GLUCAGON-LIKE PEPTIDE 1: BIOCHEMISTRY, SECRETION AND MAIN PHYSIOLOGICAL EFFECTS

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RESUMO

O "Glucagon-like peptide 1" (GLP-1), hormona produzida nas células-L intestinais por processamento diferencial do proglucagon, é libertado após ingestão de alimentos. Efeitos de GLP-1 observam-se, essencialmente, a nível gastrintestinal e pancreático resultando da sua acção directa, ligação ao receptor nas células alvo, ou indirecta, por regulação parácrina. Pelo seu papel na regulação da ingestão de alimentos e na secreção de insulina induzida por glicose, agonistas do receptor de GLP-1 são alvo de estudos para a terapia da obesidade e diabetes.

PALAVRAS-CHAVE

Hormona peptídica; "Glucagon-like peptide 1", GLP-1, Incretina, Diabetes *mellitus* tipo 2, Obesidade

ABSTRACT

The peptide hormone Glucagon-like peptide 1 (GLP-1), produced in the intestinal L-cells by differential processing of proglucagon, is secreted in response to meal intake. GLP-1 affects various systems, the gastrointestinal and pancreatic systems being the best studied, either by direct binding to the GLP-1 receptor, at the target-cells surface, or indirectly as a result of paracrine regulation. Because of GLP-1's roles, in augmenting glucose-induced insulin secretion and modulating food intake, currently GLP-1 receptor agonists are being studied for diabetes and obesity therapy.

KEYWORDS

Peptide hormone; Glucagon-like peptide 1, GLP-1, Incretin, Diabetes *mellitus* type 2, Obesity

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1. INTRODUCTION: A BRIEF HISTORY OF HOW GLP-1 CAME TO BE

Following the discovery of glucagon in the pancreas and its gluco- or counter-regulatory effect (*i.e.*, its ability to reverse the fall in serum glucose caused by insulin) (Murlin *et al.*, 1923), a series of studies identified the presence of glucagon-like substances in gastrointestinal mucosa. In 1948, based on bioassays, Sutherland and DeDuve proposed that gastric extracts may contain glucagon-like activity (Sutherland & De Duve, 1948). In 1968, Orci and colleagues reported endocrine cells of the intestinal mucosa that stained with antibodies to glucagon (Orci *et al.*, 1968), while Unger and colleagues reported that a glucagon-like immunoreactive material, physico-chemically and biologically distinct from glucagon, was secreted by the intestine in response to an oral glucose challenge (Unger *et al.*, 1968). By this time, solid evidence accumulated that up to twice as much insulin is secreted after increasing plasma glucose by ingestion of glucose than after a similar increase in plasma glucose in response to intravenous injection - the so-called "incretin effect of the gut" and evidence began to emerge that the "incretin effect" was severely blunted in type 2 diabetes *mellitus* (Perley & Kipnis, 1967). By 1985, a glucagon-like peptide (GLP), along with a second similar peptide GIP, alternately called gastrin inhibitory or glucose-dependent insulinotropic polypeptide, were seriously considered as candidate incretins. Altogether, this suggested that a supra-physiological dose of GLP might serve as a therapeutic enhancer of insulin secretion in type 2 diabetic patients who were those hyperglycemic in spite of apparently adequate insulin stores.

Here we review more recent studies characterizing the glucagon-like peptide 1 biochemically and physiologically and indicate its potential utility in the therapy of obesity as well as diabetes *mellitus*.

2. BIOCHEMISTRY AND PHYSIOLOGY OF GLP-1

2.1. PROGLUCAGON AND ITS PROCESSING BY PANCREATIC AND INTESTINAL TISSUE

Proglucagon is the main pro-hormone protein product of two distinct endocrine cell types, the pancreatic alpha-cell and the intestinal mucosa L-cell. During the maturation of these two cell types, a single proglucagon gene is activated. However with further cell differentiation, post-translationally the 160 amino acid proglucagon protein precursor undergoes differential proteolytic processing by secretory granules convertases at distinct dibasic residues (see Figure 1). The alpha-cells cleave glucagon from the region spanning amino acids (aas) 33 to 61 and then release it along with the major proglucagon fragment (MPGF) (Holst *et al.*, 1994). In contrast, L-cells cleave two structurally related GLPs from C-terminally located portions of the precursor molecule, namely GLP-1, from the region spanning aas 78 to 107, and GLP-2 from region spanning aas 126 to 158 (Mojsov *et al.*, 1986). L-cells also process and secrete glicentin from the region spanning aas 1-69, and oxyntomodulin, a C-terminally extended glucagon, from the region spanning aas 33-69 (Ørskov, 1992; Holst, 2007).

The proglucagon gene is also expressed in some neurons in the central nervous system. Cells with positive immunoreactivity for GLP-1, glucagon and glicentin have been reported in the nucleus of the solitary tract of the brainstem of some mammals, including humans (Drucker & Asa, 1988; Holst, 2007).

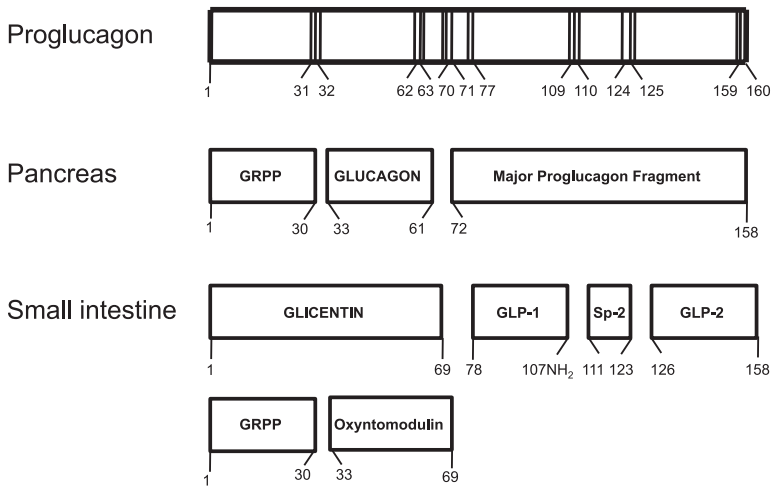

Figure 1. Posttranslational processing of proglucagon in mammalian pancreatic alpha-cells and small intestinal L-cells. The proglucagon is a 160-amino acid peptide (PG 1-160), where 1 indicates the N-terminus and 160 the C-terminus amino acid and the vertical lines indicate positions of the basic amino acid residues that are typical cleavage sites. The peptide products are represented in boxes and marked according to their position in the proglucagon sequence. (Adapted from Ørskov, 1992; Holst, 2007).

2.2. **GLP-1 DEGRADATION**

The catalytic enzyme dipeptidyl peptidase IV (DPP-IV; DP IV; CD 26) is a 766 amino acid, membrane-associated ecto-peptidase that is widely distributed in numerous tissues (*e.g.* lumenal membranes of capillary endothelial cells, the apical membranes of kidney tubule cells, the plasma membranes of hepatocytes, blood). This enzyme also exists as a soluble circulating form in plasma and significant DPP-IV-like activity is detectable in plasma from humans and rodents. DPP-IV has substrate specificity for oligopeptides with a penultimate prolyl-, analyl-, or seryl-, residue at their N-termini. In the presence of this DPP-IV the N-terminus dipeptide of a number of metabolic hormones and neuroendocrine factors are cleaved,

the order of catalytic efficiency being Neuropeptide Y (NPY) > Peptide YY (PYY) > GLP-1 > GIP > glucagon (Drucker, 2003). Since an intact N-terminus is obligatory for the biological activity of the members of the glucagon/VIP peptide family, DPP-IV inactivates these peptide hormones. In the case of GLP-1, the metabolites generated, namely GLP-1 (9-36) amide from GLP-1 (7-36) and GLP-1 (9-37) from GLP-1 (7-37), are not only inactive, they may act as competitive antagonists of the intact GLP-1 at the GLP-1 receptors (Knudsen & Pridal, 1996).

DPP-IV action is rapid and local. In experiments using isolated perfused porcine ileum, it was observed that less than 25% of the newly secreted GLP-1 leaves the gut in an intact, active form. In the liver it suffers similar degradation. Hence only about 10-15% of newly secreted GLP-1 enters the systemic circulation in an intact form (reviewed by (Holst, 2007)). GLP-1 metabolites are also cleared rapidly, mainly by the kidneys (glomerular filtration and proximal tubule uptake).

2.3. **STIMULI FOR GLP-1 SECRETION**

The secretion of GLP-1 is meal related. Although there is a basal rate of secretion, fasting GLP-1 plasma concentrations remain very low. Meal intake originates a rapid increase of GLP-1 secretion from the L-cells (Ørskov *et al.*, 1996) which is evident after about 10 min, or later than the "cephalic phase" stimulation of insulin secretion, suggesting the absence of vagal effects on GLP-1 secretion. Instead, the presence of nutrients in the gut, and probably their interaction with the L-cells, stimulates GLP-1 secretion. The L-cells response is dependent on the meal size and is highly correlated with the rate of the gastric emptying (Wachters-Hagedoorn *et al.*, 2006; Gribble, 2008). Plasma GLP-1 remains elevated for a considerable period of time after feeding cessation, indicating its continued secretion.

Mechanistically, stimulus-secretion coupling in L-cells is unclear. However, cell lines derived from L-cells depolarize, fire action potentials and display Ca^{2+}-entry dependent exocytosis of GLP-1 (Gribble, 2008). Possible routes for stimulus-depolarization coupling include Na^{+} coupled glucose and/or amino acid transport, glucose metabolism resulting in closure of ATP dependent K^{+} (KATP) channels and increased luminal osmolarity causing cell shrinkage and opening of stretch inactivated cation channels. More distally, depolarization-exocytosis coupling might be enhanced by signals from the proximal gut that may increase cytosolic cAMP and enhance the supply of release-ready GLP-1 granules (see Figure 2).

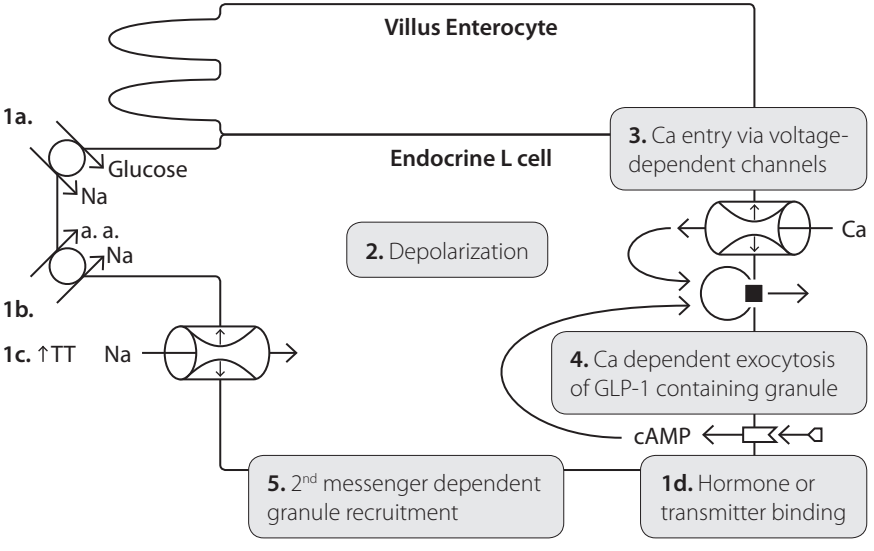

Figure 2. Two possible pathways for stimulus-secretion coupling in intestinal GLP-1 secreting L-cells. *Left:* apical glucose, amino acid and osmolar stimulation (1a-c) of depolarization (2) and triggering of Ca^{2+}-entry (3) and of Ca^{2+}-dependent exocytosis of GLP-1 containing granules (4). *Right:* basolateral synaptic transmitter or hormone stimulation of G-protein-coupled receptor (GPCR) resulting in enhancement of cytosolic [cAMP] (1d) and enhancement of depolarization-induced, Ca^{2+}-dependent exocytosis by granule recruitment (5).

2.4. GLP-1 RECEPTORS AND CELL SIGNALING

The GLP-1 receptor was first cloned in 1992 (Thorens, 1992). It is a class B GPCR, *i.e.*, one of the group of 15 receptors (in the human genome), including GIP and the glucagon receptors, that are activated by intermediate sized peptides (typically ~30-40 amino acid residues) (Mayo *et al.*, 2003). The GLP-1 receptor is coupled, functionally, to the adenylate cyclase (Drucker *et al.*, 1987) via the stimulatory G protein Gs (Mayo *et al.*, 2003; Thorens, 1992). In pancreatic beta-cells, the activation of the GLP-1 receptor leads to the increase in the cytosolic [cAMP] with a subsequent activation of the protein kinase A (PKA) and the cAMP-regulated guanine nucleotide exchange factor II (cAMP-GEFII, also known as Epac2) leading to a plethora of events (*e.g.* altered ion channel activity, intracellular calcium handling, and enhanced exocytosis of insulin-containing granules (Silva *et al.*, 2009)) that culminate in an enhancement of glucose-induced insulin secretion (for review see Holst & Gromada, 2004; Mayo *et al.*, 2003; Holst, 2007).

GLP-1 receptors were first found in pancreatic islets, stomach and lung (on rat and rat insulinoma cell-line, INS-1) (Thorens, 1992). In 2003, GLP-1 receptors were detected in hypothalamus and brain stem, heart and kidney, but not in liver, skeletal muscle or adipose tissue (Mayo *et al.*, 2003). More specifically, using fluorescence immunohistochemical microscopy, GLP-1 receptors have been selectively localized to beta-cells of the islet and pancreatic ducts, in a study on mice, rat and human tissues (Tornehave *et al.*, 2008).

2.4.1. THE GLP-1 RECEPTOR PHARMACOLOGY

Agonists for the GLP-1 receptor include GLP-1(7–37), GLP-1 (7–36)amide (K_d = 0.3 nM), the *Heloderma suspectum* peptides exendin-3 and exendin-4 (K_d = 0.1 nM) (naturally occurring Gila monster peptide from salivary secretion (Eng *et al.*, 1992)) and some labeled ligands (*e.g.* fluorescein-Trp25-exendin-4, ^{125}IGLP-1, and Tyr39-exendin-4). Structurally related members of the glucagon family such as GLP-2, glucagon, and GIP do not activate the GLP-1 receptor at physiologically relevant concentrations (Mayo *et al.*, 2003; Goke *et al.*, 1993).

Antagonists for the GLP-1 receptor include the truncated lizard peptide GLP-1 receptor antagonist exendin-(9–39) (K_d of 2.9 nM) (Goke *et al.*, 1993) and a small non-peptide ligand (T-0632), that binds the GLP-1 receptor within the micromolar range, exhibiting about ~100-fold selectivity for the human *versus* the homologous rat GLP-1 receptor (Tibaduiza *et al.*, 2001).

2.5. MAIN PHYSIOLOGICAL EFFECTS OF GLP-1

The main effects of GLP-1 are exerted on glucose homeostasis, gastrointestinal function, food intake and appetite. In the pancreatic islet of Langerhans (see Figure 3A) GLP-1 regulates glucose homeostasis by enhancing glucose-induced insulin secretion and inhibiting glucagon secretion. In beta-cells, binding of GLP-1 to GLP-1 receptors acutely activates cAMP cascade leading to enhanced glucose-induced depolarization (due to faster and greater closure of KATP channels and more intense electrical activity) (*e.g.* Holz *et al.*, 1993) and enhanced depolarization-induced insulin granule exocytosis (likely due to enhancement of the readily releasable pools of granules) (Silva *et al.*, 2009). Chronically, GLP-1 slows apoptosis and promotes proliferation of beta-cells leading to an increase in their mass (Xu *et al.*, 1999;

Mayo *et al.*, 2003; Holst & Gromada, 2004). In contrast GLP-1 inhibits glucagon secretion (Wettergren *et al.*, 1993), an effect that seems to be due to paracrine regulation of alpha-cells, in that the latter do not express GLP-1 receptors (Tornehave *et al.*, 2008). One possibility is that GLP-1 triggers the release of somatostatin from islet delta-cells, which in turn reduces the readily releasable pool of glucagon granules in neighboring alpha-cells.

In the gastrointestinal tract (see Figure 3B), GLP-1 inhibits gastrin-induced acid secretion in humans, as well as does the truncated GLP-1 (a naturally occurring peptide) being this more potent than GLP-1 (Schjoldager *et al.*, 1989). It decreases and delays gastric emptying rate by stimulating antral churning while inhibiting pyloric propulsion and duodenal peristalsis (Schirra *et al.*, 2006). It inhibits, significantly, the postprandial pancreatic secretion of trypsin and lipase, an effect that seemed to be secondary to gastric emptying as truncated GLP-1 did not affect the linear relationship that correlates pancreatic enzyme output to gastric emptying (Wettergren *et al.*, 1993).

Lastly, GLP-1 suppresses appetite either by reducing gastric emptying and inducing stomach fullness or by activating satiety centers on the arcuate nucleus of the hypothalamus or inhibiting the solitary tract nucleus of the brain stem (Holst, 2007).

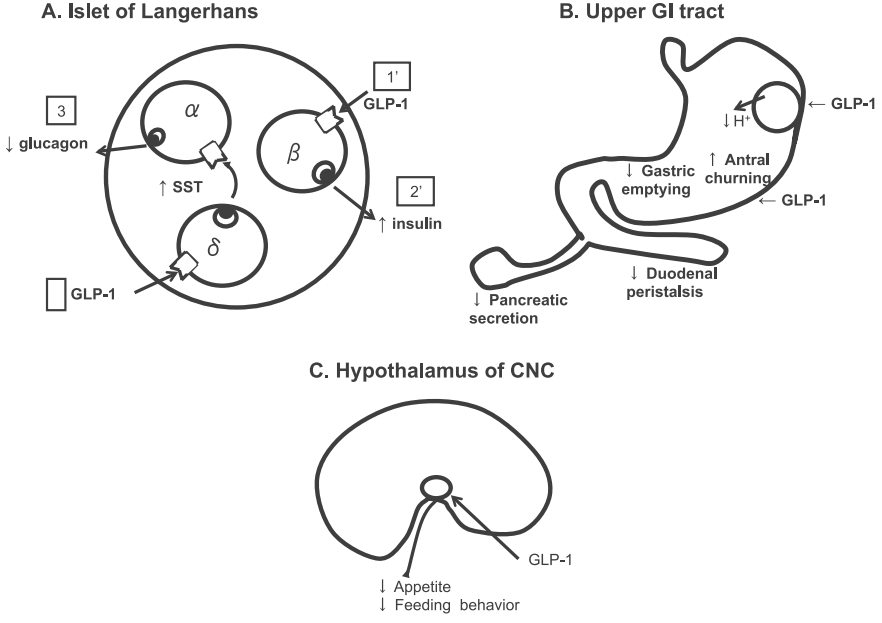

Figure 3. Actions of GLP-1 on target tissues: cells of islets of Langerhans (A), upper GI (gastrointestinal tract, stomach and duodenum) (B) and hypothalamus and brain stem of Central Nervous System (C).

3. THERAPEUTIC USE IN TYPE 2 DIABETES MELLITUS ASSOCIATED WITH OBESITY

The acute actions of GLP-1 to slow absorption of ingested glucose, "amplify" glucose-induced insulin secretion and inhibit glucagon secretion, combined with its chronic action to maintain beta-cell mass, suggest GLP-1 as a useful agent in the treatment of type 2 diabetes *mellitus* (T2-DM), where glucose insensitivity of both beta-cells and peripheral target cells produces chronic hyperglycemia and ongoing beta-cells injury. To overcome the impediment of very short (~5 min) circulating half-life of GLP-1, three new approaches are now in development / early clinical use: (i) GPL-1 agonists or mimetics = long acting recombinant GLP-1 analogues (*e.g.*, intravenous administered exenatide, a synthetic exendin-4); (ii) GLP-1 enhancers = inhibitors of DPP-IV (*e.g.*, orally administered sitagliptin); and (iii) stimulators of GLP-1 release by L-cells (AR231453, in trial). In addition, the appetite suppressing effects of GLP-1 might also treat obesity-related T2-DM, where chronically increased caloric intake, overstuffs adipocytes which chronically over-release free fatty acids (ffas). When taken up beta-cells and their peripheral targets ffas further contribute to progressive glucose-insensitivity and beta-cells failure. In therapeutic trials, twice daily subcutaneous administration of exenatide has produced 2-5 kg weight loss for up to 3 years (DeFronzo *et al.*, 2005).

4. CONCLUSIONS

Hence, by the various effects on beta-cells function, GLP-1, GLP-1 mimetics or enhancers, show improvements on glucose homeostasis in T2-DM when added to other oral hypoglycemic agents. Improved methods for administration of these agents may be the key to their expanded and more efficacious usage (Chia & Egan, 2008).

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