fatty acids have gained significant attention in the arenas of energy intake and glucose disposal, with obvious implications for the treatment of insulin resistant diabetes mellitus. The relatively specific expression of GPR40 in pancreatic islets and its coupling with Gq/11, resulting in elevation of intracellular calcium and stimulation of protein kinase C activity, suggested that the receptor may play a role in the ability of LCFAs to stimulate insulin secretion [47]. Fatty acid mediated potentiation of insulin release through GPR40 involves activation of phospholipase C, elevation of Ca^{2+} from the endoplasmic reticulum and up-regulation of Ca^{2+} entry through voltage-gated Ca^{2+} channels.

Pharmacological activation of GPR40 results in potentiation of glucose- stimulated insulin secretion. Hence development of GPR40 modulators may be an attractive approach for the treatment of diabetes.

GPR41: Recent deorphanization around GPR41 has elevated its level of interest over the last few years, mainly because of its potential as a therapeutic target. SCFAs have been demonstrated to activate GPR41 in a variety of recombinant cellular systems. GPR41 mainly initiates its signalling through coupling with the Gi/Go family of G-proteins, evidenced by the abolition of signal response when transfected cells are treated with pertussis toxin. GPR41 expression in human fat samples has been found by using mRNA detection and immunohistochemistry techniques. GPR41 implies that this receptor does play a role in monitoring energy storage and circulation, perhaps linking to diabetes. Therefore GPR41 modulators may have potential in treating diseases of glucose regulation, in particular, Type 2 (non-insulin-dependent) diabetes mellitus, and states of glucose intolerance.

GPR43: Functional activation of GPR43 by SCFAs has been characterized using a range of different assays. However, this receptor has shown interesting differences from GPR41 in intracellular signalling, ligand selectivity and tissue localization. GPR43 is mainly coupled with Gq-proteins, with some evidence for its possible coupling with Gi/Go pathways as well. GPR43 has been shown to be present in a variety of tissues, particularly in fat stores, inflammatory cells and the gastrointestinal tract. Thus, modulators of the GPR43 receptor might have therapeutic use in the treatment of numerous metabolic disorders, including diabetes mellitus and obesity. SCFA and GPR43 may control gastrointestinal motility and secretion via 5-HT mediated effects.

CONCLUSION: The science of G proteins was launched more than 30 years ago with the involvement of guanine nucleotides in the hormonal stimulation of adenylyl cyclase. Since then, the field has tremendously grown, and G protein-mediated signal transduction has turned out to be the most widely used transmembrane signaling system in higher organisms. There is probably not a single cell in a mammalian organism which does not employ several G protein-mediated signaling pathways.

Often these pathways integrate the information conveyed by several receptors recognizing different ligands. Only in recent years have we gained a more systematic insight into the role of individual G proteins on a cellular level. However, many aspects of G protein- mediated signaling still remain to be clarified. New approaches are required to determine the exact composition of individual signaling units and to define their exact cellular localization. This will probably lead to the identification of more and more proteins and nonproteins that may modulate G protein-mediated signaling.

The parallel application of genetic, genomic and of new proteomic approaches will be required to continue to define how the G protein-mediated signaling system works on a molecular, cellular, and systemic level. Such a view will provide the basis for a complete understanding of the physiological role of G protein-mediated signaling, which may allow the full exploitation of this multifaceted signaling system as a target for pharmacological interventions of diabetes and related metabolic disorders.

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