Glucagon-like peptide 1: a new promising treatment for type 2 diabetes

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Glucagon-like peptide 1 (GLP-1) is a gut-derived peptide hormone secreted from intestinal endocrine L-cells after a meal. GLP-1 is a powerful incretin hormone: it increases insulin response to oral glucose, and thus constitutes a potential target for the treatment of type 2 diabetes. The biological actions of GLP-1 have several insulinotropic effects including potentiation of glucose-stimulated insulin secretion, enhancement of pancreatic β-cell growth, and inhibition of glucagon release. GLP-1 also inhibits food intake and gastric emptying. Unlike other incretin hormones, GLP-1 retains its insulinotropic effect when administered to persons with type 2 diabetes. These antidiabetic effects of GLP-1 have led to intense interest in the use of the GLP-1 signaling system as a mechanism for the treatment of patients with type 2 diabetes. The GLP-1 receptor agonist Exendin-4 is now approved for the treatment of type 2 diabetes in U.S.A., and several degradation resistant GLP-1 analogues as well as agents that retard the degradation of native GLP-1 are in the clinical trials. In this mini review we summarize the recent advances in our understanding of the biology of GLP-1.

INTRODUCTION

Diabetes is a chronic disease that occurs when the pancreas does not produce enough insulin, or alternatively, when the body cannot effectively use the insulin it produces. The World Health Organization (WHO) estimates that more than 180 million people worldwide have diabetes. This number is likely to more than double by 2030. There are two types of diabetes: type 1 and type 2 diabetes. Type 1 (also known as insulin-dependent Diabetes) is characterized by a lack of insulin production. Without daily administration of insulin, type 1 diabetes is rapidly fatal. Type 2 diabetes (also called non-insulin-dependent diabetes) results from the body’s ineffective use of insulin. Type 2 diabetes accounts for 85-90% of patients with diabetes mellitus around the world, and is largely the result of excess body weight and physical inactivity. WHO projects that diabetes related deaths will increase by more than 50% in the next 10 years without urgent action. Most notably, diabetes deaths are projected to increase by over 80% in upper-middle income countries between 2006 and 2015. Patients with diabetes have approximately a threefold increased risk for all cardiovascular diseases and their relative risk of death from all causes is increased by 75%. Type 2 diabetes mellitus is characterized by insulin resistance and pancreatic beta-cell dysfunction, which lead to high blood glucose concentrations. Loss of metabolic control is also attributed to defects in neuroendocrine control systems that affect hepatic glucose output, gastrointestinal motility, peripheral insulin sensitivity, and energy expenditure. An increased incidence of vascular complications is the leading cause of morbidity and premature mortality associated with type 2 diabetes (http://www.who.int/en/). Diet and/or exercise, oral anti-diabetic agents, and insulin are

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currently used to correct hyperglycaemia in patients with type 2 diabetes. The most commonly prescribed treatments for type 2 diabetes are sulfonylurea (increase insulin secretion), metformin (increases insulin sensitivity and decreases glucose production by the liver.), and thiazolidinediones (improve insulin sensitivity). These agents are effective for the treatment of type 2 diabetes, however, many of them have limiting side effects including weight gain and hypoglycaemia. These clinical shortcomings highlight the need for alternative interventions in the long-term treatment of type 2 diabetes. A new class of drugs based on the Glucagon-like peptide-1 (GLP-1) system is showing remarkable results in correcting hyperglycaemia in patients with type 2 diabetes without risk of hypoglycaemia or weight gain. It has long been known that circulating insulin levels are significantly higher after oral glucose ingestion than when glucose is administered intravenously. Subsequently, factors released from the gastrointestinal (GI) tract that regulate insulin secretion were identified and termed incretins [1]. Currently, there are two known incretin hormones: Glucose-dependent insulintropic polypeptide (GIP) and Glucagon-like peptide-1 (GLP-1). GIP is produced by endocrine K cells localized in the proximal small intestine, while GLP-1 is produced by endocrine L cells localized in the distal ileum and colon. GIP and GLP-1 act on specific G-protein coupled receptors that are expressed in pancreatic islets to enhance glucose stimulated insulin secretion [1]. Although, GIP and GLP-1 share similar beneficial effects on glucose homeostasis, GIP was ineffective in enhancing insulin secretion in diabetic patients while the effect of GLP-1 was preserved in these patients [2,3]. Conversely, GLP-1 was found to be very effective in correcting hyperglycaemia in diabetic patients. Currently, a GLP-1 receptor agonist (exendin 4) has been approved for the treatment of type 2 diabetes and several products affecting the GLP-1 system are currently in different stages of clinical trials for the treatment of type 2 diabetes [4].

**GLP-1 synthesis and secretion**

The proglucagon gene is expressed and transcribed into proglucagon (117 amino acid precursor protein) in both pancreatic alpha cells and endocrine L cells of the distal ileum and colon [5]. Proglucagon is differentially processed into active peptides in alpha cells and L cells (Figure 1). The tissue specific processing of proglucagon is dictated by the differential expression of prohormone convertase isoforms. Pancreatic alpha cells express the prohormone convertase 2 (PC2) that cleaves the proglucagon molecule into glucagon, glicentin-related pancreatic peptide (GRPP), and the major proglucagon fragment. Enteroendocrine L cells express the prohormone convertase 2 (PC2) that cleaves the proglucagon molecule into glucagon, glicentin-related pancreatic peptide (GRPP), and the major proglucagon fragment. Enteroendocrine L cells express the prohormone convertase 1 (PC1) that cleaves the proglucagon molecule into GLP-1 and GLP-2 as well as glicentin and oxyntomodulin (Figure 1) [5-7]. Enteroendocrine L cells that synthesize and secrete GLP-1 are localized in the distal small intestine and colon. The L cell is an open-type intestinal epithelial endocrine cell that directly contacts luminal nutrients through its apical surface (Figure 2). The principal physiological stimulus of GLP-1 secretion is food ingestion [5,
Among nutrients, fat and glucose were found to be the potent stimulators of GLP-1 secretion [5,8,9]. GLP-1 secretion in response to nutrient intake is biphasic, with an initial rapid increase in circulating GLP-1 occurring 15-30 min after a meal, followed by a second smaller peak at 90-120 min [11].

Figure 2: Immunofluorescence staining using GLP-1 specific antibody in mouse distal ileum and visualized by fluorescence microscopy, showing enteroendocrine L cells (green).

Since GLP-1 cells are localized in the distal part of the small intestine, the rapid increase in plasma GLP-1 after food ingestion (15-30 min) is not initiated by the direct effect of nutrients on L cells. It was suggested that the initial peak of postprandial GLP-1 secretion is mediated by an indirect neuro/endocrine pathway, rather than through direct contact of the luminal contents with L-cells [11]. The role of the vagus nerve as an important mediator of postprandial GLP-1 secretion has been established by studies in rats in which bilateral subdiaphragmatic vagotomy completely blocked fat-induced GLP-1 secretion [11] indicating the importance of the vagus nerve in relaying nutrient-induced GLP-1 secretion signals. The role of muscarinic receptors in nutrient-stimulated GLP-1 secretion was reported in both humans and rodents. In humans, administration of atropine, a non-specific muscarinic-receptor antagonist, reduced glucose-stimulated GLP-1 secretion [8,12]. Similarly, we demonstrated that in rats, atropine and pirenzepine (M1 muscarinic-receptor antagonist) completely inhibit fat-induced GLP-1 secretion [8,12]. Using primary fetal rat intestinal cells (FRIC) in culture, we demonstrated that M1 and M2 muscarinic-receptor agonists stimulate GLP-1 secretion (8, 12). The same results were also found using a human L cells line [8,12] indicating the importance of muscarinic receptors in the early phase of GLP-1 secretion. GRP is also a potent stimulator of intestinal secretion in vitro and in vivo in rodents and in humans [13]. The second phase of GLP-1 secretion occurs 90-120 min after nutrients ingestion and is likely caused by direct contact of nutrients with intestinal L cells [14,15]. Circulating GLP-1 levels are reduced in obese individuals (16-18). Since obesity is accompanied by an increase in the adipocyte derived hormone leptin, we hypothesized that leptin may be involved in regulating GLP-1 secretion. We demonstrated that the leptin receptor (Ob-Rb) is expressed in rodent and human L cells (19). Administration of leptin to rats and mice in vivo stimulated GLP-1 secretion, and leptin was also able to stimulate GLP-1 secretion from a human L cell line in vitro through an increase in Signal Transducer and Activator of Transcription-3 (STAT-3) phosphorylation. In mice, leptin resistance induced by a high-fat diet is associated with reduced basal and nutrition-induced GLP-1 secretion (19). These results established for the first time the existence of an adipose-entero-insular axis, and that leptin resistance occurs also at the level of the L cell. Several studies have shown that GLP-1 levels are reduced in obese patients as well as in some patients with type 2 diabetes [5,10]. More studies are needed to elucidate the mechanisms regulating GLP-1 secretion in healthy and diabetic subjects. Understanding these mechanisms will allow the enhancement of GLP-1 secretion in type 2 diabetic patients as mean to correct their hyperglycaemia. The predominant GLP-1 circulating form is GLP-1 (7-36)NH2 which is the bioactive form, and The half life of GLP-1...
in the circulation is very short (1-2 min, [20]).
GLP-1 is very quickly inactivated by the
removal of the two first amino acids from the
amino terminus, by the proteolytic enzyme
dipeptidyl peptidase-IV (DPP-IV) (Figure 3)
[20].

**Biological actions of GLP-1**

The GLP-1 receptor belongs to the 7-
transmembrane-spanning, heterotrimeric G-
protein coupled family of receptors [21].
Activation of GLP-1 receptor can lead to
increases in Ca2+, adenylate cyclase,
phospholipase C, and activation of Protein
kinase A. Protein kinase C and
phosphatidylinositol-3 kinase [22]. Exendin 4 is
a naturally occurring component of the saliva of
Gila monster (*Heloderma suspectum*). It has
53% homology with mammalian GLP-1 and has
a very high affinity for the GLP-1 receptor.
Exendin 4 is resistant to DPP-IV because of a
key difference in amino acid sequence: the
glycine at position 2 instead of the alanine [23].

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Its long half life and its high affinity to the GLP-
1 receptor made Exendin-4 an ideal candidate to
use for the treatment of type 2 diabetic patients
(Figure 3).

**Effects on pancreatic beta cells:**

Administration of GLP-1 to animals and humans
increases insulin secretion when blood glucose
increases above basal levels. The effect of GLP-
1 on insulin secretion was observed using
concentrations similar to those observed in the
circulation after food ingestion, and it is
assumed that this represents a physiological
action of GLP-1. GLP-1 receptor is expressed in
pancreatic beta cells and its activation induces
insulin dependent insulin secretion. GLP-1
induces the activation of adenylate cyclase and
subsequent production of cAMP [24]. Activation
of the GLP-1 receptor in beta cells interferes
with different pathways that lead to the
potentiation of glucose-induced insulin
secretion. GLP-1 induces a direct inhibition of
K-ATP channels, which leads to beta cell
membrane depolarization, increases in
intracellular Ca2+ levels, increases
mitochondrial ATP [25]. GLP-1 has also a direct
effect on beta cell insulin storage granules [25].
GLP-1 also stimulates insulin gene transcription,
mRNA stability, and biosynthesis in a glucose
dependent manner. GLP-1 receptor agonists
maintain beta cell insulin stores and secretory
capacity by increasing glucose-induced insulin
biosynthesis at the transcriptional level [25].
GLP-1 was found to enhance glucose sensitivity
to glucose-resistant beta cells by up-regulating
the expression of glucose transporters and
glucokinases, key molecules of the beta cell
glucose sensor [25]. Recently, GLP-1 was found
to stimulate beta cell proliferation and
neogenesis and inhibit beta cell apoptosis,
resulting in an increase in beta cell mass [26].
Treatment of pancreatic ductal cell lines with
GLP-1 or GLP-1 receptor agonists promotes
their conversion into islets like cells.
Experiments in rodent models as well as *in vitro*
studies in beta cell lines demonstrate an increase
of beta cell mass after long-term administration
of GLP-1 or GLP-1 receptor agonists due to a
stimulation of islet cell neogenesis from
precursor cells and to an inhibition of apoptosis
of beta cells [25]. Beta cell mass cannot be
directly quantified without invasive surgical
techniques in humans. However, the restoration
of some β-cell function parameters can be
detected indirectly from the increased insulin
secretory capacity in humans receiving GLP-1.
In isolated human islets, glucose-dependent
insulin secretion and islet cell morphology is
significantly improved, when the islets are
incubated with GLP-1. The reason for the
expansion of β-cell mass by GLP-1 is due to the
inhibition of apoptotic signaling pathways and
the stimulation of signaling pathways leading to
a proliferation of β-cells [25]. The mRNA levels
for Bcl-2 and caspase 3 (markers for apoptotic
activity) are down regulated in human islets
incubated with GLP-1 [27].

**Effect on glucagon secretion:**

In type 2 diabetes, excessive glucagon secretion
in relation to the plasma glucose stimulates
hepatic glycogenolysis and therefore contributes
to fasting hyperglycemia. GLP-1 was found to
inhibit glucagon secretion *in vitro* and *in vivo*. In
type 2 diabetic patients, infusions of GLP-1 lead
to a significant suppression of glucagon
secretion together with a normalization in fasting plasma glucose. GLP-1 administration however, does not impair the glucagon counter-regulatory response to hypoglycemia [1].

**Effect on food intake**

Rodent studies demonstrate that central or peripheral administration of GLP-1 and Exendin 4 reduce short-term food and water intake and decreases body weight [1]. GLP-1 receptors are densely expressed in the subfoveal organ and the area postrema, which have close neuroanatomical connections with hypothalamic areas involved in appetite control [28,29]. In the area postrema, the blood-brain barrier is “leaky” and allows small peptides from the periphery access to the brain. This is consistent with the potential role of circulating GLP-1 in the central regulation of appetite. Conversely, GLP-1R agonists could modify food intake indirectly by virtue of their ability to inhibit gastric emptying [1]. In humans, a continuous subcutaneous infusion of GLP-1 over six weeks lead to significant weight loss due to reduced caloric intake attributable to increased feelings of satiety [31]. Whether the effects of GLP-1 on satiety in humans are mainly mediated by the retardation of gastric emptying through a feedback loop or are centrally mediated is still under investigation.

**GLP-1 and the treatment of type 2 diabetes**

Recent studies have indicated that GLP-1 secretion is impaired in type 2 diabetic patients. Exogenous administration of GLP-1 is effective in patients with type 2 diabetes, increasing insulin secretion and normalizing both fasting and postprandial blood glucose when given as a continuous intravenous infusion, even in subjects with advanced type 2 diabetes long after sulfonylurea secondary failure [31]. The multiple actions of GLP-1 constitute a new and attractive therapeutic alternative for the treatment of type 2 diabetes by improving the postprandial metabolic situation and eliminating hypoglycemic events. The risk of hypoglycemia observed in patients treated with GLP-1 is minimal because GLP-1 only stimulates insulin secretion under hyperglycemic conditions.

**Figure 3:** GLP-1 (7-36) amide is the active circulating form of GLP-1. GLP-1 (7-36) is very quickly inactivated by the removal of the two first amino acids by the proteolytic enzyme dipeptidyl peptidase-IV (DPP-IV) generating an inactive form of GLP-1 (GLP-1 (9-36)) (A). Exendin 4 is a naturally occurring component of the saliva of Gila monster (*Heloderma suspectum*). It has 53% homology with mammalian GLP-1 and has a very high affinity for the GLP-1 receptor. Exendin 4 is resistant to DPP-IV because of a key difference in amino acid sequence: Glycine at position 2 instead of Alanine (B). Exendin 4 constitutes a promising treatment for type 2 diabetes.

**Figure 4:** Glucagon like peptide 1 (GLP-1) is synthesized and secreted by enteroendocrine L cells localized in the distal ileum, after nutrient ingestion. GLP-1 acts on pancreatic beta cells to enhance glucose-stimulation of insulin secretion. It also increase beta cell mass. GLP-1 acts on the stomach to decrease gastric emptying and on the hypothalamus to reduce food intake. The multiple actions of GLP-1 constitute a new and attractive therapeutic alternative for the treatment of type 2 diabetes by improving the postprandial metabolic situation and eliminating hypoglycaemic events. GLP-1 related therapies
provide new as well as complementary options for the treatment of type 2 diabetes.

Additionally, intravenous infusions of GLP-1 are able to normalize plasma glucose in patients with type 2 diabetes. Furthermore, hepatic glucose production is lowered by GLP-1 due to the inhibition of glucagon secretion. A 6-week continuous subcutaneous infusion of GLP-1 significantly decreased glucagon levels and improved glycemic control, HbA1c decreased by 1.3%. Interestingly, the patients treated with GLP-1 lost approximately 2 kg in weight [20]. The very short half life of native GLP-1 has lead to the development of DPP-IV-resistant peptides that bind to the GLP-1 receptor and show GLP-1-like biological effects (GLP-1 mimetic) or substances inhibiting the DPP-IV. Long acting GLP-1 analogues as well as several DPP-IV inhibitors are now tested in clinical trials or already being introduced into clinical practice for the treatment of type 2 diabetes [1,32,33]. In comparison to DPP-IV inhibitors, GLP-1 mimetic agents have more pharmacological specificity but require subcutaneous injections. In conclusion, GLP-1 related therapies, whether they function to increase endogenous GLP-1 levels (DPP-IV inhibitors) or elicit actions similar to GLP-1 (GLP-1 mimetics) provide new as well as complementary options for patients with type 2 diabetes.

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