The Role of incretin-based therapies in maintaining glucose homeostasis and management of diabetes mellitus

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Abstract

Multiple organs work together to achieve a balance between glucose entry into blood circulation (from the liver, glucose is absorbed through intestinal mucosa after meals) and peripheral tissue glucose uptake (mainly brain and skeletal muscles). Blood glucose is controlled and maintained within a narrow range under normal physiological conditions, which is referred to as glucose homeostasis. Endogenous insulin is secreted when blood glucose levels rise. Its release can be aided by other factors such as amino acids and hormones released from the gut following food consumption, a phenomenon known as the incretin effect. Diabetes mellitus is caused by defects in insulin secretion, insulin action, or both, resulting in chronic hyperglycemia caused by disturbed carbohydrate, fat, and protein metabolism. The current review discusses the role of incretin-based therapies in the management of diabetes mellitus and the maintenance of glucose homeostasis.

Keywords: Diabetes; Incretin; DPP-4 inhibitors; GLP-1 agonist

1. Introduction

1.1. Glucose homeostasis

In normal physiological conditions, blood glucose is controlled and maintained within a narrow range which is termed glucose homeostasis. This is vital for continuous glucose supply to the central nervous system. Multiple organs coordinate together to achieve a balance between the glucose entry into blood circulation (from the liver, glucose absorbed through intestinal mucosa after meals), and the glucose uptake by peripheral tissues (mainly brain and skeletal muscles). After carbohydrate containing meal, pancreatic islets maintain normal blood glucose levels (BGL) via suppression of glucose production by liver, stimulation of glucose uptake by liver and peripheral tissues (Pearson & McCrimmon, 2013).

The pancreatic islets (islets of Langerhans) are the pancreatic areas which comprise hormone producing cells. The islets percentage is about 4.5% of the total pancreatic size and receive nearly 10% to 15% of pancreatic blood flow (Langerhans, 1869). Histologically, islets of Langerhans are distributed as density routes throughout the healthy pancreas of adult human. A thin fibrous capsule separates the islets from the surrounding pancreatic tissues. This capsule is composed of connective tissue that is continuous with the interwoven connective tissue throughout the rest of the pancreas. Islet clusters are super structures composed of small islets that surround large blood vessels (Feldman, Friedman, & Brandt, 2010; Ionescu-Tirgoviste et al., 2015).

Islets cyto-architecture varies among species. The cluster of human islets show beta and alpha cells in close correlation, while rodent islets cluster are characterized by a major part of beta cells in the core and by limited alpha, gamma and delta cells in the periphery (Brissova et al., 2005; Cabrera et al., 2006).   In rat islets, endocrine cells are categorized into glucagon producing cells (Alpha cells, 20% of total islet cells), insulin and amylin producing cells (Beta cells, 70%), somatostatin producing cells (Delta cells, <10%), pancreatic polypeptide producing cells (Gamma cells, <5%), ghrelin producing cells (Epsilon cells, <1%). Paracrine and autocrine communication are present in-between islets. The pancreatic islets paracrine feedback system comprise glucose-insulin system which stimulates beta cells and inhibits alpha cells, glycogen-glucagon system that stimulates alpha cells which triggers beta cells and delta cells and somatostatin which inhibits both beta and alpha cells (Elayat, el-Naggar, & Tahir, 1995).

1.2. Insulin secretion and regulation

Secretion of insulin, glucagon and somatostatin from islets of Langerhans are regulated by large number of G protein-coupled receptors (GPCRs) regulate the secretion of insulin, glucagon and somatostatin from islets of Langerhans. Some of these GPCRs are potential targets of drugs used in treatment of diabetes such as dipeptidyl peptidase-4 enzyme (DPP-4) inhibitors and glucagon-like peptide-1 (GLP-1) receptor agonists (Amisten, Salehi, Rorsman, Jones, & Persaud, 2013).

Insulin secretion from pancreatic beta cell is regulated mainly by glucose, although amino acids, gastrointestinal peptides, various nutrients, ketones, and neurotransmitters also stimulate insulin secretion. Insulin synthesis is stimulated by glucose levels >70 mg/dL, mainly by increasing protein translation and processing. Glucose-stimulated insulin secretion begins with its transport via a facilitative glucose transporter into the beta cell. Glucokinase-mediated glucose phosphorylation is the rate limiting step that controls insulin secretion. More metabolism of glucose-6-phosphate by glycolysis generates ATP, which hinders the action of an ATP-sensitive K+ channel. This channel has 2 separate proteins: one is an inwardly rectifying K+ channel protein; the other is the binding target for certain oral hypoglycemic (e.g., meglitinides, sulfonylureas). Beta cell membrane depolarization occurs as a result of inhibition of this K^+ channel, which opens voltage-gated calcium channels leading to calcium influx which induces insulin secretion. A pulsatile pattern of insulin release is seen, with small secretory ones taking place each ten minutes and later ones about 80–150 min that superimposed upon greater amplitude oscillations (Powers, 2015).

About 50% of insulin is degraded and removed by the liver, as soon as it is released into the portal venous system. Remaining insulin moves in the systemic circulation where it binds to its receptors in target sites, which leads to receptor autophosphorylation by intrinsic tyrosine kinase and recruitment of insulin receptor substrates (IRS), intracellular signaling molecules; IRS with other adaptor proteins begin a cascade of phosphorylation and dephosphorylation, resulting in the mitogenic and widespread metabolic effects of insulin. As an example, stimulation of the phosphatidylinositol-3′-kinase pathway induces glucose uptake by skeletal muscle and fat by translocation of Glucose transporter type 4 (GLUT4), a facilitative glucose transporter; Other insulin receptor activation pathways stimulates glycogenesis, lipogenesis, protein synthesis (Powers, 2015).

2. Diabetes mellitus

Chronic hyperglycemia is caused by disruptions in carbohydrate, fat, and protein metabolism caused by defects in insulin secretion, action, or both. Diabetes mellitus is the medical term for this metabolic disorder (Alberti & Zimmet, 1998). Diabetes mellitus is divided into four broad types: type I (TID), type II, gestational diabetes, and other specific types (David, Gardner, & Dolores, 2011). According to the International Diabetes Federation (IDF), there are approximately 415 million diabetic patients worldwide, with nearly 41.5 million cases (10% of the total) suffering from TID. Furthermore, the IDF predicts that this figure will rise to 642 million by 2040. Diabetes affects more than 35.4 million people in the Middle East and North Africa region, with that figure expected to rise to 72.1 million by 2040. In Egypt, there were over 7.8 million diabetic patients in 2015 (IDF Diabetes Atlas, 2015).

Type I diabetes is commonly thought to be caused by an immune-mediated, if not directly immune-mediated, destruction of pancreatic cells (insulin-producing cells). However, some argue that beta cells are only rendered inoperable rather than completely destroyed. At the onset of TID symptoms, autoimmune destruction is thought to be caused by chronic inflammation of pancreatic islets (In't Veld, 2011). Another widely held belief is that in patients with long-term diabetes, the pancreas is devoid of insulin-producing cells, and the remaining cells are incapable of regeneration (Butler, Meier, Butler, & Bhushan, 2007). Despite the fact that most patients with long-term TID have few β cells, some claim that evidence for -cell regeneration exists in infants and very young children but not in adults (Gregg et al., 2012).

In the past, TID was mainly considered a childhood disorder, but this belief has changed in the recent decade, the age is no longer a limiting factor. The classic trio of diabetes symptoms (Polydipsia, polyphagia, and polyuria) along with obvious hyperglycemia remain diagnostic criteria in children, and to a lesser extent in adults. (Bluestone, Herold, & Eisenbarth, 2010; Todd, 2010). TID patients require insulin therapy for the rest of their lives. To avoid diabetes-related acute and late complications, the majority of them require two or more insulin injections per day, with doses determined by blood glucose levels. Diabetes complications are reduced and delayed by strict blood glucose control (Cleary, Dahms, Goldstein, Malone, & Tamborlane, 2001).

Insulin therapy has advanced significantly since its discovery by Banting et al. in 1921. Human insulin is now biosynthesized using recombinant techniques, and the presence of highly purified human insulin preparations has reduced immunogenicity significantly, virtually eliminating therapeutic complications such as localized lipoatrophy at the injection site, immune insulin resistance, and insulin allergy (Hirsch, 2005).

The goal of insulin therapy in TID is to mimic pancreatic insulin secretion. To achieve glucose homeostasis, the pancreas secretes continuous, background insulin, basal insulin, to maintain euglycemia in the fasting state, as well as short bursts of insulin, bolus insulin, in response to BGL elevations above 80-100 mg/dL, such as those that occur immediately after a meal. As a result, an insulin replacement regimen for TID includes both basal and bolus insulin components (Figure 1), as well as any additional insulin required to bring blood glucose back into the target range when hyperglycemia occurs (Ferrannini, 2012). Current treatments do not resemble pancreatic endogenous insulin, and all have risks of poor control, ketosis, and hypoglycemia. (Abdelhamid, Abdelaziz, & Salem, 2018; Nathan et al., 2009).
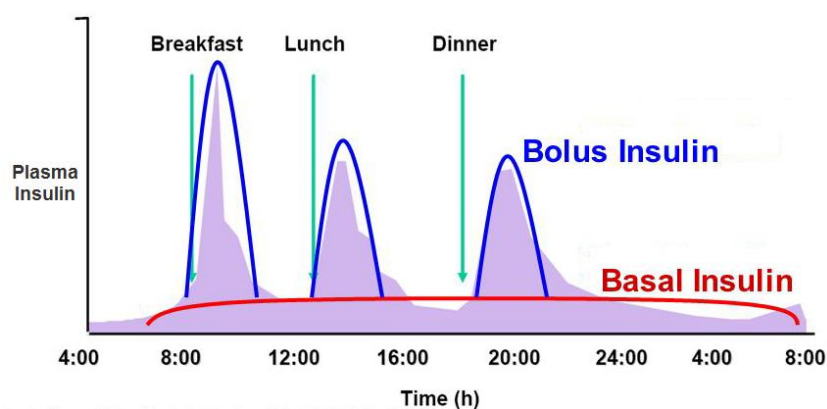

Figure 1: Ideal insulin replacement strategy. Insulin regimen that includes long-acting insulin for basal insulin coverage and three shots of short or rapid acting insulin for glycemic control at each meal(White Jr, Campbell, & Hirsch, 2003).

3. Incretin hormones

Endogenous insulin is secreted as a result of blood glucose rising. Its release can be augmented by other factors like amino acids and hormones released from the gut after food intake in a phenomenon known as incretin effect (Pearson & McCrimmon, 2013). The incretin effect, Figure 2, is an augmented insulin secretion provoked by oral when compared with intravenous administration of the same amount of glucose. Glucose dependent insulinotropic polypeptide (GIP) and glucagon-like peptide (GLP-1) are potent insulinotropic hormones. GLP-1 induces gene expression, stimulates insulin secretion, and β-cell growth (Fehmann, GÖKE, & GÖKE, 1995; Jens Juul Holst, 2016). Although individuals with TID have normal incretin response to meals, injection of exogenous GLP-1 decreases postprandial glucose peak by 45%. (Kielgast, Holst, & Madsbad, 2011).

Glucose dependent insulinotropic polypeptide (GIP) is a 42 amino acids peptide that belong to the glucagon secreting family of peptides. Receptor of GIP is formed in the pancreatic islets as well in the gut, heart, adipose tissue, adrenal cortex, pituitary, and many regions of the brain (Usdin, Mezey, Button, Brownstein, & Bonner, 1993). K cells is specific endocrine cells with largest mass in the duodenum but present in the small intestinal mucosa that is responsible for secretion of GIP (Mortensen, Christensen, Holst, & Orskov, 2003). Absorbable carbohydrates and lipids stimulate its secretion. GIP secretion is therefore significantly increased in

response to meals. Binding of GIP with its receptor on the β-cells causes elevation of cAMP levels, which sequentially rises the intracellular calcium concentration and results in exocytosis of insulin-containing granules (Jens Juul Holst & Gromada, 2004).

Glucagon-like peptide (GLP-1) is a glucagon gene product. It is expressed in the L-cells of the intestinal mucosa as well as in pancreatic α-cells (Mojsov et al., 1986). Its secretion is stimulated by existence of food in the gut, and the release of insulin throughout the day is highly correlated to GLP-1 secretion. GLP-1 exceeds GIP in stimulation of insulin secretion as it is one of the most powerful insulin-releasing substances recognized (Ørskov, Wettergren, & Holst, 1996). Most of the following effects appear to be secondary to accumulation of cAMP which results from binding of GLP-1 with a GPCRs on the β-cells (Jens Juul Holst & Gromada, 2004).

Thus, both hormones are important for incretin function. However, GIP is less potent than GLP-1 as it is only insulinotropic at raised levels of glucose. But its concentrations in blood is up to ten folds much higher than GLP-1 (Vilsbøll, Krarup, Madsbad, & Holst, 2003).
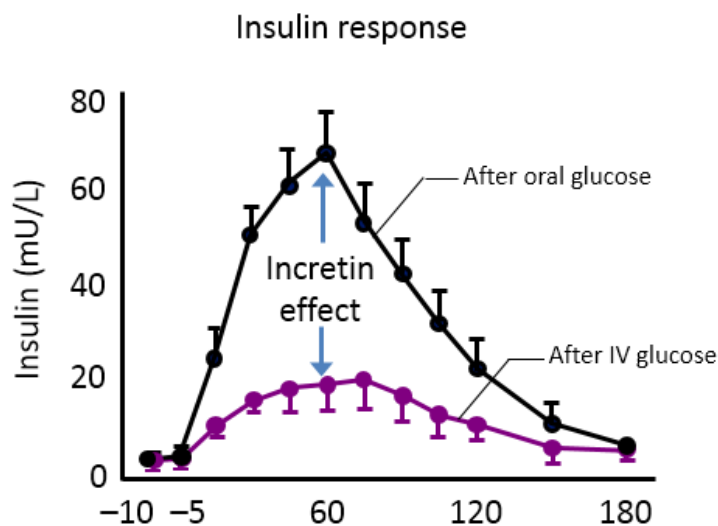

Figure 2: Difference in the Insulin Response to Oral vs. IV Glucose (Nauck et al., 1986)

4. Incretin based treatment approaches

4.1. *Inhibition of GLP-1 degradation (DPP-4 inhibitors):*

Dipeptidyl peptidase-4 (DPP-4) enzyme, a highly specialized amino-peptidase, presents in intestinal brush-border membranes, plasma, kidney and on a subset of T lymphocytes, the surface of capillary endothelial cells and hepatocytes (Deacon, Johnsen, & Holst, 1995). As a therapeutic application, DPP-4 inhibitors prolong the effect of the GIP and GLP-1 in type II diabetes, which has several benefits compared with GLP-1 analogues and conventional therapies; For example, DPP-4 inhibitors are able to potentiate the actions of both GLP-1 and GIP and can be taken orally. In addition, GLP-1 elicits insulin secretion in a glucose dependent manner (Deacon, 2004); thus, DPP-4 inhibitors have lower risk to induce hypoglycemia when compared with traditional treatments such as thiazolidinediones and sulfonylureas.

Due to great DPP-4 mediated degradation of GLP-1 in diabetic patients, its use was proposed (J J Holst & Deacon, 1998). Protection of endogenous and exogenous GLP-1 from degradation was demonstrated using the available DPP-4 inhibitors and thereby significantly improve its insulinotropic action (Deacon, Hughes, & Holst, 1998). Many following studies have showed that use of DPP-4 inhibitors significantly improved glucose intolerance in animal models.

Administration of *Novartis-DP728* inhibitor 2 to 3 times daily for four weeks significantly lowered HbA$_{1c}$, fasting and postprandial plasma glucose levels (Ahrén et al., 2002); Novartis pharmaceutical company has consequently presented another inhibitor, *LAF237* that was marketed under the name Vildagliptin, which can be taken once-daily as it has a longer duration of action. This compound was administrated as a single dose and showed

an efficacy similar to *DP728* administered 2 to 3 times daily (Ahrén et al., 2004). DPP-4 inhibitors alone or in combination with Pioglitazone significantly also reduced blood glucose levels and HbA$_{1c}$., increased serum insulin levels and decreased serum glucagon levels in type I diabetes rats. Also, it showed strong anti-oxidant activity with marked improvements in β-cells architecture in treated groups (Abdelhamid et al., 2018; Amir Mohamed Abdelhamid, Rania Ramadan Abdelaziz, Hoda Atef, 2017).

4.2. Treatment with native GLP-1:

In type II diabetes, intravenous administration of GLP-1 can control fasting BGL, even in patients with poor residual β-cell capacity treated with insulin for long term (Nauck, Holst, & Willms, 1997). In patients with the highest fasting BGL, GLP-1 administration possesses the highest glucose lowering effect; on the other hand, many hours are required for normalization and even after seven hours euglycemia was not reached but BGL were still declining. It can be concluded that in very little residual β-cell capacity patients, euglycemia can't be induced by GLP-1 infusion in the fed state (Toft-Nielsen, Madsbad, & Holst, 2001). However, administration of GLP-1 overnight and during the following meals has shown to complete normalize plasma glucose (Rachman, Barrow, Levy, & Turner, 1997).

Glycated hemoglobin (HbA$_{1c}$), plasma glucose concentrations, and free fatty acids were markedly lowered, and the patients had a gradual and significant decrease in body weight. Furthermore, insulin secretion capacity greatly was enhanced and insulin sensitivity nearly doubled (Zander, Madsbad, Madsen, & Holst, 2002). GLP-1 subcutaneous administration may not be ideal; as during infusion very high levels of the GLP-1 amide metabolite are produced, and although insulin-independent glucose-lowering properties have been discussed for this metabolite (Deacon, Plamboeck, Møller, & Holst, 2002), it has also a GLP-1 receptor antagonist properties (Knudsen & Pridal, 1996).

4.3. GLP-1 receptor agonists:

The intrinsic strength of GLP-1 receptor agonist, Exendin-4, toward the GLP-1 receptor appears to be as that of GLP-1. It could have other actions besides those provoked by activation of the GLP-1 receptor (Idris, Patiag, Gray, & Donnelly, 2002). *In vivo*, however, it is greater in potency than GLP-1 due to slower breakdown. Exendin-4 is resistant to DPP-4 as amino acid number 2 is replaced with Glycine while GLP-1 has Alanine. Thus the half-life of the molecule in the body increased from 2 min to ∼4 min (Deacon et al., 1998).

Exendin-4 seems to act in a way identical to GLP-1 in humans (Edwards et al., 2001). Exendin-4 administration in patients already treated with sulfonylurea, metformin, or both caused a 0.7–1.1% decrease in HbA$_{1c}$ in all groups. The most common side effect was mild to moderate nausea. In about 33 % of the diabetic subjects also treated with sulfonylurea, mild hypoglycemia was reported. These incidences may also illustrate the power of this combination (Gutniak et al., 1996). The most remarkable outcome was significant decrease in body weight (Baron et al., 2003), where over a period of 26 weeks, a gradual weight loss was observed. Exendin-4 provided significant extra BGL control, even in patients uncontrolled with oral anti-diabetic agents, and resulted in weight loss, which can suggest additional enhancements of metabolism (Markovic et al., 1998). A more gradual and pronounced decrease in HbA$_{1c}$ was detected, but the decrease flattened out during the last three months, which may show that mean 24 hours BGL dropped more than fasting BGL. Exendin-4 has not been clinically assessed as monotherapy, but only as add on to existing therapies.

Another approach to achieve slow elimination kinetics of GLP-1 is to bind this molecule to albumin that make the molecule unaffected by DPP-4 and allow the bound part to escape renal elimination. This increased half-life to 10–12 hours following a single subcutaneous injection in healthy and type II diabetic subjects (Juhl et al., 2002). In addition, side effects such as nausea caused by less long-lived analogs, are likely to be avoided in chronic treatment (Ribel et al., 2002).

The analog itself is as potent as GLP-1 at the cloned human GLP-1 receptor. Due to its long half-life, the analog is suitable for studies in rodents. In rats with β-cell deficiencies, Liraglutide had noticeable anti-hyperglycemic effects and significantly delayed development of diabetes. There were also great effects on food intake and β-cell mass (Sturis et al., 2003). It also induced reversible and lasting decrease in body weight in both normal and obese rats (Larsen, Fledelius, Knudsen, & Tang-Christensen, 2001). These analogs, like Exendin-4 or GLP-1, inhibited apoptosis in primary β-cells induced by fatty acid and cytokine (Bregenholt, Moldrup, Blume, Knudsen, & Petersen, 2001; Bregenholt, Moldrup, Knudsen, & Petersen, 2001). In clinical studies, it effectively reduced both fasting and prandial glycemia (12 h post-injection) by increasing insulin secretion, suppressing prandial glucagon secretion and delaying gastric emptying (Juhl et al., 2002).

The FDA approved the first GLP-1 receptor agonist exenatide in 2005. An extended-release (ER) formulation of exenatide is approved and can be administered once weekly which offers substantial convenience for patients who have high medication burden and poor medication (Mann & Raskin, 2014; Syed & McCormack, 2015). Currently, there are many injectable GLP-1 receptor agonists including, the twice-daily exenatide (McCormack, 2014), the once daily liraglutide (Garber et al., 2011) and lixisenatide (Leon, LaCoursiere, Yarosh, & Patel, 2017), the once weekly dulaglutide (Smith et al., 2016), semaglutide (Smits & Van Raalte, 2021) and exenatide ER (Mann & Raskin, 2014); in addition to the oral semaglutide tablet which was approved by the FDA in 2019 (Hughes & Neumiller, 2020).

5. Summary:

Incretin-based therapies have emerged as potential anti-diabetic agents with high efficacy and a favorable safety profile. They are currently approved for management of type II diabetes and the current preclinical studies showed that they are may be promising in type I diabetes too. They also demonstrated pleotropic effects in addition to their known antihyperglycemic effects. Finally, incretin-based therapies are novel therapeutic approaches with numerous advantages.

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