

Biological actions of GLP-1 analogues: Recent advancements and development

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Glucagon-like peptide-1 (GLP-1) an incretin released from the gut stimulates insulin secretion in response to food ingestion. GLP-1 acts via its G protein-coupled receptor to produce its biological actions. The GLP-1 and its receptor have been characterized in pancreatic islets, brain, heart and the gastrointestinal tract. GLP-1 not only regulates insulin secretion but also functions as a neuropeptide in the brain to modulate appetite, gastrointestinal tract and cardiovascular system. Because of its ability to regulate insulin secretion, a GLP-1 receptor agonist, exenatide was approved for the treatment of type 2 diabetes mellitus. Newer GLP-1 analogues like liraglutide, taspoglutide, albiglutide, BMS-686117, GSK-716155, AVE-0010 and others are being developed with good safety profile, long duration of action, with potential to decrease body weight and blood pressure. GLP-1 analogues are likely to become an important class of drug to treat type 2 diabetes mellitus.

Introduction

Glucagon-like peptide-1 (GLP-1) is a 30-amino acid peptide produced in the intestinal epithelial endocrine L-cells by differential processing of proglucagon gene expressed in these cells. GLP-1-producing L-cells have been recognized in the jejunum, ileum and colon, with ileum and colon being claimed to have highest cell densities¹. The mammalian proglucagon gene encodes two glucagon-like peptides (GLPs), GLP-1 and GLP-2 that exhibit approximately 50% amino acid identity to pancreatic glucagon². In the periphery, GLP-1 occurs as a truncated and amidated form, GLP-1₇₋₃₆. These peptide hormones first came into the picture to explain the larger and longer-lasting insulin response after oral compared to intravenous glucose administration in normal humans. An array of activities accounted to GLP-1 include augmentation of insulin response, lowering of glucagon levels, delaying of gastric emptying, stimulation of biosynthesis of (pro)insulin, and reduction in food intake upon intracerebroventricular (ICV) administration in animals^{2, 3}.

GLP-1 is emerging as a regulatory factor with widespread actions allied to substrate and energy metabolism. With the recent advent of medications based on GLP-1 receptor signaling for treatment of diabetes, further application of

this system for the treatment of other conditions such as cardiovascular disease open up the new avenues in drug development.

A. Secretion, Regulation and Degradation

GLP-1, an incretin hormone is now extensively portrayed as vital exponent of glucose homeostasis. It is released primarily in response to food intake which causes a rapid increase in L-cell secretion. Escalation in plasma levels of GLP-1 is detectable within 10 min of meal intake, and persists for up to several hours, depending on both the nutritional composition of the meal and the calorific value of the nutrients. Under fasting condition concentration in plasma is very low but a measurable basal level is always maintained in body^{2,3}.

Although relation between meal intake and GLP-1 release from L-cells of intestine has been well established, little is known about the mechanisms explaining influence of various nutrients on GLP-1 secretion. Sugiyama *et al.* have shown that blockade of the luminal sodium/glucose cotransporter, SGLT-1, in canine ileum with phlorizin inhibited GLP-1 secretion, signifying that absorption of glucose may be a critical requirement⁴. A second school of thought suggests the involvement of neurohormonal mechanisms in the rapid postprandial onset of secretion^{5,6,7}. Experimental evidences both *in vivo* and *in vitro* exist to underscore the role of glucose-dependent insulinotropic peptide (GIP). It has been shown in rats that GIP stimulates GLP-1 secretion via activation of a neural pathway which involves the vagus nerve⁵. Isolated perfused rat ileum and colon demonstrated

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stimulated GLP-1 secretion when administered with muscarinic cholinergic agonists⁶.

The half-life of GLP-1 is very short, owing to extremely rapid proteolytic cleavage, producing an inactive or even antagonistic fragment, GLP-1₉₋₃₆ amide. The enzyme dipeptidyl peptidase IV (DPP IV)^{9,36} metabolizes GLP-1 at such a fast rate that the hormone is inactivated sooner than it escapes out of the gut. The rapid DPP IV-mediated inactivation of GLP-1 can be exploited fruitfully to prolong GLP-1 biological half life leading to sustained lowering of blood glucose *in vivo*. Diabetic rodents are shown to have improved glucose tolerance with DPP IV inhibitors^{8,9}. In addition to DPP IV, GLP-1 is also a substrate for another enzyme, neutral endopeptidase².

B. Physiological actions

One of the core functions of GLP-1 is to act as an incretin hormone i.e. to stimulate insulin secretion and to inhibit glucagon secretion, thereby collaborating with other hormones of plasma glucose regulation to limit postprandial glucose excursions. The other diverse actions of GLP-1 include the proliferation, differentiation, and protection from apoptosis of pancreatic cells and the induction of satiety. GLP-1 also improves learning, stimulates afferent sensory nerves, and has neuroprotective functions. Furthermore, GLP-1 receptors have been reported to have cardiac and vascular actions in rodents and humans that include effects on contractility, blood pressure, cardiac output, and cardioprotection.

GLP-1 receptors: The GLP-1 receptor is widely distributed in pancreatic islets, brain, heart and the gastrointestinal tract. The receptor was first cloned by Bernard Thorens (1992) and it has now been established to be a G protein-coupled receptor. It was subsequently found that the GLP-1 receptor belongs to the same family as the glucagon receptors. GLP-1 receptor activation elevates the levels of intracellular cAMP via a stimulatory G protein^{10,11}. Nearly all of the actions of GLP-1 are derived from the formation of cAMP by adenylate cyclase. Elevated levels of intracellular cAMP result in the activation of protein kinase A and the cAMP-regulated guanine nucleotide exchange factor II (cAMP-GEFII, also known as Epac2). Consequence of this is a plethora of events including altered ion channel activity and elevation of intracellular calcium concentrations in the target cells¹².

Despite numerous attempts made to identify subtypes of GLP-1 receptors, all the organs whether the brain, the stomach, or the pancreas have been found to express a single GLP-1 receptor¹³. However, Lankat-Buttgereit *B et al.* in an experiment involving molecular cloning of a cDNA encoding for the GLP-1 receptor expressed in rat lung, showed that the pulmonary receptor differ from the islet receptor with respect to electrophoretic mobility¹⁴. However,

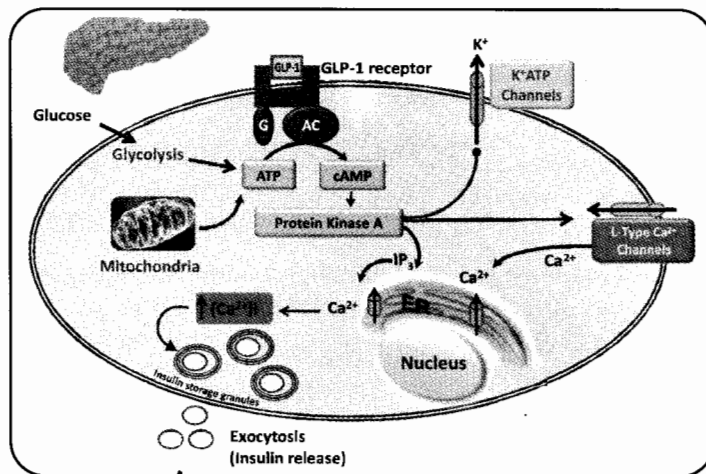


Fig 1 Cellular actions of GLP-1 that lead to stimulation of insulin secretion

since the primary sequence was identical, it was concluded that glycosylation patterns might differ. Ramifications of such differences are still alien to the researchers.

Pancreas: The effects of GLP-1 on pancreas can be divided into two distinct categories: first, effects on the pancreatic secretions, insulin in particular and second, effect on pancreatic cells.

GLP-1 stimulates glucose-dependent release of insulin via increasing the levels of cAMP in β cell of islets of pancreas and insulin gene transcription¹⁵. Uniqueness of GLP-1 in this regard derives from the fact that contrasting to other hypoglycemic agents such as the sulfonylureas, β cell GLP-1 receptor signaling is dependent on glucose. Acting in concert with glucose, GLP-1 facilitates membrane depolarization resulting in the closure of ATP-sensitive K^+ (K^+ ATP) channels. This enhances Ca^{2+} influx and exocytotic release of insulin¹⁶. Till now, sufficient clinical data have been generated to support the insulin releasing effect of GLP-1. After 30 weeks of therapy with exenatide, a GLP-1 agonist, 10 mcg twice daily, insulin secretion rates were shown to increase by up to 72%¹⁷. Whether the improvements observed in clinical studies are the direct effects of GLP-1 agonists on beta-cell function or are a result of improved glycemia remains to be elucidated.

The specific functions of GLP-1 in pancreatic cells include proliferation, differentiation and inhibition of apoptosis of cells. In neonatal diabetic rats with partial pancreatectomy, administration of GLP-1 or exendin-4 for 10 days stimulated expansion of cell mass via islet neogenesis and stimulation of islet proliferation¹⁸. In addition, exendin-4 proved to be protective against cytokine-induced apoptosis in the pancreatic beta cell line INS-1¹⁹. A striking demonstration of antiapoptotic actions of GLP-1 in beta-cell was provided by Li *et al.* who were able to demonstrate that GLP-1 receptor knockout mice were highly susceptible to beta-cell apoptosis induced by streptozotocin. Treatment with

the GLP-1 agonist exendin 4 reduced streptozotocin-induced beta-cell apoptosis in mice²⁰. GLP-1 mediated stimulation of islet cell proliferation occurs via a PI3-kinase-dependent pathway²¹.

Food intake: GLP-1 also appears to be a physiological regulator of appetite and food intake. It regulates nutrient consumption via effects on gastrointestinal motility and secretion and short-term regulation of feeding behavior²². ICV administration of GLP-1 resulted in decreased food intake in rodents whereas when the GLP-1 antagonist exendin₉₋₃₉ was administered food intake was stimulated². Coinciding effects are seen in humans, with decreased secretion of GLP-1 leading to the development of obesity and exaggerated secretion resulting in postprandial reactive hypoglycemia. However, the detailed mechanism underlying this effect is largely unknown. It has been hypothesized that communication with sensory neurons in the gastrointestinal tract or the hepatoportal bed may be involved³. There are many studies which contradict the role of GLP-1 in food intake and satiety and suggest that GLP-1 is not essential for physiological control of nutrient intake and body weight regulation *in vivo*²³.

Brain: In addition to gut, specific receptors for GLP-1 have been shown in a variety of organs, including lungs, kidneys, heart and CNS. Brain has been identified as a potential target organ for the actions of GLP-1. Considerable amounts of GLP-1 receptors have been found in the Nucleus Tractus Solitarius and GLP-1 has been shown to serve many critical functions in the CNS²⁴. Reports show that stimulation of central GLP-1 receptor leads to slight increase in blood pressure and heart rate and activation of autonomic regulatory neurons in rats, resulting in downstream activation of cardiovascular responses²⁵. Effects of GLP-1 and its analogs on cardiovascular parameters have been discussed in detailed in the next sections.

Many studies have documented that GLP-1 may also be involved in the regulation of the activity of the hypothalamic–pituitary axis. ICV administration of GLP-1 in rats stimulates the secretion of LH, TSH, corticosterone and vasopressin. However GLP-1 does not appear to be a critical factor for the regulation of hypothalamic–pituitary axis, as GLP-1 receptor knockout mice are fertile and demonstrate normal basal levels of plasma osmolarity, corticosterone, thyroid hormones, estradiol, and testosterone^{2, 3, 26}. ICV administration of GLP-1 has been found to improve learning in rats and also proclaimed neuroprotective effects, and thus GLP-1 is now viewed as a promising neurotrophic agent for neurodegenerative diseases³.

Cardiovascular system: As discussed in the preceding sections, GLP-1 receptors are found widely distributed from gut to CNS. Now it turns out that in the heart also, there are GLP-1 receptors as cardiovascular effects of GLP-1 have been evidently defined in many studies^{27, 28}.

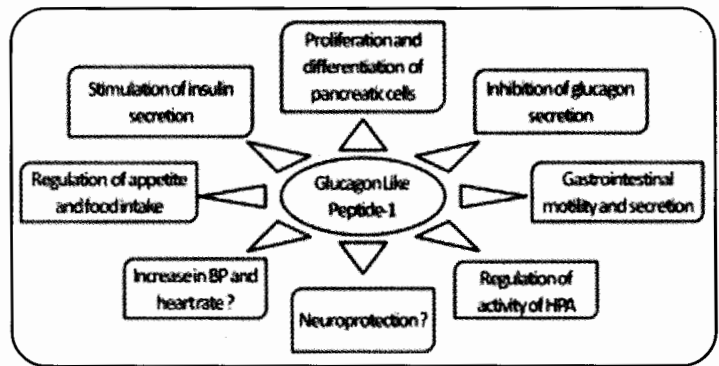


Fig 2 Physiological Functions of GLP-1

GLP-1 increases blood pressure and heart rate regardless of whether administered peripherally or centrally. However differences in the duration of action with different routes of administration was seen as pressor response induced by intravenous route lasted for less than 1 min whereas that induced by central route was more prolonged (5–30 min). Although not backed by any consolidated proof, it has been speculated that it may be a consequence of a rapid degradation of the peptide in the plasma by dipeptidyl peptidase IV. These effects are specifically antagonized by exendin^{9,29}. It is of interest that biphasic pressor response is observed with low doses: a moderate rise followed by a fall in the blood pressure²⁷. The mechanisms explaining such contrasting effects are not yet established.

In addition to nucleus tractus solitarius, GLP-1 receptors are also expressed in the subfornical organ and area postrema of the brain. Not protected by the blood–brain barrier these structures are responsive to regulatory substances present in the blood, thus canvassing the thought that they could be involved in mediating the effects of GLP-1²⁷. Interestingly, it has been found that the pressor response to intravenous GLP-1 does not engage either the vagus nerve or adrenergic receptors, suggesting that with intravenous injection GLP-1 is mediating most of its cardiovascular effects directly through peripheral GLP-1 receptors whereas, response to centrally injected GLP-1 is mediated through central nicotinic receptors and vasopressin systems²⁸. On the other hand, involvement of vasopressin in the pressor effect of GLP-1 has been disputed²⁷. In other study, it was found that both intravenous and ICV administration of GLP-1 receptor agonists increase blood pressure and heart rate which appears to be mediated through the catecholamine neurons in area postrema. It has been further proposed that central GLP-1 system represents a regulator of the sympathetic outflow leading to downstream activation of cardiovascular responses^{25, 29}. Some contrasting results relating to blood pressure were obtained when different animal models were employed for studying the cardiovascular effects of GLP-1 analogs. In a study conducted in Dahl salt sensitive rats, GLP-1 agonist was found to decrease blood pressure³⁰. The reasons for

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the differences in the result is not known, however, it may be due to different animal model, hypertensive Dahl salt sensitive rats versus normotensive rats or related to differences in acute versus chronic administration of GLP-1 agonist.

It is clear from literature that the action of GLP-1 cardiovascular parameters is under dual control in CNS and in peripheral structures and it produces an increase in blood pressure and heart rate in rat; however some interesting results were reported when GLP-1 analogs were recruited in clinical trials. Liraglutide is an analog of human GLP-1 with 97 % homology to endogenous human GLP-1³¹. In a 14 week monotherapy clinical trial liraglutide reduced fasting and post prandial glucose concentration, glycosylated hemoglobin HbA_{1c}, weight and a very low risk of hypoglycemia. In a 52 week randomized, double blind parallel treatment trial comparing liraglutide versus glimeperide monotherapy it was found that liraglutide leads to greater reduction in hemoglobin HbA_{1c}, weight and blood pressure compared to glimeperide. Interestingly data from LEAD (Liraglutide Effects and Actions in Diabetes) studies show that liraglutide produced a consistent, significant reduction in systolic blood pressure³². The mechanisms for this effect are undetermined but initial analyses suggest it may precede, and thus may be unrelated to weight loss.

Future Directions

There is extensive research being conducted in GLP-1 targeted therapies and several products are in various stages of development. The driving interest in GLP-1 targeted therapies includes lack of hypoglycaemia and weight gain along with indication of blood pressure lowering effects. The first drug developed in this area was exenatide by Amylin Pharmaceuticals was approved for marketing in the US in 2005. Exenatide, a GLP-1 receptor agonist, is a synthetic exendin-4 with a half life of 2.4 hours requiring twice daily administration before meals. It has been shown to reduce HbA_{1c}, fasting plasma glucose and body weight. Another GLP-1 product liraglutide being developed by Novo Nordisk is under review and is likely to get marketing approval in US in the first half of 2009. Liraglutide is GLP-1 receptor agonist with a long half life (13 hours) given once a day produces decrease in HbA_{1c}, fasting plasma glucose, body weight and blood pressure. There are several other GLP-1 diabetes products in the pipeline like taspoglutide (R-1583) from Roche, albiglutide from Human Genome Sciences and Glaxo Smith Kline, BMS-686117 from Bristol-Meyers Squibb, GSK-716155 from Glaxo Smith Kline, AVE-0010 from Sanofi-Aventis. Once weekly exenatide is also being developed by Amylin and Lilly using a marketed formulation and they plan to file application with FDA in the middle of 2009. The observation that lowering

of blood pressure occurs with liraglutide is likely to foster extensive research in this area and might lead to better understanding of biological actions of GLP-1 analogues and treatment of Type-II diabetes mellitus.

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