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Glucagon-like peptide-1 and glucagon-like peptide-2

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The glucagon-like peptides (glucagon-like peptide-1 (GLP-1) and glucagon-like peptide-2 (GLP-2)) are released from enteroendocrine cells in response to nutrient ingestion. GLP-1 enhances glucose-stimulated insulin secretion and inhibits glucagon secretion, gastric emptying and feeding. GLP-1 also has proliferative, neogenic and antiapoptotic effects on pancreatic β-cells. More recent studies illustrate a potential protective role for GLP-1 in the cardiovascular and central nervous systems. GLP-2 is an intestinal trophic peptide that stimulates cell proliferation and inhibits apoptosis in the intestinal crypt compartment. GLP-2 also regulates intestinal glucose transport, food intake and gastric acid secretion and emptying, and improves intestinal barrier function. Thus, GLP-1 and GLP-2 exhibit a diverse array of metabolic, proliferative and cytoprotective actions with important clinical implications for the treatment of diabetes and gastrointestinal disease, respectively. This review will highlight our current understanding of the biology of GLP-1 and GLP-2, with an emphasis on both well-characterized and more novel therapeutic applications of these peptides.

Key words: diabetes; obesity; food intake; intestinal disease; cell proliferation; apoptosis; insulin secretion.

Glucagon-like peptide-1 (GLP-1) is an incretin hormone that regulates blood glucose level through its combined actions on the stimulation of glucose-dependent insulin secretion and the inhibition of glucagon secretion, gastric emptying and food intake.[1] GLP-1 also increases β-cell mass via a stimulation of β-cell proliferation and neogenesis, and an inhibition of β-cell apoptosis.[2] The observation that the pharmacological

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administration of GLP-1 and related analogues can reduce elevated fasting and postprandial blood glucose levels in diabetic human subjects has generated intense interest in the development of GLP-1R agonist-based therapies for the treatment of diabetes mellitus.

GLP-2 is a 33 amino acid peptide that regulates energy homeostasis via acute and chronic effects on gut motility, and nutrient ingestion and absorption. In rodents, exogenous GLP-2 administration promotes the growth and survival of epithelial cells within the small and large bowel mucosa via an inhibition of apoptotic cell death and a stimulation of cellular proliferation.[1] GLP-2 also enhances barrier function and increases the resistance to and recovery from a variety of experimental models of gut injury.[1] The reported actions of GLP-2 have fostered the initiation of clinical studies to assess the ability of GLP-2 to improve nutrient absorption and epithelial integrity and restore functional bowel mass in human patients with intestinal disease.

The actions of GLP-1 and GLP-2 are mediated via unique G protein-coupled receptors. The GLP-2 receptor (GLP-2R) is expressed in a highly tissue-specific manner, predominantly in the gastrointestinal tract and brain.[3] In contrast, the GLP-1 receptor (GLP-1R) has a more widespread distribution and is expressed in a number of tissues, including the pancreas, intestine, stomach, central nervous system (CNS), heart, pituitary, lung and kidney.[4–6]

Despite the potential therapeutic benefits of GLP-1 and GLP-2, the durations of their action are limited owing to a rapid inactivation of these peptides by the ubiquitous protease dipeptidyl peptidase-IV (DPP-IV). Consequently, the inhibition of DPP-IV activity or the development of DPP-IV-resistant glucagon-like peptide analogues offers additional therapeutic options for treating human disease.

SYNTHESIS, SECRETION AND METABOLISM OF GLP-1 AND GLP-2

GLP-1 and GLP-2 are co-encoded within the proglucagon gene, which, in mammals, gives rise to a single mRNA transcript that is expressed in the α-cells of the endocrine pancreas, in the enteroendocrine L-cells of the intestine and in the hypothalamus and brainstem in the CNS.[7,8] The proglucagon mRNA is translated into a single 160 amino acid precursor protein that undergoes tissue-specific post-translational processing to produce several biologically active proglucagon-derived peptides (PGDPs), including glucagon in the pancreatic α-cells and glicentin, oxyntomodulin, GLP-1 and GLP-2 in the intestine and brain (Figure 1). Glucagon is the major counter-regulatory hormone to insulin and is essential for maintaining blood glucose levels in the physiological range during the post-absorptive state. Oxyntomodulin has inhibitory effects on gastrointestinal secretion and motility, and stimulatory effects on pancreatic enzyme secretion and intestinal glucose uptake.[9] More recently, it has been demonstrated that oxyntomodulin can reduce food intake in both rodents and humans.[10,11] In contrast, the physiological actions of glicentin are poorly defined. GLP-1 and GLP-2 exhibit trophic effects in the pancreas and intestine, respectively, and play important roles in the regulation of nutrient assimilation and energy homeostasis (see below).

The PGDP sequences within the proglucagon precursor are flanked by pairs of basic amino acids, and the post-translational processing of proglucagon is carried out by prohormone convertases, which are endoproteolytic enzymes that cleave C-terminal to paired basic amino acid residues.[12] Although the prohormone convertase enzymes responsible for the production of GLP-1 and GLP-2 in the CNS have not been

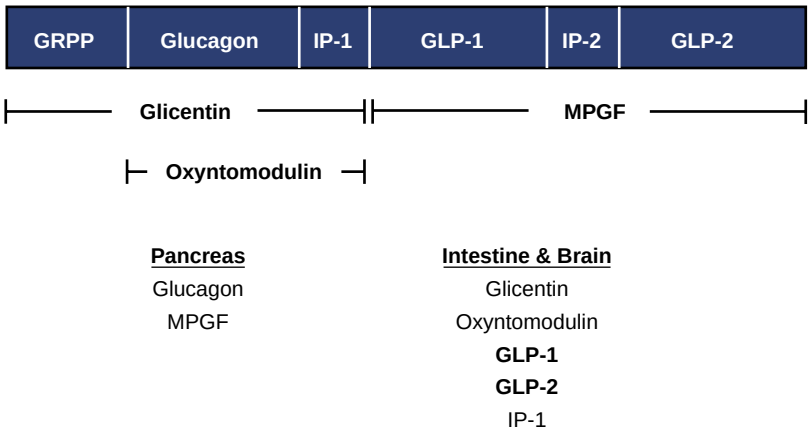

Figure 1. Mammalian proglucagon structure and tissue-specific post-translational processing of the proglucagon-derived peptides. GRPP, glicentin-related polypeptide; IP-1 and IP-2, intervening peptide-1 and -2; MPGF, major proglucagon fragment; GLP-1 and GLP-2, glucagon-like peptide-1 and -2.

definitively established, prohormone convertase 1/3 has been localized to intestinal L-cells and is both necessary and sufficient for the post-translational processing of proglucagon to GLP-1 and GLP-2.[13] In contrast, prohormone convertase 2 is expressed in the islet α-cell and is essential for the generation of 29 amino acid pancreatic glucagon.[14]

GLP-1 and GLP-2 are secreted in a 1:1 ratio from intestinal L-cells[15], the majority of which are located in the distal ileum and colon.[16] The major stimulus for GLP-1 and GLP-2 secretion is the ingestion of nutrients, including glucose, fatty acids and dietary fibre.[17] Fasting plasma levels of the biologically active forms of GLP-1 and GLP-2 in healthy humans are 5–10 and 15–20 pM, respectively, and increase 2–5-fold following food ingestion, the absolute peak level being dependent on the size and nutrient composition of the meal.[18,19] When nutrients are ingested, the release of GLP-1 and GLP-2 into the circulation occurs in a bi-phasic manner, consisting of a rapid (within 10–15 minutes) early phase followed by a more prolonged (30–60 minutes) second phase.[20] The distal location of most L-cells that produce GLP-1 and GLP-2 makes it unlikely that the rapid nutrient-stimulated increase in plasma levels of these peptides is due to a direct effect of nutrients on the L-cell. Indeed, studies in rodents and humans clearly indicate that the vagus nerve, the neurotransmitter gastrin-releasing peptide and the hormone glucose-dependent insulinotropic peptide all contribute to the rapid release of GLP-1 and GLP-2 from distal L-cells in response to nutritional stimuli.[17] In contrast, the second phase of peptide secretion probably results from a direct stimulation of the L-cell by digested nutrients.[21] Thus, nutrient-induced stimulatory signals are transmitted to intestinal L-cells indirectly, via neural and endocrine effectors, and also by direct interaction with these cells, to mediate the first and second phase, respectively, of GLP-1 and GLP-2 secretion.

The half-life of circulating biologically active GLP-1 is less than 2 minutes[22], whereas GLP-2 is more stable, with a half-life of approximately 5–7 minutes.[23,24] The relatively short circulating half-lives of the bioactive forms of these peptides can be attributed to renal clearance and enzymatic inactivation. DPP-IV, a serine protease that cleaves dipeptides from the amino terminus of oligopeptides or proteins that have a proline or

alanine residue in the penultimate position[25], is a critical determinant of GLP-1/GLP-2 degradation. DPP-IV cleaves GLP-1 and GLP-2 at the alanine residue in position 2, yielding the inactive peptides GLP-1 (9–37/36NH_2) and GLP-2 (3–33). DPP-IV expression is fairly widespread and is found on the surface of circulating white blood cells and in cells constituting the vascular endothelium of the small intestine, adjacent to the sites of GLP-1 and GLP-2 secretion.[25,26] Thus, the majority of GLP-1 and GLP-2 entering the portal circulation has already been inactivated by DPP-IV prior to entry into the systemic circulation. The kidney provides the major route of clearance for both GLP-1 and GLP-2[27], and patients with uraemia or chronic renal insufficiency have elevated levels of circulating GLP-1 relative to healthy control individuals.[28,29]

GLP-1 AND GLUCOSE HOMEOSTASIS

GLP-1 elicits multiple actions in the pancreas and in extra-pancreatic tissues that lead to the reduction of blood glucose (Figure 2). The first physiological action to be described for GLP-1 was the augmentation of glucose-stimulated insulin secretion.[30–32] GLP-1 binds to its specific receptor on the pancreatic β-cell and stimulates insulin secretion through mechanisms that involve an inhibition of ATP-sensitive K^+ channels (K_{ATP}) and subsequent β-cell depolarization, elevations in intracellular Ca^{2+} level, an inhibition of voltage-dependent K^+ channels and direct effects on the β-cell exocytotic machinery.[33,34] The intracellular signalling events that modulate GLP-1-regulated insulin release include activation of the cAMP/protein kinase A (PKA), cAMP/guanine-nucleotide exchange factor and phosphatidylinositol-3 kinase (PI-3K)/protein kinase C (PKC)ζ pathways.[34–36] Unlike other insulin secretagogues, GLP-1 also promotes insulin gene transcription, mRNA stability and biosynthesis, and thus has the capacity to replenish depleted β-cell insulin stores.[37,38]

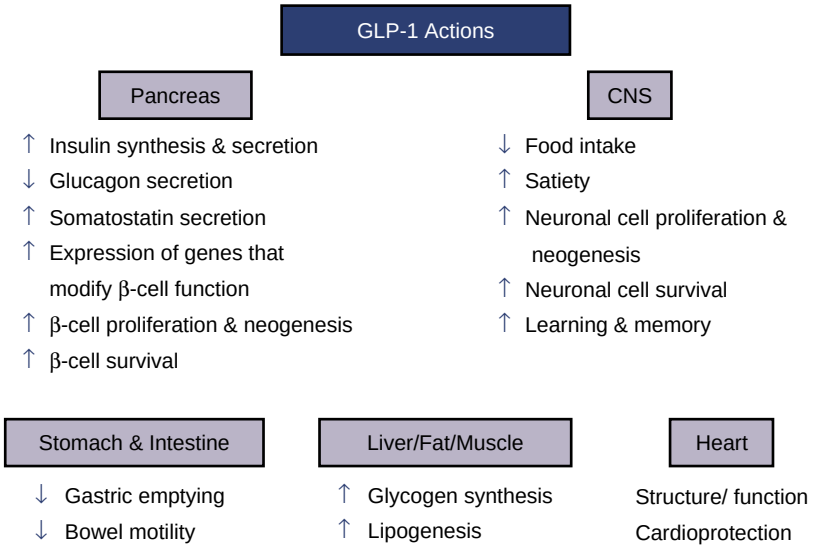

Figure 2. Pancreatic and extra-pancreatic glucagon-like peptide-1 receptor agonist-dependent actions. CNS, central nervous system.

In addition to its ability to stimulate glucose-dependent insulin secretion by direct interaction with the pancreatic β-cell GLP-1R, GLP-1 may also stimulate insulin secretion indirectly via neural mechanisms. It has been estimated that more than half of the GLP-1 secreted from intestinal L-cells is inactivated by DPP-IV, while the majority of the remaining intact peptide is inactivated as it passes through the liver.[26,39] It is thus likely that only small amounts of bioactive GLP-1 actually reach the pancreas intact. Studies using ganglionic blockers in rats or chemical denervation experiments in mice following capsaicin treatment suggest that endogenously released GLP-1 can stimulate insulin secretion in part by a sensory neural reflex that probably initiates in the hepatoportal system.[40,41]

GLP-1 can also increase the steady-state levels of mRNA transcripts for key components of the molecular machinery involved in β-cell exocytosis, such as the sulphonylurea receptor and the inwardly rectifying potassium channel (Kir 6.2), molecular subunits of the β-cell K_{ATP} channel. Similarly, GLP-1 also regulates β-cell K_{ATP} channel function via the prevention of glucose-dependent inhibition of K_{ATP} channel activity.[42]

Studies in both rodents and humans reveal that GLP-1 can improve the ability of the β-cell to sense and respond to glucose, thereby conferring glucose sensitivity to previously resistant β-cells.[43,44] A potential mechanism whereby GLP-1 could restore β-cell glucose responsivity is suggested by studies demonstrating that GLP-1 can upregulate the expression of glucose transporters and glucokinases, components of the β-cell glucose sensor.[45,46]

GLP-1 also lowers blood glucose levels by inhibiting the secretion of glucagon.[47] The inhibitory effects of GLP-1 on glucagon secretion may occur through direct interaction with GLP-1 receptors on pancreatic α-cells[48] or indirectly through GLP-1-mediated stimulation of insulin and/or somatostatin secretion.[49]

Both animal and human studies demonstrate that GLP-1 delays gastric emptying and intestinal motility, thereby slowing the transit of nutrients from the stomach to the small intestine and attenuating meal-associated elevations in plasma glucose levels.[50,51] The inhibitory effects of GLP-1 on the gut are likely to involve both CNS- and intestinal-derived GLP-1.[52] The mechanism whereby peripheral GLP-1 inhibits gastrointestinal motility appears to involve either direct interaction with CNS centres that regulate visceral motility or an indirect mechanism via vagal afferent pathways.[52]

GLP-1 may also regulate glucose disposal through peripheral actions on liver, skeletal muscle and adipose tissue. GLP-1 has been shown to increase glucose incorporation into glycogen in isolated rat hepatocytes and skeletal muscle[53], and enhance insulin-stimulated glucose metabolism in 3T3 L1 adipocyte cultures and isolated rat adipocytes.[54,55] However, subsequent studies have failed to support a direct extra-pancreatic role for GLP-1 in liver, muscle or adipose tissue.[56,57] Whether GLP-1 has direct effects on glucose disposal independent of changes in the levels of islet hormones in humans remains unclear. A number of studies in healthy and diabetic humans suggest that GLP-1 can increase glucose disappearance, independent of insulin and glucagon[58–60]; in contrast, other studies indicate that GLP-1 has no direct influence on glucose disposition.[61–65] Recent evidence suggests that the effects of GLP-1 may involve a suppression of endogenous glucose production rather than an increase in peripheral glucose disposal.[66] The mechanism whereby GLP-1 could mediate these extra-pancreatic effects in humans, independent of changes in the insulin:glucagon ratio, remains uncertain. There is no consistent evidence from rodent or human studies that GLP-1 receptors are present in liver, fat or muscle tissues.[67–71] Since GLP-1R binding,

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