Glucagon-like peptide-1: a major regulator of pancreatic β-cell function
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Abstract
Glucagon-like peptide-1 (GLP-1) is a gut hormone synthesized by post-translational processing in intestinal L-cells, and it is released in response to food ingestion. GLP-1 stimulates insulin secretion during hyperglycemia, suppresses glucagon secretion, stimulates (pro)-insulin biosynthesis and decreases the rate of gastric emptying and acid secretion. GLP-1 has also been shown to have a pro-satiety effect. In addition, it has been demonstrated that a long-term infusion with GLP-1, or exendin-4, a long-acting analog of human GLP-1, increases β-cell mass in rats. In conclusion, GLP-1 appears to regulate plasma glucose levels via various and independent mechanisms. GLP-1 is an excellent candidate option for the treatment of patients with type 2 diabetes mellitus.

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Introduction
The potential use of hormones, other than insulin, for the treatment of diabetes is a relatively new avenue of clinical investigation and therapy. In response to the oral ingestion of food, both insulin and insulin counter-regulatory hormones are released into the circulation (as well as acting in a paracrine fashion) (1). Insulin secretion is influenced by serum concentrations of the absorbed products of digestion, such as glucose, amino acids and fatty acids. In addition, insulin secretion is modulated by secretagogue hormones, termed incretins, which are produced by the intestinal enteroendocrine cells and constitute one arm of the enteroinsular axis (2). The major incretins, the glucose-dependent insulinitropic polypeptide gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) account for about 20 and 80% respectively, of the intestinal incretin effect (3). In non-diabetic subjects, there is a greater insulin response to the oral administration of glucose rather than i.v. administration, for equivalent serum glucose levels, and this is related to the response and action of these two incretins to oral glucose. GIP, but not GLP-1, tends to lose its effectiveness in patients with type 2 diabetes (4), thereby making GLP-1 an attractive candidate treatment for this patient population.

In addition to inducing the secretion of insulin, GLP-1 has been shown to have other profound biological effects on the function of the β-cells. The aim of this article is to critically review the experimental evidence that makes GLP-1 an excellent candidate for the treatment of diabetes.

Gene and protein structure
GLP-1 is a 30 amino acid derivative of proglucagon (PG), a 160 amino acid prohormone (Fig. 1). Mammalian PG gives rise to glucagon, two glucagon-like sequences, named GLP-1 (PG(78–107)) and GLP-2 (PG(126–158)), and other peptide sequences (IP-1 and IP-2) of yet unknown biological activity (1). The amino acid sequence of GLP-1 is 100% homologous in all mammalian species (1, 3) and highly homologous in many lower vertebrates (5), implying that it plays a critical physiological role. The preproglucagon gene, which is a 9.4 kb polynucleotide composed of six exons and five introns (1, 5), located on the long arm of chromosome 2 (5), is expressed in the pancreas and the intestine. This sequence gives rise to preproglucagon, which is then reduced to PG. In the pancreas, the post-translational processing of PG occurs in the α-cells of the islets of Langerhans and gives rise to glucagon (PG(33–61)), glicentin-related pancreatic polypeptide (GRPP), which corresponds to PG(1–30), and a large peptide, major PG fragment (MPGF), which corresponds to PG(72–158) (1, 3, 6). This processing also produces trace amounts of the biologically inactive (7), non-truncated GLP-1(1–36) (5). The L-cells of the ileum, colon and rectum produce glicentin (PG(1–69)), oxyntomodulin (PG(33–69)), GLP-1 (PG(78–107)),

and GLP-2 (PG(126–158)) (1, 5, 6). GLP-1 is cleaved to form the bioactive GLP-1(7–37) molecule, which is then C-terminally truncated and amidated to form the GLP-1(7–36) amide (5). In the gastrointestinal tract, GLP-1(7–36 amide) is secreted from enteroendocrine producing cells (L-cells) in ileal mucosa, including colonic and rectal mucosa, where its secretion is stimulated by intraluminal glucose (1, 3, 8). The plasma half-life of GLP-1 is about 5 min, and the metabolic clearance rate is about 12–13 min (1, 3). GLP-1 is degraded in the plasma by the actions of the enzyme dipeptidyl peptidase IV, whereby the GLP-1 molecule loses its two N-terminal amino acid residues, becoming GLP-1(9–36 amide), which has no known biological activity (3).

**Mechanisms of action**

GLP-1 action is mediated by its binding to a cell surface receptor. GLP-1 receptors (GLP-1-R) are highly expressed on the cell membranes of pancreatic β-cells (3) and the lung, and are also detectable (although in a much lower amount) in the brain, liver, skeletal muscle, adipose tissue and kidney (Fig. 2) (5). The receptor consists of 463 amino acids and belongs to the seven-transmembrane G-protein-coupled receptor family, and is a part of the glucagon/secretin/vasoactive intestinal peptide receptor superfamily (3, 5, 9, 10). The coding sequence of the GLP-1-R is interrupted by 12 introns (11) and is located on the long arm of the human chromosome 6 (5). The binding of the GLP-1-R is highly specific (3, 10), and through the activation of the adenyl cyclase pathway, it causes glucose-dependent insulin secretion (3, 9, 10, 12).

The stimulation of insulin secretion begins with the binding of GLP-1 to its receptor on the β-cell, which stimulates the formation of the second messenger, cAMP (9, 10, 13). The post-receptor signaling pathway involves the activation of cAMP-dependent protein kinase and phosphorylation of key proteins in the control of insulin secretion (9). The phosphorylation of the receptor itself, causing homologous desensitization and internalization of the receptor, occurs at three serine doublet sites that are all located in a 33 amino acid segment of the cytoplasmic tail of the receptor (12).

**GLP-1 analogs**

The short half-life of biologically active GLP-1 has prompted the search and discovery of analogs that may provide an extended GLP-1-like biological activity (Table 1). The peptide with the highest degree of sequence homology has been isolated from Heloderma-idae venom (Gila monster), and is called exendin-4 (5, 14). This peptide contains 35 amino acids, and it avidly binds to and activates the GLP-1-R in β-cells (3, 5), inducing cAMP formation (14). Exendin-4, like GLP-1,
is a potent insulinotropic agent with a much longer in vivo half-life than GLP-1 itself (15), making it a strong candidate for treatment of type 2 diabetes. Exendin-4 has also been shown to increase β-cell mass by both differentiation and neogenesis of precursor cells and by replication of pre-existing β-cells (15). The antidiabetogenic effects of exendin-4, through both increased insulin secretion and the neogenesis of β-cells in the pancreas, show strong promise for exendin-4 to be used in the treatment of type 2 diabetic patients.

Exendin(9–39), a N-terminal truncated product of exendin-4, is a specific antagonist of the GLP-1-R on β-cells (5, 14). It specifically inhibits GLP-1-mediated insulin secretory responses to intraduodenal glucose and the oral intake of nutrients. Extendin(9–39) also inhibits GLP-1-induced cAMP production (14), and it enhances food intake in mice (16). This peptide is of value in scientific studies as a potent inhibitor of GLP-1 action.

Human GLP-1(1–36), a precursor of the biologically active GLP-1, is a GLP-1 antagonist, as well as GLP(9–36), the catabolic product of GLP-1 itself. GLP(9–36) results from the cleavage of GLP-1 in position 7–9 by dipeptidyl peptidase-IV. It has been proposed, although not yet shown, that in human physiology, the loss of the first two amino acids of GLP-1 is likely to play a fundamental role in abolishing the biological action of this very powerful hormone.

**Insulin secretory activity**

GLP-1 is the most potent insulinotropic hormone secreted from the intestinal mucosa in response to food (Table 2) (1, 3). Fasting levels in normal-weight humans are 5–10 pmol/l, rising to approximately 25 pmol/l after eating. The prandial rise of GLP-1 is the major determinant of the early insulin secretory response to a mixed-meal intake, and it represents the so-called ‘incretin effect’ of gastro-intestinal hormones.

The incretin hormones were first identified by Unger and Eisentraut, whose study of the secretory response of islet β-cells demonstrated that 50% of post-prandial insulin release was triggered by the enteroinsular axis (17, 18). The increased insulin response resulting from oral administration of glucose, when compared with the response elicited by an isoglycemic i.v. infusion, has been called the ‘incretin effect’ (18). The incremental difference between the glucose-dependent insulin-response curves in the two situations was attributed to the intestinal release of humoral factors, which were referred to as incretins. This research established the
importance of the enteroinsular axis hormones in the augmentation of insulin secretion (17, 18).

Of the various peptide hormones secreted by the gastro-intestinal tract in response to food, GLP-1, in coordination with GIP, accounts for more than 80% of the whole ‘incretin effects’. In vivo, it has been shown that GLP-1-R knockout mice are glucose-intolerant (19), showing the importance of this one hormone on glucose metabolism. A major limitation of GIP is presented by the observation that, unlike GLP-1, it does not augment insulin secretion in type 2 diabetes (4). In fact, after administration of i.v. GLP-1, the insulin secretory response in non-diabetic and diabetic subjects is remarkably similar (Fig. 3) (20). However, there is a reduced incretin effect when glucose is given orally to type 2 diabetic subjects (21). When administered by s.c. injection for 4 h to subjects with type 2 diabetes, whose fasting blood glucose was poorly controlled on diet and sulfonylurea therapy, GLP-1 was found to normalize the fasting glucose levels (8). When euglycemia was approached, insulin and C-peptide levels decreased, implying that the glucose threshold for insulinotropic action was intact. Additionally, it has been shown in glucose-intolerant rats, that when dipeptidyl peptidase IV is inhibited by isoleucine thiazolidide, therefore preventing the enzymatic degradation of GLP-1, insulin secretion is dramatically increased and glucose tolerance is restored to near-normal levels (22). These findings provide valuable information for the future treatment of type 2 diabetes, suggesting that even long after sulfonylurea secondary failure, GLP-1 therapy is still an option for the treatment of type 2 diabetes (23). So far, genetic studies have not uncovered any inherited defects of the GLP-1-R that may be associated with diabetes (24, 25). Therefore, GLP-1 has potential use in all type 2 diabetic subjects (4, 26, 27).

In patients with type 1 diabetes, infused GLP-1 reduces the fasting blood glucose levels, decreases the calculated isoglycemic meal-related insulin requirements, and significantly increases glucose utilization (8, 27, 28). As this cannot be ascribed to the increase of insulin secretion, it is almost certainly due to other mechanisms for controlling blood glucose concentrations, such as gluconeogenesis inhibition, decreased gastric motility and glucagon suppression.

**Other mechanisms for GLP-1 hypoglycemic activity**

GLP-1 regulates the concentration of glucose in the plasma by mechanisms other than stimulating insulin secretion (Table 2, Fig. 2). These include the inhibition of glucagon secretion (1, 3, 4, 6, 27) and the inhibition of gastric motility (16, 29).

By inhibiting the α-cells of the pancreas, GLP-1 decreases glucagon concentrations, which, in turn, decreases hepatic glucose production by inhibiting gluconeogenesis and glycogenolysis (3, 8, 30). This antiglucagon activity of GLP-1 is preserved in subjects with both type 1 (29, 31) and type 2 diabetes (4, 32).

GLP-1 has been shown to be responsible for the so-called ileal brake effect, which is characterized by the inhibition of gastric motility and secretion. i.v. infusion of GLP-1 delays gastric emptying in both diabetic (29, 32) and non-diabetic subjects (16), with a profound effect on both the lag phase and the emptying rate of liquids and solids (29). It has also been shown to

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**Table 2 GLP-1 biological action in health and disease.**

<table>
<thead>
<tr>
<th>Target tissue</th>
<th>Biological action(s)</th>
<th>Health</th>
<th>Type 1 diabetes</th>
<th>Impaired glucose tolerance</th>
<th>Type 2 diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreas</td>
<td>Stimulation of insulin secretion</td>
<td>Present (7, 20, 54)</td>
<td>Absent</td>
<td>Preserved (22, 37)</td>
<td>Preserved (24, 25)</td>
</tr>
<tr>
<td></td>
<td>Inhibition of glucagon secretion</td>
<td>Present (4, 7, 20, 54)</td>
<td>Absent</td>
<td>Preserved (37)</td>
<td>Preserved (4, 20, 23, 32)</td>
</tr>
<tr>
<td></td>
<td>Transcription of islet-specific genes</td>
<td>Present (37)</td>
<td>Absent</td>
<td>Preserved (37)</td>
<td>Not-determined</td>
</tr>
<tr>
<td></td>
<td>Increase of islet cell mass</td>
<td>Not-determined</td>
<td>Likely present</td>
<td>Preserved (39, R Perfetti, unpublished observations)</td>
<td>Not-determined</td>
</tr>
<tr>
<td>Gastrointestinal tract</td>
<td>Inhibition of gastric acid secretion</td>
<td>Present (6, 35)</td>
<td>Absent</td>
<td>Not-determined</td>
<td>Not-determined</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>Inhibition of gastric motility</td>
<td>Present (6, 29, 35)</td>
<td>Absent</td>
<td>Not-determined</td>
<td>Not-determined</td>
</tr>
<tr>
<td>Other tissues (fat, muscle, liver)</td>
<td>Promotion of satiety Increase of insulin sensitivity/glucose disposal</td>
<td>Present (41, 42)</td>
<td>Not-determined</td>
<td>Preserved (19)</td>
<td>Preserved (16, 29, 32)</td>
</tr>
</tbody>
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decrease small intestine transit by inhibiting the action of the smooth muscle directly (33), via the GLP-1-R (34). This near-inhibition of the gastro-intestinal motility reduces the availability of nutrients for absorption, which diminishes the meal-induced glucose excursions and lowers the need for rapid insulin secretory responses (1, 4, 8, 27). GLP-1 also inhibits gastric acid secretion (6, 35), which delays enzymatic breakdown and absorption of nutrients (3). All of these actions are beneficial for both type 1 and type 2 diabetic patients, who have little or no insulin supply to maintain metabolic balance. Because the reduction of meal-induced glucose excursion is also lowered in type 1 diabetic patients, it proves that the GLP-1 effect in these subjects is independent of \( \beta \)-cell stimulation and insulin secretion.

**Regulation of \( \beta \)-cell-specific genes**

GLP-1 has been shown to regulate the expression of islet \( \beta \)-cell-specific genes, both in vitro (36) and in vivo (37). Using an insulinoma cell line, Wang et al. (36) demonstrated that in addition to inducing an increase in insulin secretion in a glucose-dependent fashion, GLP-1 enhances the mRNA levels for the various \( \beta \)-cell-specific genes. These included insulin and the two major regulators of glucose utilization, the glucose transporter (GLUT-1) and the glucose-phosphorylating enzyme (hexokinase-1). GLP-1 was shown to regulate the gene transcription of GLUT-1 and hexokinase-1 and the mRNA stability of insulin (36).

Using a different in vitro experimental model, I also proposed an effect of GLP-1 on the transcription of the insulin gene (R Perfetti, unpublished observations). A recent report demonstrating that GLP-1 promotes the interaction between the insulin transcription factor IDX-1 and the promoter region of the insulin gene itself further supports the significance of GLP-1 action on the regulation of gene transcription (38). Taken together, these data demonstrate that GLP-1 enhances glucose transport and glucose metabolism via a novel mechanism by which the response of \( \beta \)-cells to glucose could be improved.

The assessment of the biological effect of treating glucose-intolerant aging Wistar rats with GLP-1 strongly supports the in vitro data (37). In this study, GLP-1 was shown to potentiate the glucose-dependent insulin secretion and reverse the glucose intolerance associated with ‘normal’ aging in Wistar rats (Fig. 4). An increase in the total cellular mRNA for insulin, GLUT-2 (the \( \beta \)-cell glucose transporter) and glucokinase (the rate-limiting enzyme for glucose utilization by the \( \beta \)-cell) resulted from the treatment with GLP-1. These effects were inhibited by the treatment with a GLP-1-R antagonist in conjunction with GLP-1 (37). Similar effects were observed by treating glucose-intolerant sub-pancreatectomized rats with the GLP-1 agonist exendin-4 (39).
These findings suggest that GLP-1 may be able to induce changes in the functional activity of β-cells that appear much more profound than those associated with the well-documented effect on insulin secretion. It remains to be determined whether the same phenomena can occur in humans.

**Effects on β-cell mass**

Recent studies using a long-term infusion of GLP-1 or a single-dose injection with the long-acting analog of GLP-1, termed exendin-4, have demonstrated a novel biological function of these peptides in glucose-intolerant and diabetic rats. In sub-pancreatectomized rats (39) and in aging rats (R Perfetti & H Hui, unpublished observations), exendin-4 and GLP-1 respectively were able to stimulate both the replication and the differentiation of islet cells, and this induced a dramatic improvement in glucose tolerance. These changes resulted from an increase in β-cell mass deriving from the proliferation of cells within the islet and from the differentiation of the ductal epithelium into insulin-secreting cells.

Indirect evidence that GLP-1 may promote the expansion of the β-cell mass is provided by a recent study investigating the changes observed in pancreatic islet cells after treating rodents with streptozotocin (40). As the animals develop diabetes, due to the toxic effect of streptozotocin, a compensatory regeneration of islet cells occurs and this is associated with the synthesis of GLP-1 by the surviving α-cells. The ability of the α-cells to utilize the preproglucagon molecule to synthesize GLP-1 rather than glucagon may play a role in the attempt to expand and/or regenerate the mass of β-cells.

This observation substantially enriches, and somehow reframes, the general understanding of the role of GLP-1 in the physiology of islet cells. The possibility of increasing β-cell mass in insulinopenic subjects has obvious relevant implications for the treatment of individuals with diabetes. Indeed, an absolute or a relative insufficiency in β-cell mass are common biological features of type 1 and type 2 diabetes. The correction/improvement of this pathogenesis that leads to hyperglycemia with a gastro-intestinal hormone that is physiologically expressed in humans has two major
implications in the biology of diabetes: (i) islet cells are capable of proliferating in mature individuals, even in a diabetic setting; (ii) the islet cell mass can be increased by exposure to local growth factors.

**Extrapancreatic effects**

GLP-1 has also been proposed to exert a biological effect in tissues other than the gastro-intestinal system (Fig. 2). GLP-1 and its specific receptors are also present in the hypothalamus (5, 16, 41) and several target tissues for insulin action. The biological effect of GLP-1 in these tissues has been suggested to be involved with decreased food intake (hypothalamus) and increased insulin sensitivity (skeletal muscle and adipose tissue).

The GLP-1-R located in the hypothalamus have been shown to have an affect on satiety. In rats, i.c.v. GLP-1 injection powerfully inhibits feeding, an effect that is reversed by the GLP-1-R antagonist, extendin(9–39) (41). This GLP-1 injection stimulated neuronal activation exclusively in the paraventricular nucleus of the hypothalamus and central nucleus of the amygdala, regions of primary importance in the regulation of feeding (41). This effect is also seen in healthy (42) and overweight humans, where i.v. GLP-1 infusion increased satiety and decreased caloric intake (16). In a recent study (43), a 48 h continuous s.c. infusion of GLP-1 in type 2 diabetic patients decreased hunger and prospective food intake and increased satiety (43). These effects were absent in GLP-1-R knockout mice (19), suggesting that this receptor plays a critical role in promoting satiety. These effects are of profound significance for the treatment of type 2 diabetic patients, where caloric intake and weight management are of paramount importance.

It has been shown that GLP-1, at physiological concentrations, directly stimulates glycogen synthesis in rat skeletal muscle (44) and rat adipose tissue (45), which is accompanied by an increase in glycogen synthase-α activity (44), which will lower plasma glucose levels. Glycogen synthase-α activity is also increased in hepatocytes from normal and diabetic rats (46). However, other studies were not able to reproduce the findings (47), and to date, this controversy has not yet been solved. A recent study showed that at high, but not low, insulin levels, GLP-1 increases glucose utilization in vivo using depancreatized dogs (48). However, a previous study (49) showed no effect of GLP-1 on glucose metabolism in healthy dogs. In humans, GLP-1 has been shown to have no effect on insulin sensitivity in non-diabetic (50) and NIDDM subjects (51); however, GLP-1, at physiological concentrations, has been shown to increase glucose disposal (50, 52), perhaps by stimulating glycogen synthesis in hepatocytes and muscle cells. The receptor that mediates these extrapancreatic activities of GLP-1 has been proposed to be different from the classic GLP-1-R present in the β-cells. Alternatively, a coupling of the classic GLP-1-R with a different G-protein has been postulated for tissues other than the endocrine pancreas. Both of these hypotheses would be compatible with the observation that in those tissues, the activation of the receptor does not increase cAMP content (44). The controversial results in muscle and adipose tissue, along with the lack of increase in cAMP levels, could also be due to the ability of GLP-1 to bind to other receptors in these tissues, especially at pharmacological concentrations.

In addition to the muscle, fat and brain tissue, GLP-1 mRNA has been detected in the kidney, heart and liver (53). It is unknown what the function of these receptors is in these tissues.

**Summary and conclusions**

The unique biological features of GLP-1 make this peptide hormone an ideal candidate agent for the treatment of diabetes. The ability of lowering post-prandial hyperglycemia, via three independent mechanisms of action (increased insulin secretion, inhibition of glucagon release, inhibition of gastrointestinal motility), provides an unprecedented advantage when compared with any pharmacological agent currently available for the treatment of diabetes. Perhaps even more importantly, it is the observation that the insulin secretory action of GLP-1 is regulated by the plasma concentration of glucose, virtually preventing the possibility of developing reactive hyperglycemia while inducing the release of insulin. Finally, it is of significant clinical relevance that GLP-1 retains its glucose-lowering activity in patients with diabetes, even many years from its clinical onset and when islet β-cells are no longer responsive to pharmacological insulin-secreting agents.

In addition to these very well-characterized physiological properties, novel biological actions of GLP-1 have been recently proposed. These include the regulation of islet cell-specific genes, the proliferation of pancreatic cells, the regulation of appetite, and, finally, the potentiation of insulin action at the level of its major target tissues (i.e. muscle, fat and liver). The investigation of these findings (which are independent from the well-characterized insulin secretory activity) is still at its early stage, and the significance in normal human physiology has not yet been fully elucidated. It is, however, unquestionable that they directly indicate the great scientific interest and the potential impact that the use of GLP-1 may have in the pharmacological treatment of diabetes.

Despite this promising amount of data, some limitations for the possible use of GLP-1 in humans are clearly evident. The major drawback of GLP-1 is its short biological half-life. Even when given s.c., its peak concentrations have returned to baseline within 90 min (54). However, when GLP-1 is given continuously to subjects with type 2 diabetes, blood glucose is normalized (55), appetite is reduced (43) and, more importantly, post-prandial glucose excursions are also
blunted (56), all with no apparent side effects (43). These findings demonstrate the importance of improving the methods of GLP-1 delivery, as the benefits strongly support the time and effort involved in finding a solution to its short half-life. In order to delay the degradation of GLP-1, the properties of the injectable form need to be modified. Possibilities include the preparation of GLP-1 with protamine or zinc, as has been done with the insulin molecule. Similarly, altering the properties of one or more of the amino acids of GLP-1, such as by acylation, may prolong its action.

In the past 3–5 years, the pharmacological treatment of type 2 diabetes has witnessed an unprecedented flourishing of new drugs with numerous capabilities: (i) potentiating the secretory activity of the β-cell, (ii) limiting the absorption of carbohydrates, (iii) inhibiting the glucose production by the liver, and (iv) enhancing the action of insulin at the level of target tissues. This complex repertoire of synthetic agents is likely to profoundly change the way we manage diabetes, and, consequently, its natural course. GLP-1 deserves to be considered as the best candidate of the new class of naturally occurring biological agents that may improve the metabolic control of diabetes in a more physiological manner.

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