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ORIGINAL ARTICLE

Physiological Interactions of Pancreatic Hormones and Incretins in Type 2 Diabetes Mellitus

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ABSTRACT

It is known that after meal ingestion two incretins on its active form; glucose-dependent insulinotropic polypeptide (GIP [1-42]) and glucagon like peptide-1 (GLP-1 [7-36]) are released from duodenal K-cells and ileum-colon L-cells, respectively. GIP and GLP-1 induce insulin release from pancreatic β-cells in order to increase glucose tissue uptake (muscles, liver and adipose cells), and they also induce the suppression of glucagon from pancreatic α-cells avoiding hepatic glucose release, it is due to an indirect local effect of insulin in islet pancreatic cells. These insulinotropic effects maintain euglycemia. However GIP and GLP-1 are rapidly degraded by their hydrolytic enzyme, dipeptidylpeptidase-4 (DPP-4) which inactives incretins leading them like GIP [3-42 amide] and GLP-1 [9-36 amide] both de-amidated peptides act like antagonist to their own receptors in pancreatic cells.

In diabetic subjects, incretin effect is abnormal because the incretin secretion is impaired or the effect is lost. GIP secretion is normal but its effect is low, while GLP-1 levels are low but its physiological action is not affected.

Recent advances in the knowledge of physiological abnormalities associated with type 2 diabetes mellitus have lead to the development of novel incretin – based therapies offering greatest promise related to the currently available. Treatments against type 2 diabetes mellitus include GLP-1 intravenous infusions (such as incretin mimetics and analogues), DPP-4 inhibitors and bariatric surgery to reach the equilibrium between incretins and anti-incretin factors are being successfully used.

Key words: Type 2 diabetes mellitus, glucose-dependent insulinotropic polypeptide (GIP), glucagon like peptide-1 (GLP-1), Insulin, Glucagon, Bariatric surgery

Introduction

Diabetes mellitus is a common disease worldwide. Currently, 246 million people worldwide have diabetes and this will grow to more than 420 million by 2025 [1]. This disease is characterized by insulin resistance caused by a wrong glucose-induced insulin secretion, contributing to an impairment of insulin action that feedback to a further progressive deterioration

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in islet cell function [2-4]. Specially structure and function deterioration almost in 50% of β–cell mass is observed in type 2 diabetes mellitus, but also incretin metabolism is abnormal because there is an evident decrease on incretin effect [5, 6], that is the reason of the reduction in nutrient-mediated secretion of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1) [7], this effect leads to an inappropriately elevated glucagon concentrations that results in hyperglycemia [3, 4].

Digestion, absorption and assimilation of ingested nutrients especially carbohydrates provokes GLP-1 release, but fats and protein ingestion are excellent stimulus for GIP secretion, these two incretins suppress glucagon release and promotes insulin effects. This interaction between incretins, insulin and glucagon restore plasma glucose levels into normal limits [7 - 9].

In last years, the role of incretins has been extensively studied in experimental models and humans in health and disease, because the use of these peptides as therapeutic agents offers new approach to the treatment of type 2 diabetes mellitus. Here, some aspects on the physiology of pancreatic hormones and incretins are reviewed.

Pancreatic Hormones

The endocrine activity is centered in cellular groupings called islets of Langerhans, which contains three types of cells, α-cells, β-cells and δ-cells. α-Cells secrete glucagon in response to low blood glucose, glucagon stimulates liver to release glucose through glycogenolysis and gluconeogenesis and it also stimulates adipose tissue to release fatty acids through lipolysis; β-cells produce insulin that stimulate muscle, liver and adipose cells to store glucose for later use by synthesizing glycogen, protein and fats (Figure 1), δ-cells secrete somatostatin that inhibits insulin and glucagon release from their islet cells [10, 11].

Insulin and glucagon have been the most studied hormones, both hormones are involved in the affection of type 2 diabetes mellitus when there is a decrease in the insulin secretion which provoke an increase of blood glucose levels.

Insulin

This peptide has many functions not fewer important but most studies have focused to the best known action, increase glucose uptake in most peripheral tissues, consequently lowering the level of glucose in blood [13].

After the insulin is secreted by β-cells of the islets of Langerhans in the pancreas, it is transported to all the tissues with insulin receptors. When insulin and its receptor interact, they produce the complex and liberation the tonic inhibition of tyrosinkinase, beginning the transduction of the hormonal signs. The subunit β of receptor binds adenosine triphosphate that generates autophosphorylation, promoting cascade of phosphorylation of intracellular substrates.

The circulating insulin joined to its receptor, increases glucose uptake from muscle and adipose tissue, inhibits hepatic glucose production, stimulates glycolysis, lipogenesis, glycogenesis and protein synthesis and also inhibit β-oxidation of fatty acids, glycogenolysis and proteolysis.

Glucagon

Glucagon is a peptide hormone of 22 amino acids secreted by pancreatic α-cells, includes 33-61 amino acids whose MW is 3500 Da [7].

Glucagon binds to its receptors in the plasma membranes of adipocytes, activating (via a Gs protein) adenylyl cyclase, during fasting state glucagon increases blood glucose via hepatic glycogenolysis or gluconeogenesis and it stimulates adipose tissue to release fatty acids through lipolysis. Some hormones act by inhibiting adenylyl cyclase, lowering cAMP levels, and suppressing protein phosphorylation [11, 14, 15].

In non-diabetic subjects after carbohydrate ingestion, glucagon release is inhibited and the stimulation of insulin secretion establishes an equilibrium that maintains euglycemia.

Somatostatin

Somatostatin is a polypeptide hormone secreted by pancreatic δ-cells and hypothalamus where inhibits grown hormone (GH) release [15]. It regulates the secretion of insulin by interfering with G-proteins in the b-cell membrane rather than interacting with K^+ -ATP channel [16] and glucagon is regulated when somatostatin binds to his receptor leading to activation of an inhibitory G protein, or Gi, structurally homologous to Gs protein that inhibits adenylyl cyclase and lowers cAMP, therefore counterbalancing the effects of glucagon [11, 15]. Chronic infusion of somatostatin has not effect on food intake and body weight [13].

Incretins (GLP-1 and GIP)

In order to consider a substance like incretin, two specific criteria must be fullfilled: 1) released molecule after oral nutrient ingestion, especially glucose, 2) which reaches physiological concentrations *in vivo* to cause insulin release [7]. GIP and GLP-1 are two incretins members of the glucagon peptide super family and share considerable amino acid identity [17].

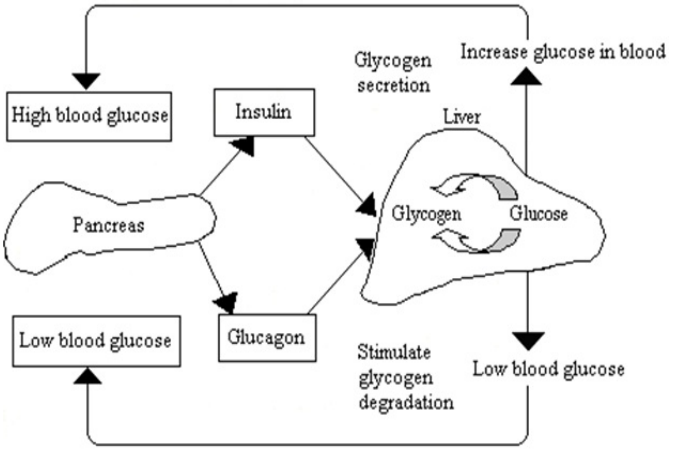

Fig. 1: Insulin and glucagon secretions from pancreas act directly in the liver where it takes place the glycogenolysis. (Adapted from reference 12)

GIP is a peptide of 42-chain amino acid (GIP 1-42NH_2, MW 4984 Da) [7] (Figure 2) which comes from the GIP gene that is mainly expressed in specific endocrine cells, called K cells, that are found in the highest density in the duodenum (proximal small intestine) [7]. Its direct precursor is ProGIP [17, 18].

GLP-1(Figure 2) is a single 30-chain amino acid peptide (GLP-1 7-36NH_2, MW 3298 Da) [7], derived from proglucagon gene which also contains the information for glucagon peptides and GLP-2 [2, 17]. This gene is expressed not only in pancreatic α-cells also in L- cells of the intestinal mucosa [18] hypothalamus [19] and predominantly in ileum and colon.

Proglucagon gene, leads to the production of proglucagon peptide (1-185 amino acid) which exist in pancreas and ileum/colon/brain, through 2 enzymes; proconvertase 2 and proconvertase 1 there is the production of glucagon and GLP-1 respectively. GLP-1 is produced as an inactive 37-amino acid peptide whose C-terminal contains glycine, by post-translational cleavage of six amino acids from the N-terminal of GLP-1(1–37) to form the amidated form as GLP-1 (7–37) which conserves the C-terminal of the glycine to produce another amidation at position 37 leads to production of GLP-1 (7–36) NH_2, the major circulating form, however GLP-1 (7-37) and GLP-1 (7-36) both have identical biological properties as incretins [7,19,20].

Incretin receptors (GLP-1R and GIP-R) are located within several organs (Figure 3) [21, 22, 23]. These specific receptors belong to the G protein-coupled receptor superfamily, they are located in the membrane of pancreatic b-cell as well as in extrapancreatic sites. Glucagon, GIP, pituitary adenylate cyclase-activating polypeptide, secretin and vasoactive intestinal peptide receptors also belong to this receptor superfamily.

Kinetics of incretin secretion

GIP and GLP-1 secretion start 15 minutes after carbohydrate ingestion, they reach circulating concentrations up 100 pmol/l of GIP and below 50 pmol/1 of GLP-1 between 30-45 minutes. The two incretin concentrations return to basal values by 2-3 hours [7, 18, 24]

Factors affecting incretin secretion

As it was mentioned, GIP and GLP-1 release comes from the stimulus of the nutrients consumed, so the factors that limit or promote incretin secretion are: Composition of nutrient intake, intestinal luminal factors like rate of nutrient entry into the small intestine determined by gastric emptying, absorption of nutrients, osmotic phenomena or distension, glucagon and also circulating free fatty acids.

GIP secretion is suppressed by glucagon and not by free fatty acids but GLP-1 secretion is suppressed by free fatty acids [7].

Physiological incretin action

GLP-1

GLP-1 secretion from intestinal L-cells has non-insulinotropic physiological actions to which they can be called inhibitory effects or glucoregulatory effects as described below [8, 25]:

1. GLP-1 reduces α-cell secretory activity by inhibiting glucagon secretion [2, 4, 8,17, 19, 25] because there are no receptors for GLP-1 on the α-cell, but intra-islet insulin released from b-cells in response to GLP-1 locally inhibits glucagon secretion [7, 26].
2. Potent effects on gastrointestinal motility therefore generates gastric emptying to decrease

Fig. 2: Structures of GLP-1 (A) and GIP (B) and its precursors proglucagon and ProGIP. The amino acids underlined are similars in both incretins. ProGIP gene leads to the production of preProGIP of 153 amino acids from which GIP of 42 amino acids marked in preProGIP structure is derived.

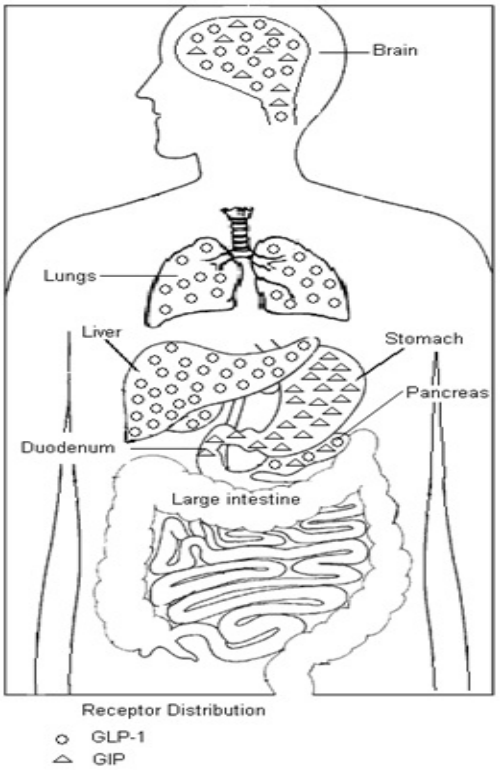

Fig. 3: GIP receptors are expressed in pancreatic b and α cells, stomach, adipose tissue, brain and bone (osteoblasts) [21] and osteoclasts [7, 14] GLP-1 receptors are found on b and δ-cells of the pancreas, parietal cells of the stomach, pylorus, adipose tissue, brain, in the lungs (lung membranes, mucous glands of the trachea and vascular smooth muscles of the pulmonary arteries) [22, 23] and in bone (osteoblasts) [7, 10]

the rate of nutrient absorption [2, 7, 8, 17, 19, 20, 25] by stimulation of GLP-1 receptors in the pyloric sphincter that has influences in the distension of the stomach and peripheral satiety signals [7, 27].

3. GLP-1 stimulates reduction of appetite and food intake [2, 4, 8, 20] and it has been found in nerve fibers in the brain and in high concentration in hypothalamic areas, where it serves as a neurotransmitter, that is closely involved in the control of food intake and appetite by influencing the neuropeptide Y (NPY) pathway [7, 10, 11, 18]. When it is given over a prolonged period (approximately six weeks) by continuous subcutaneous infusion results in significant weight loss [3, 4, 28].

GIP

The primary action of GIP is to stimulate glucose-dependent insulin secretion [2]. GIP unlike GLP-1 does not inhibit glucagon secretion but it has less effect on the release of glucagon [4, 17, 29]. GIP neither has effect on gastric emptying or food intake, but may have a role in lipid metabolism [4, 17, 30]:

GIP promotes lipolysis partly by stimulating lipoprotein lipase receptors on adipocytes in order to regulate fat metabolism in those cells. But GIP can also stimulate adipose tissue lipogenesis which is further enhanced by its insulinotropic property [7, 17, 20, 31-33].

The insulinotropic effects of GLP-1 and GIP is mediated by the receptor activation that starts with a cascade where the first events are the increases in intracellular cAMP with subsequent activation of protein kinase A (PKA). Furtherly it is an alteration in ion channel activity and intracellular calcium handling that induce the stimulation of exocytosis of insulin-containing granules [2, 4, 8] (Figure 4).

GIP and GLP-1 work together to realize two important functions, they stimulate insulin secretion in a glucose-dependent manner (only in the presence of raised blood glucose) [20] and promotes expansion of b-cell mass [17].

β-cells proliferation

Neogenesis of the b-cells is a topic that has been studied a lot to be able to find different solutions that regulated between b-cells proliferation and death to avoid serious damages. It is known in patients with T2DM there is an increase in b-cell mass apoptosis and reduction in the proliferation of these cells [34]. The defects that can appear in b-cells can be genetic and generate hyperglycemia acquired as a result of obesity [35]. This can be reversible with help of a great variety of hormones as: GLP-1, GIP, IGF-I, IGF-II, insulin, glucagon, gastrin, growth hormone (GH), prolactin (PRL), placental lactogen (PL), leptin and nutrients as glucose, amino acids and free fatty acids [35,36].

It has been shown that GH, PRL and PL can stimulate b-cell proliferation, glucose-induced insulin release, and insulin gene expression and biosynthesis in fetal, newborn and adult rat islets [35].

Studies realized in mice show that gene IPF1/PDX1 is required for maintaining the hormone producing phenotype of the β-cell by positively regulating insulin, islet amyloid polypeptide, glucagon and GLUT2 expression [37].

There is a family of transcription factors called signal transducers and activators of transcription (STAT), that are activated by GH and PRL [35].

There is a balance between b-cells proliferation and b-cells death in healthy subjects but in patients with T2DM exist an increment in b-cells death that generates problems in insulin released.

Incretin degradation

The main enzyme responsible for GLP-1 and GIP degradation is dypeptidylpeptidase - 4 (DPP-4), this is a serine protease located in the membranes brush border of the intestine, kidney, in surfaces of capillaries and also in soluble form in circulation that cleaves at the position 2 alanine, a dipeptide from the N-terminus of these two incretins, which thereby is inactivated [4, 17, 18]. Both hormones differ in the response of inactivation [2, 4, 18]. These two hormones are unable to realize their functions but they have antagonist effects on their respective receptors [8, 17].

After the inactivation GIP is transformed in GIP 3-42NH_2 and GLP-1 is transformed in GLP-1 9-36NH_2 these metabolites are small impotent peptide fragments eliminated by the kidney [2].

Analogues resistant and DPP-4 inhibitors have demonstrated to be workforce in protecting the GLP-1 and GIP of the degradation.

GLP-1R agonist restores first phase insulin secretion in type 2 diabetic subjects in response to glucose [38].

The therapeutic use DPP-4 inhibitors as antihyperglycemic drug prevents the N-terminal degradation of the GLP-1 turning out to be an increase of the insulinotropic effect of this incretin for increase its half life.

Incretins in healthy subjects

In fasting, there is a decrease in glucose levels, pancreatic α-cells release glucagon and pancreatic b-cells decrease insulin production in order to avoid glucose tissue uptake.

Glucagon increases plasmatic glucose levels by the stimulation for glucose release from the liver. This process results in a decrease of blood glucose.

After food intake there is an increase on glucose levels (hyperglycemia), GIP and GLP-1 are released from duodenum, ileum and colon respectively, GIP and GLP-1 have two effects on pancreatic hormones, they cause insulin release from pancreatic b-cells and glucagon suppression from pancreatic α -cells (Figure 5).

Insulin reduces hepatic glucose production and it increases glucose uptake by the peripheral tissues (muscles and adipose cells). This process also results in the decrease of blood glucose.

Incretins in type 2 diabetes mellitus

In type 2 diabetics the effect of GLP-1 and GIP is reduced or absent compared to healthy subjects, this is reason by which studies on the action of GIP and GLP-1 in this type of pathology have been focused.

It is known that excessive glucagon secretion stimulates hepatic glycogenolysis and therefore contributes to fasting hyperglycemia [19]. In type 2 diabetic patients, infusion of GLP-1 lead to a significant suppression of glucagon secretion together with normalization in fasting plasma glucose [30]. GLP-1 administration however, does not impair the glucagon counterregulatory response to hypoglycemia, since the glucagon secretion is glucose-dependent [30].

GIP secretion in type 2 diabetic subjects is normal but its response is defective because there is a lost of its insulinotropic potency, unlike GIP, secretion of GLP-1 is reduced in type 2 diabetes but it preserve its response [17,19].

Bypass in the control of incretin production

Perspectives

Gastric bypass (GBP) and biliopancreatic diversion (BPD) are the most effective treatment for obese patients with type 2 diabetes mellitus [39, 40]. The procedure is effective because guarantees weight

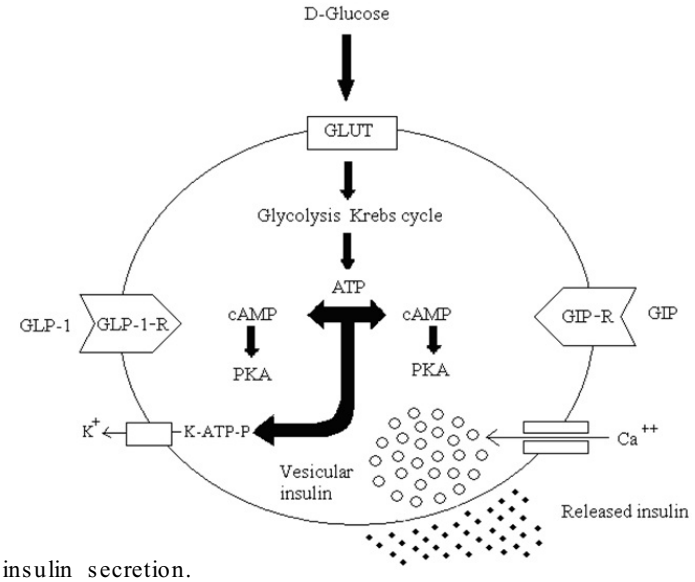

Fig. 4: Cascade of insulin secretion.

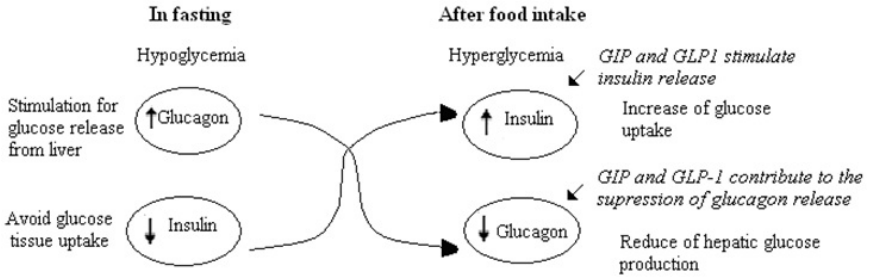

Fig. 5: Glucose control only occurs while there are GIP and GLP-1 in plasmatic circulation in their active form, GIP 1-42NH_2 and GLP-1 7-36NH_2, but in few minutes they are degraded by DPP-4, resulting GIP (3-42) and GLP-1 (9-36) in an inactive form. GIP and GLP-1 release occur until finish food intake.

loss, it helps in the control of hypertension and normal insulin levels [41]. In both procedures there is an exclusion of duodenum and at least part of the jejunum from the transit of food. GBP and BPD obviously presents an incomplete digested food early to the ileum, duodenum and jejunum and as consequence a change in the secretion of gastrointestinal hormones [42].

GBP induce an overall improvement of serum lipid profiles that implicates a marked fall of serum triglyceride level with a relatively small decrease in serum total cholesterol. BPD is very effective in patients with type 2 diabetes mellitus because is reversing dyslipidemia that represents a major component of the metabolic syndrome [39, 43, 44].

With this technique can come near to a balance between the incretins (located in the region of the duodenum, jejunum and ileum) and the unknown factor anti- incretin effect (located in the part of the ileum), which allows that GLP-1 and GIP response again and secrete insulin in a normal way [39, 42].

Conclusions

There is a complex and delicate equilibrium between insulin, glucagon, somatostatin and incretins, each one has specific effects on blood glucose control, but they also have a deficient effect when exist cell damage and the result is the incapacity of the pancreas to maintain euglycemia.

Incretins GLP-1 and GIP allows the regulation of the insulin in healthy subjects but there are complications in patients with type 2 diabetes mellitus that can lead to the disease to levels more serious. Therefore known about the affections of each patient is important to establish treatments adapted to their needs.

Exist the possibility of taking the patient to surgery as the Bypass that will help to restore the levels of GLP-1 and GIP in the organism but there is another type of alternatives as the DPP-4 inhibitors, and a new class of antidiabetic oral agents as well as drugs that act like incretins.

Several investigations are being realized to obtain effective solutions, there are many different alternatives due to this problem, and they will be possible as the knowledge of how diabetes is developed [45, 46].

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